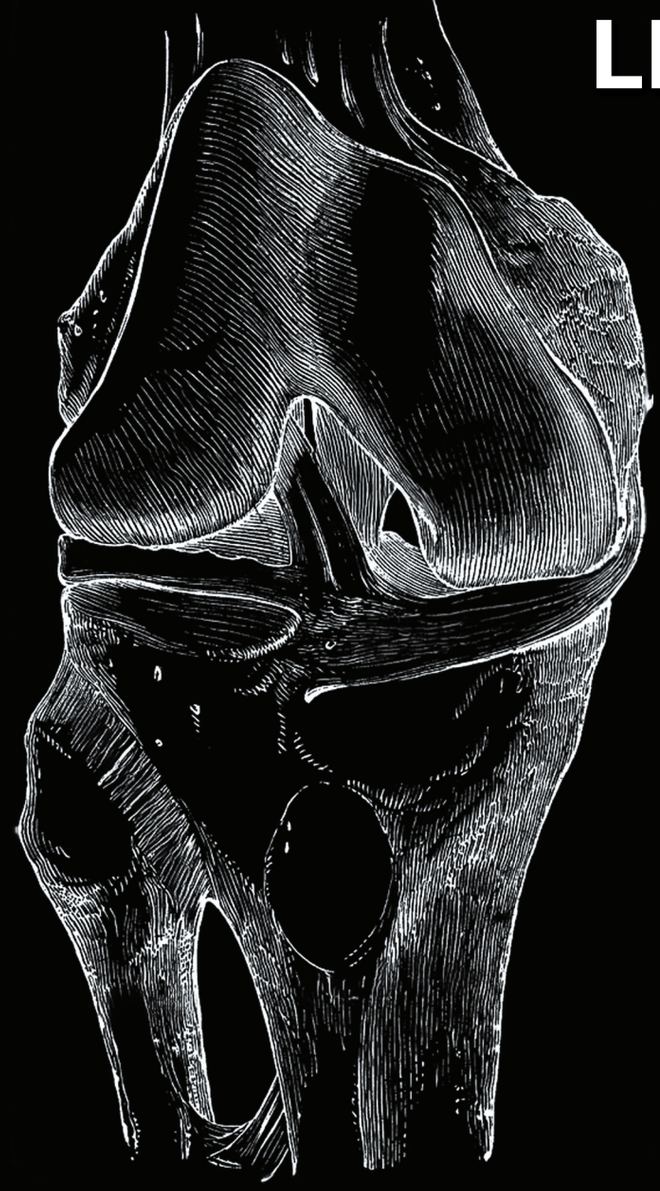
Knee Joint Preservation Throughout Life

RALPH JEUKEN

KNEE JOINT PRESERVATION THROUGHOUT LIFE



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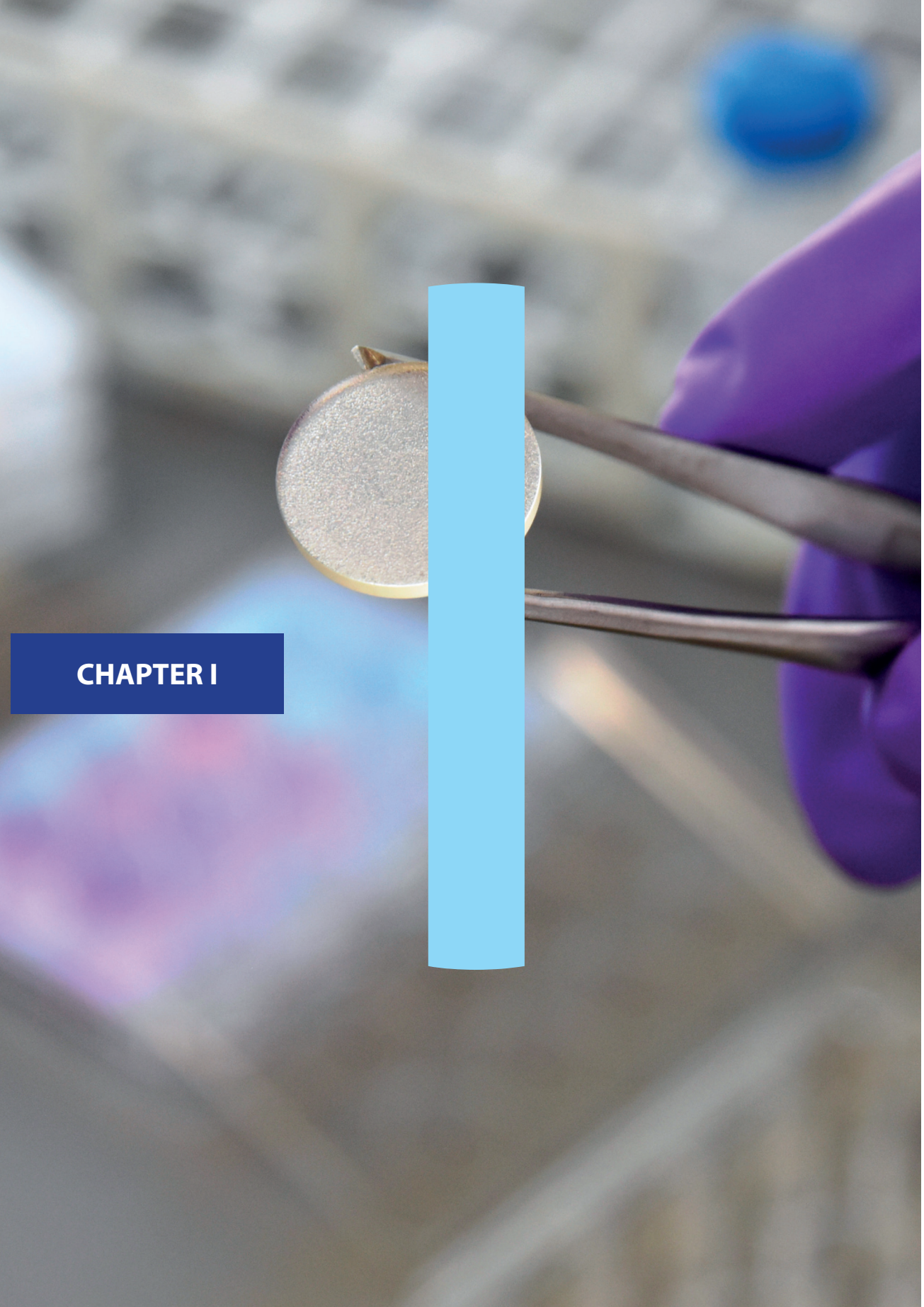

CHAPTER I

OUTLINE AND AIMS OF THIS THESIS

Chapter I – Outline and Aims of this Thesis

Physical activity is paramount for people's health and quality of life. Participation in physical activity improves mental health at all ages and delays the need for care in adults over 65 years of age. Physical inactivity on the contrary, has been linked to coronary heart disease, colon and breast cancer, type 2 diabetes and premature all-cause mortality (1, 2).

Diseases of the knee joint, such as cartilage defects and osteoarthritis (OA), can lead to decreased levels of physical activity, dropout from sports, kinesiophobia, chronic pain, and decreased quality of life (1, 2).

Articular cartilage is a specialized connective tissue, important to allow pain-free, almost frictionless motion of major joints such as the knee. Articular cartilage consists of a dense extracellular matrix predominantly composed of type II collagen fibers, proteoglycans and chondrocytes. Articular cartilage is hypocellular, aneural, avascular, and relies on diffusion from the synovial fluid for nutrient and waste product exchange. All these characteristics contribute to its limited intrinsic regenerative capacity (3). Focal articular cartilage defects, from here on referred to as 'cartilage defects', are defined by damaged articular cartilage, leading to lesions which sometimes also involve the subchondral bone (4). Cartilage defects can give rise to pain and functional impairment (4, 5), comparable to patients with end-stage OA scheduled for total knee arthroplasty (TKA) (6). Cartilage defects can develop at any age. In children, cartilage defects are often seen as part of juvenile osteochondritis dissecans (OCD), or in rare occasions resulting from traumatic shear-off injuries. In adults, cartilage defects can result after an acute trauma such as a twisting injury. Cartilage defects in adults are also seen in repeated micro-trauma, for instance after repetitive patellar (sub-) dislocations or repetitive overload of the inner side of the knee due to congenital varus (bow leg, outward bending) knees (4, 5). Similar as in children, adults can also suffer from OCD. By themselves, cartilage defects are an important risk factor for OA development (4). Particularly large and deep cartilage defects and untreated OCDs carry a high OA risk (7, 8).

There is a plethora of treatments available for cartilage defects aiming to improve function and reduce the pain. Conservative therapies such as physiotherapy and weight loss are generally considered first for small defects and low demand patients. For larger symptomatic defects, treatments include the regenerative bone marrow stimulation (BMS) technique 'microfracture' (MF), regenerative cell-based techniques such as autologous chondrocyte implantation (ACI), and substitutive bone-based techniques such as osteochondral auto- and allografting. High tibial osteotomies (HTO) are often considered as an extra-articular salvage procedure, but becomes more and more prevalent as adjunct to cartilage repair. Salvage procedures, such as unicompartmental or total knee arthroplas-

ties (UKA and TKA), are considered when cartilage defects have progressed to end-stage OA (3, 9). The choice is dependent on patient and defect characteristics, surgeons' preference, availability of treatments and their reimbursement (9).

A patient's age is not yet a strong discerning factor in contemporary treatment algorithms, while increasing age has been found to be a negative prognostic factor for cartilage repair outcomes for the frequently used regenerative treatments such as MF (9, 10). Differences in the treatment selection are evident at the ends of the age spectrum: in children, the regenerative potential is theoretically high, life expectancy long, and thus all efforts should be made to preserve the native knee joint. In the low-demanding older patient, the opposite applies, and TKA is a good treatment choice. The matter becomes complex and challenging in middle-aged patients (40-60 years). The middle-aged form the largest group treated for cartilage defects in international literature (11-14), but at the same time their regenerative potential starts to deteriorate at an unknown pace, potentially leading to inferior outcomes after regenerative cartilage repair (15, 16). On the other hand, arthroplasties in middle-aged patients have a high risk for dissatisfaction (10-25%) and a high lifetime risk of revision (up to 35%) (17-20). Revision knee arthroplasties are notorious for less functional outcomes, more complications and high costs (17, 18). Hence, orthopedic surgeons describe a treatment gap for middle-aged patients (21). Despite this knowledge, at present, patients between 45 and 55 years of age are the fastest-growing 'users' of TKAs (22, 23).

Knee joint preservation in this thesis is defined as all treatments, conservative and operative, that improve knee symptoms and thereby function by postponing or eliminating the need for an arthroplasty before the age of 60. Although not often appreciated, postponing the first TKA by only 5 years leads to 17% less revisions later in life (24).

Focal Knee Resurfacing Implants (FKRIs) are specifically designed for the middle-aged patient suffering from cartilage defects (25-27). During an FKRI procedure, the defect site is prepared by drilling, removing all the affected cartilage and subchondral bone. Then, the FKRI is implanted, with the implant replacing both the cartilage and bone at the defect site. By only replacing the affected site, the rest of the joint is preserved including the opposing knee compartment (3, 9). As its name already implies, the focal approach of FKRIs is what these implants distinguishes from UKAs and TKAs, in which the whole compartment or the whole knee is replaced, including removing bone on both the femur and tibia and often also removing cruciate ligaments.

Current commercially available FKRIs are either completely or partially made of metals, such as titanium and cobalt/chromium, raising concerns due to the mechanical property mismatch with the surrounding and opposing native host tissues (28-31). The elastic

modulus of these implant materials shows great disparities relative to native cartilage and bone tissue and increased friction when articulating with native cartilage. In turn, this could lead to damage to the opposing cartilage and osteolysis due to stress shielding (32). Polymer FKRIs could potentially overcome the drawbacks of metal FKRIs and could thus become an important treatment for improving joint preservation treatments.

The aims addressed in this thesis are designed to facilitate orthopedic surgeons to preserve patients' native knees as long as possible, keep patients moving and improve their quality of life. The aims of this thesis are as follows: first, to reflect on the effect of advancing age on the outcomes of various cartilage repair procedures and how this influences treatment selection; second, to reflect on biomaterial selections for cartilage repair procedures considering the needs of various patient groups; third, to investigate the feasibility of a novel non-degradable polymer FKRI. The main focus in the animal trials of this thesis is the optimization of the osseointegration the novel polymer FKRIs.

Cartilage Repair in Children: Refixation of Chondral Fragments

Children have a high regenerative potential, thanks to fully potent chondrocytes and mesenchymal stem cells (33). Children also have a longer expected remaining lifetime than adults and will have a higher physical demand in their remaining lifetime.

It is thus of utmost importance to utilize the high regenerative potential in children to optimize treatment, prevent early degeneration and preserve the native joint.

Cartilage defects in children can be the result of trauma, OCD or osteonecrosis (33). Children and adolescents are especially susceptible to shear-off lesions, which are also known as osteochondral fracture (33, 34). This is due to the fact that in the developing joints, the articular cartilage is anchored by relatively weak interdigitating fingers of uncalcified cartilage penetrating into the subchondral bone, while in mature joints, cartilage is anchored in the subchondral bone through a well-defined calcified cartilage layer (33). Research has shown that after high shear forces were applied to bovine cadaveric osteochondral plugs, the plane of separation occurs along the tidemark in mature samples, leaving the osteochondral junction intact. However, in samples obtained from immature joints, failure occurs primarily in the bony tissue (33, 34). Ex vivo studies showed that the energy required to shear-off cartilage from subchondral bone was much lower in immature joints in comparison to mature joints (33, 34). Besides the subchondral anatomy differences, children also tend to have more laxity in their joints, which leads to higher shear forces (34). If the shear forces exceed the threshold for rupture, the cartilage is sheared off the underlying bone, resulting in a cartilage defect and loose fragment, i.e., corpus liberum. The amount of bone attached to this cartilage corpus liberum is very thin

and these 'fractures' are consequently often missed on plain radiographs. The resulting cartilage defects are often large and have been described up to 8 cm^2 (35).

The loose chondral fragments are frequently deemed unviable and therefore discarded (33). Children are consequently treated using established techniques such as ACI. If, however, loose chondral fragments could be reattached to the subchondral bone of the defect site and remain viable over time, then this would render a relatively cheap one-step repair technique. Survival of such autograft resurfacing in terms of viability and attachment to the subchondral bone would also contradict the dogma – at least in children – that loose cartilage is unviable and cannot be reattached. It would be testament to the high regenerative potential of children.

As a proof of concept, in the first chapter of this thesis, a novel repair strategy dubbed the 'modified hedgehog technique' was used for the first time in children to treat three patients with a shear-off lesion and a loose chondral fragment. The survival of this 'autograft resurfacing' is monitored using magnetic resonance imaging (MRI). The objective of chapter III is:

To assess if a loose pure chondral fragment will remain in place and appear viable on MRI 1 year after refixation using the "Modified Hedgehog Technique" in children.

Treatment of Cartilage Defects in Middle-aged: The Treatment Gap

There are no official international age cut-offs for the term 'middle-aged', but the age between 40 and 60 or sometimes somewhat broader from 35 to 65, is generally accepted as 'middle-aged' (5, 36). Large database studies from the US and Germany show that most patients that undergo cartilage repair surgery are middle-aged (12, 13, 37).

Due to changing demographics including higher sports participation, the obesity pandemic and changing demands, there is a projected rise in the middle-aged patient seeking help for cartilage damage (38, 39). An increase in sports participation will not only translate to sport-related knee injuries, but also higher physical demands in older individuals (38, 39). Moreover, the middle-aged patient is not someone who is on the verge of approaching old age nowadays. but a much more active person than decades ago (38). Hence, orthopedic surgeons are facing a growing middle-aged population seeking help for cartilage damage and the desire to remain capable of fully participating in life (23).

Advancing age has been shown to lead to an impaired regenerative potential (10, 40). This fundamental insight seems to be neglected when it comes to cartilage repair. Most clinical studies evaluating outcomes of cartilage repair include an upper age limit as inclusion criteria, which is usually around 40-50 years (41). The average age of subjects

in those studies is consequently even lower and clinical outcomes cannot be considered to be representative for the typical middle-aged patient (10). It is also conceivable that cartilage defects in middle-aged are already an expression of early osteoarthritis, indicating that these defects are surrounded by an impaired joint homeostasis (42). Combined with the inherent suboptimal environment for tissue regeneration, due to the absence of blood supply and low cellular number in cartilage, it is imaginable that age plays a crucial role in cartilage repair.

UKAs or TKAs are often considered when patients are in the older range of middle-aged or when the disease process is more on the osteoarthritis side of the disease spectrum. Unfortunately, still 10-20% of patients remain dissatisfied after TKA. One of the prognostic factors for dissatisfaction is implantation at an age < 65 years (17). Receiving an UKA or TKA at a young age is also a risk factor for revision later in life. Revision surgeries carry the risk of lower functional outcomes, and are notoriously known for high complication rates and higher costs (43, 44). Although the drawbacks of arthroplasties at young age are widely known, the rise in TKAs is expected to be the largest in the < 65 years group, due to the aforementioned changing demographics and patient demand (22, 39). This trend is set to continue with predictions that over 50% of TKAs in the United States will soon be undertaken in patients aged < 65 years (20).

Orthopedic surgeons consequently face a treatment gap for middle-aged patients suffering from cartilage damage (21). On the one hand, there are concerns that cartilage repair is negatively influenced by an increasing age. On the other hand, these patients are considered too young for a durable knee arthroplasty, with a high risk for revision or dissatisfaction. Indeed, when asked, orthopedic surgeons perceive a need for better treatments in patients < 60 years (21).

It is not known if advancing age affects all cartilage repair treatments equally, or if some treatments are more susceptible for age-related changes than others. In chapter IV, the scientific evidence on the effect of advancing age on outcomes after different cartilage repair treatments are systematically retrieved from appropriate databases and consequently evaluated. The results of the included studies are combined with insights from fundamental research on the effects of ageing and put in appropriate perspective. The objective of chapter IV was:

To systematically review available literature on knee cartilage repair in middle-aged patients and include studies comparing results between middle-aged and young patients.

The Netherlands do not have a dedicated cartilage registry. It is largely unknown how most patients are treated. Dutch experts in the field have composed guidelines and it is at least the intention that Dutch orthopedic surgeons conform to these guidelines. The latest Dutch Cartilage Consensus Statement was published in 2019, succeeding the 2011 consensus statement.

Chapter V provides a nationwide survey on cartilage repair strategies amongst knee specialist orthopedic surgeons in the Netherlands. General questions posed are related to the surgeon's experience, characteristics of typically treated patients, defect type, utilization of available therapies, and application of concomitant treatments. There is a special emphasis on the treatment of loose chondral fragments, patients in different age categories and the perceived effect of aging. Comparison of actual practices to the 2019 Dutch Cartilage Consensus Statement is an integral part of this study. The objective of this study was:

To provide insight in the applied cartilage repair techniques with special emphasis on age and the adherence of Dutch orthopedic surgeons to the Dutch Cartilage Consensus Statement in the Netherlands.

Novel Treatments in Cartilage Repair and the Role of Biomaterials.

Research groups around the world are constantly trying to develop new cartilage repair techniques that are truly capable of restoring articular cartilage to its native structure and composition. Treatments are either potential improvements of established treatments or are completely new approaches to the matter. For instance, efforts have been made to improve the results of MF by providing a biomaterial scaffold or by the means of orthobiologics, i.e. adding active organic materials to enhance biological response (45). Both aim to improve the cellular response, i.e. chondrogenesis. Other research aims to improve the single-surgery cell-based therapies. An example is minced cartilage implantation, in which non-weight bearing healthy cartilage and debrided cartilage defect edges are minced intra-operatively and placed back in the defect site using fixatives such as fibrin glue (46).

A different approach, in contrast to the aforementioned approaches, are the FKRIs. A FKRI is composed of a non-degradable top-layer which has a smooth surface to articulate with the opposing meniscus and cartilage, and a stem that is able to osseointegrate in the subchondral bone for durable fixation (27). Hence, FKRIs mainly rely on osseointegration rather than chondrogenesis for achieving successful treatment. This is similar to osteochondral auto- and allografts, but without their drawbacks of geometry matching, shelf life and availability issues. Most of the investigated FKRIs are partially or completely composed of established orthopedic metals such as titanium and cobalt-chrome. Con-

cerns were raised about damage to the opposing cartilage and short treatment longevity following the clinical introduction of the first generation FKRIs (47). There is a large disparity in elastic modulus between metal and cartilage and the coefficient of friction (COF) between metal and cartilage is also much higher than the COF for cartilage on cartilage (28, 48-50). High stiffness metals also show a large disparity with the subchondral bone and could lead to stress shielding (51). Routine follow-up of the intra-articular tissues of the knee using magnetic resonance imaging (MRI) could also be hampered by metal artefacts (52).

In order to develop a new cartilage treatment, an in-depth understanding of currently used biomaterials and potential future biomaterials is mandatory. Polymers in particular have the capacity to be tailored in terms of properties to match the physiochemical properties of intended use site. Both degradable as well as non-degradable polymers are increasingly being applied in bone and cartilage tissue engineering. The objective of chapter VI is:

To provide an overview of cartilage physiology, repair considerations and polymers in clinical and preclinical cartilage repair research

The Development of a Novel, Polycarbonate-urethane, Focal Knee Resurfacing Implant

The following and experimental part of this thesis is part of the development of a non-degradable polycarbonate-urethane (PCU) FKRI. It is designed to overcome the aforementioned drawbacks of metal FKRIs. The two chapters are the result of the SyCaP (Synthetic Cartilage Plug) project, part of the multi-disciplinary collaboration Chemelot Institute for Science and Technology (InSciTe) Biomedical. InSciTe Biomedical is a public-private research and technical validation institute partnering Maastricht University, Maastricht University Medical Center, Eindhoven Technical University, Royal DSM and the Province of Limburg.

In chapter VII, as a proof-of-concept study, we designed a simple bi-layered PCU FKRI. The implant is composed of a hard-grade PCU bottom layer, Bionate® (DSM Biomedical BV, Geleen, The Netherlands) Shore Hardness 75D (B75D), for the purpose of osseointegration in the subchondral bone. The top consists of a softer PCU layer, Bionate® 80A (B80A), with mechanical properties mimicking the native cartilage to articulate with the opposing cartilage. As opposed to metal implants, polymers have a less fortunate history in terms of osseointegration due to their physical properties, with many papers describing a fibrous tissue interface between the implant and bone (53). Therefore, chapter VII starts with the development of a surface modification to enhance the osseointegration of the

B75D. For this purpose, different coatings were introduced on B75D discs and tested *in vitro*. The first objective of chapter VII was:

To assess the biocompatibility of B75D, its surface modifications and if these modifications enhance its osteoconductive properties in vitro in comparison to untreated B75D.

In the second part of chapter VII, the best performing *in vitro* surface modification was applied to the B75D stem of bi-layered PCU implants. The osseointegration of this implant was subsequently tested *in vivo* in a goat model. Uncoated and titanium implants serve as control group. All implants were implanted in the knees of Dutch milk goats. Animals were followed for 3 months after which bone histomorphometry was performed for osseointegration evaluation. The second objective of chapter VII was

To assess if the application of a biphasic calcium phosphate coating to a B75D implant enhanced its in vivo osseointegration in comparison to uncoated B75D and titanium implants.

The ultimate assessment of osseointegration is still preferably quantitatively analyzed using bone histomorphometry. Inherent to bone histomorphometry is the need to use laboratory animals. Particularly if the osseointegration process needs to be evaluated at different time points, this inadvertently demands more animals in the same study. With the increasing efforts to limit the use of animals in trials and if used, to expand their usability(reduce, refine and replace) (54), methods to predict osseointegration in a non-destructive manner could limit the number of required laboratory animals. In the third part of chapter VII, the potential of positron emission tomography PET/CT-scanning in predicting osseointegration was evaluated. The Dutch milk goats with the implants *in situ* were PET/CT scanned at 3 and 12 weeks and the results were compared with the bone histomorphometry. The third objective of chapter VII was:

To visualize the bone metabolism surrounding to the implant using in vivo PET/CT-scanning and to determine whether this modality has a predictive discriminating potential for ultimate osseointegration

Chapter VIII describes the second large animal trial study of this thesis. Mimicking the mechanical properties of native bone tissue was important for an implant's osseointegration. A composite of zirconium oxide (ZrO2) and hard-grade PCU (Bionate 75D) (B75D-ZrO_2) material was developed to closely approximate the mechanical properties of the distal femoral trabecular bone. In the first part of this second animal trial the mechanical properties of this novel biomaterial were characterized. The first objective of chapter VIII was:

To assess if the B75D-ZrO_2 composite approximates the mechanical properties of subchondral bone better than B75D

The second objective of chapter VIII was:

To assess the biocompatibility and osteoconductive properties of the B75D-ZrO_2 in vitro and compare it to the untreated B75D.

In the third part of chapter VIII the *in vivo* osseointegration of the B75D-ZrO_2 composite biomaterial was evaluated. Mushroom-shaped implants were manufactured with an articulating soft-grade PCU top layer (B80A) and a B75D stem or B75D-ZrO_2 stem. Surface modification was applied to both the B75D and B75D-ZrO_2 stems. Metal implants served as control. All implants were implanted in the knees of Dutch milk goats. Animals were followed for 6 months after which bone histomorphometry was performed for osseointegration evaluation. The third objective of chapter VIII was:

To assess and compare the B75D-ZrO_2 composite in vivo osseointegration with the B75D and titanium implants after 6 months.

Chapters IX and X present the general discussion and impact analysis, integrating the findings from the individual studies of this thesis. For a comprehensive background on knee anatomy, alignment, and joint homeostasis, as well as an overview of cartilage defects, osteoarthritis, and current treatment options, the reader is referred to Chapter II.

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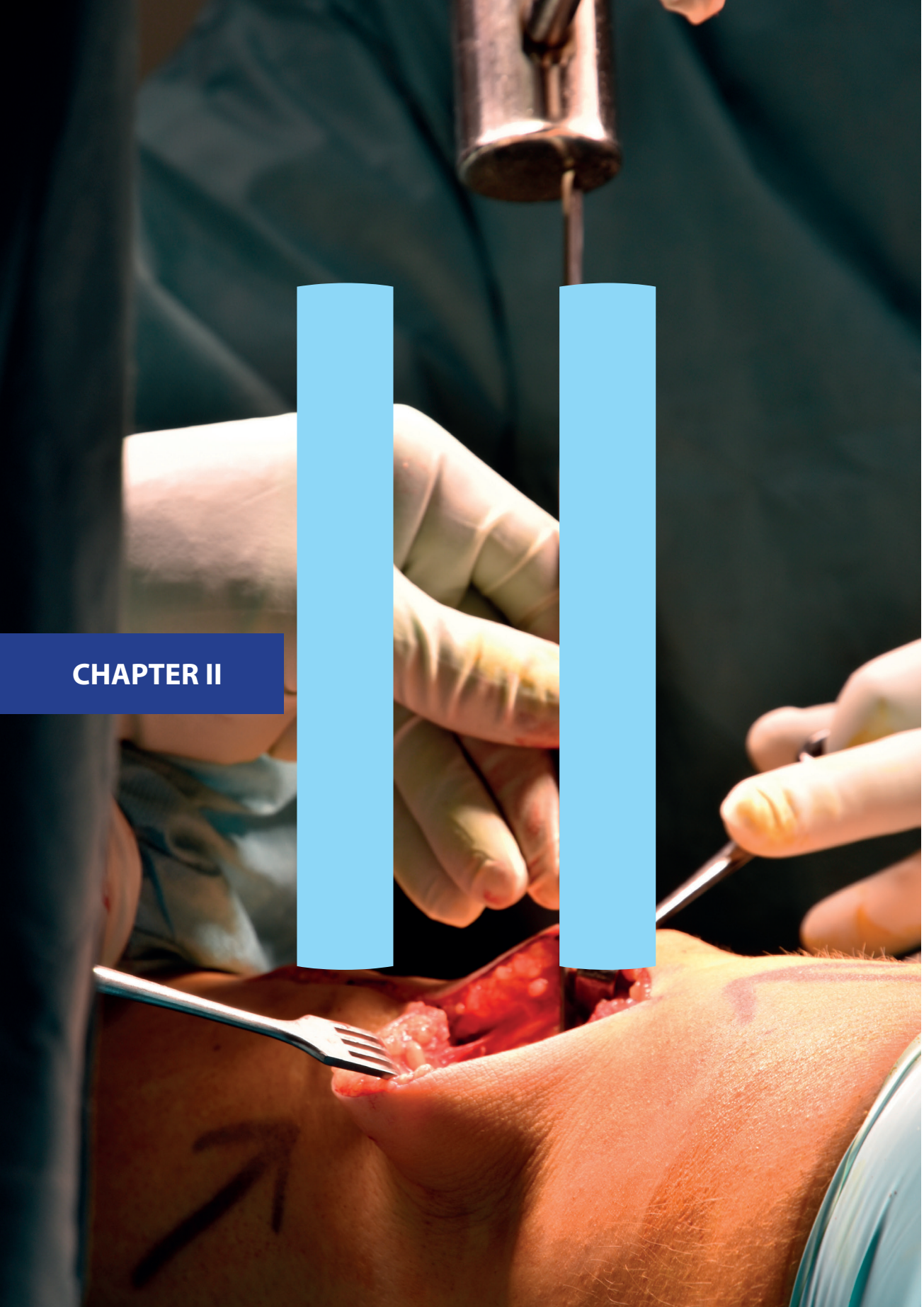

CHAPTER II

BACKGROUND INFORMATION

Chapter II – Background Information

The Knee Joint

Basic Anatomy and Histology

The knee joint is a complex synovial joint that allows for articulating between the femur (thigh bone), the tibia (shin bone) and patella (knee cap), and is responsible for weight-bearing, stability, and mobility in the lower limb. It is one of the largest and most commonly injured joints in the body. A combination of structural components, including bones, cartilage, ligaments, tendons, and muscles, work together to provide stability and facilitate movement of the knee joint (1).

The synovium is a specialized tissue that lines the inner surface of the joint capsule. It plays important roles in joint lubrication, immune responses, and tissue repair (2).

Hyaline articular cartilage covers the articulating ends of the femur, tibia and patella and facilitates low-friction movement. Articular cartilage is composed of a dense extracellular matrix (ECM) with sparse chondrocytes, see Figure 1. The ECM is rich in primarily type II collagen fibers. The collagen fibers are oriented horizontally in the superficial layer and vertically in the deep layer, forming arch-like structures (3).Collagen fibers provide the tensile strength of cartilage, for instance at the superficial layer where the horizontal oriented fibers resist shear forces (3). The ECM also contains proteoglycans, which are large molecules made up of a core protein and long chains of glycosaminoglycans (3). The proteoglycans are responsible for attracting and retaining water. Collagen fibers provide resistance to excessive swelling and leakage of proteoglycans out of the ECM, by providing a strong and organized network of fibers. Under load, the proteoglycans are compressed together, but repelled from each other due to their negative charge, giving articular cartilage its characteristic resilience to compressive load (4). Healthy articular cartilage has no innervation (nerve ingrowth) nor a direct vascular supply (3).

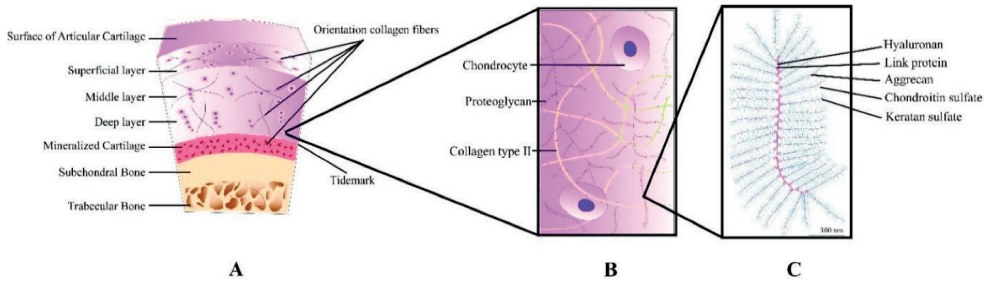

Figure 1: Schematic representation of articular cartilage and its contents: (A) Normal view of cartilage as osteochondral unit with specific zones; (B) Magnification of middle zone and its content; (C) Representation of typical proteoglycan structure. Reprinted with permission from Marjolein M. J. Caron (5)

The menisci are crescent-shaped fibrocartilaginous structures, that locate between the femur and tibia, and act as load distributors (1), see Figure 2 and 3. The medial meniscus is C-shaped and is solidly attached to the medial joint capsule. On the other side, the lateral meniscus is more O-shaped and loosely attached to the joint capsule (1).

There are four major ligaments that provide stability in the knee, see Figure 2: the anterior cruciate ligament (ACL), posterior cruciate ligament (PCL), medial collateral ligament (MCL), and lateral collateral ligament (LCL), see Figure 2 and 3. The ACL and PCL cross inside the knee joint, preventing excessive forward and backward movement of the tibia relative to the femur. The ACL and PCL also play a role in resisting rotation and side-to-side (varus/valgus) movement. The MCL and LCL provide stability on the inner and outer sides of the knee, respectively, preventing excessive varus/valgus movement (1).

Large muscles end, originate or cross the knee joint including: quadriceps, hamstrings, popliteus, the group of adductors, gluteal muscles inserting on the tibia via the iliotibial tract, and the lower leg muscles such as the gastrocnemius and soleus (6). These muscles have a delicate interplay during normal motion. Proprioception is the sensory system that enables the body to monitor the position of a joint and its movement in space (7, 8). For this purpose the joint tissues contain mechanoreceptors which are mainly found in the ligaments such as the ACL, PCL, MCL and LCL and muscle tendons (7). Well-functioning proprioception has been linked to good subjective performance of the knee and prevention of injury (9, 10). For example, the proprioception is impaired in ACL-deficient knees and this is one of the reasons for lowered subjective knee scores (11). Many surgeons believe that better functional outcomes are typically observed after unicompartmental knee arthroplasty UKA than after total knee arthroplasty (TKA), as the cruciate ligaments are retained with UKA (12). These examples highlight the importance of maintaining as much native anatomy as possible for preserving native knee function and proprioception for any surgical intervention in the knee joint.

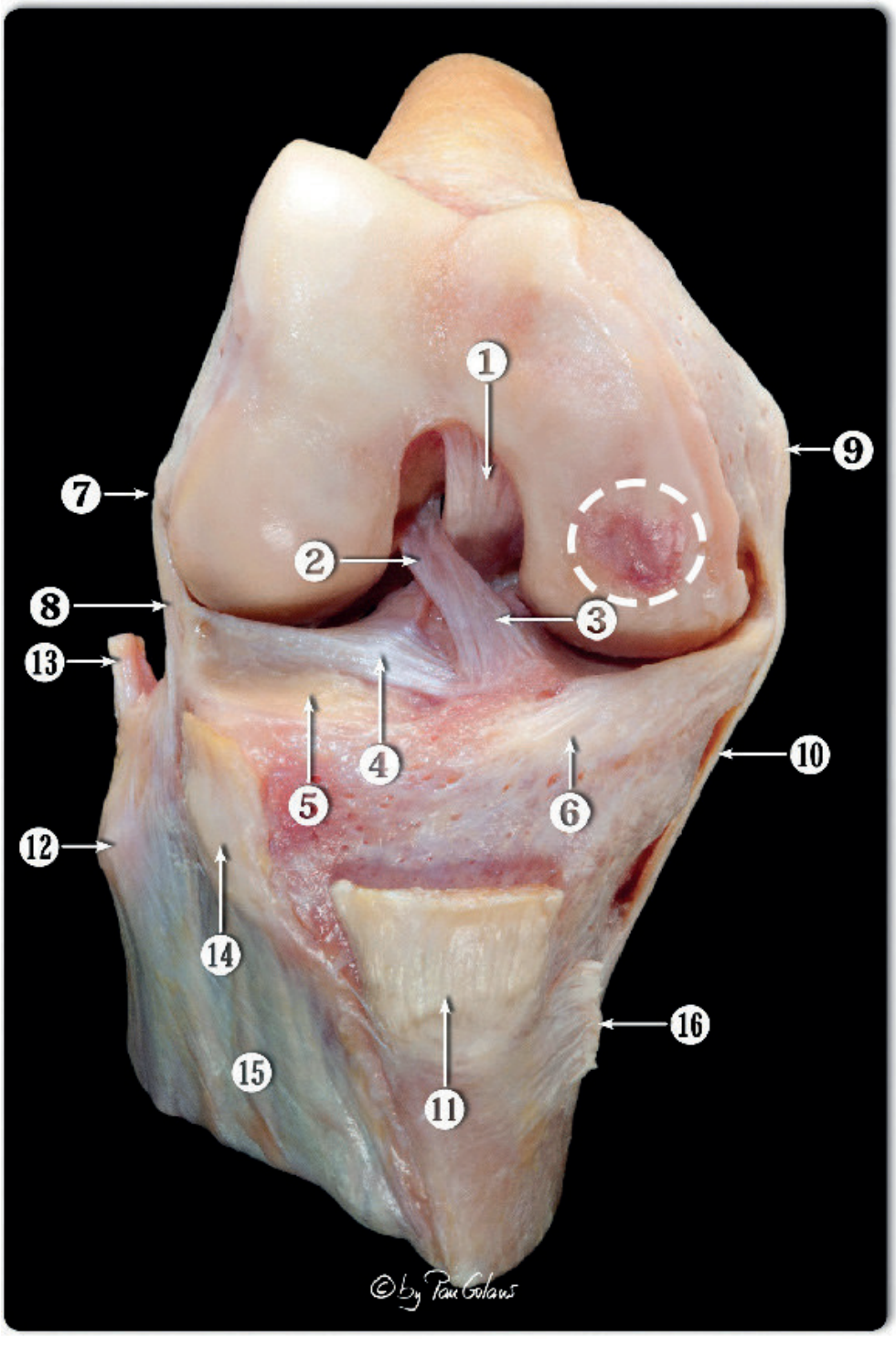
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Figure 2: Anterior view of the osteoarticular dissection of the right knee joint. Knee in 90º of flexion. 1=Femoral insertion of the posterior cruciate ligament, 2=anterior cruciate ligament (posterolateral), 3=anterior cruciate ligament (anteromedial bundle), 4=anterior horn of the lateral meniscus, 5=coronary ligament (meniscotibial capsule), 6=anterior horn of the medial meniscus, 7=lateral epicondyle, 8=lateral collateral ligament, 9=medial epicondyle, 10=medial collateral ligament, 11=patellar tendon (cut), 12=head of the fibula, 13=biceps femoris tendon (cut), 14=iliotibial tract insertion in the anterior tubercle or Gerdy's tubercle (cut), 15=anterior compartment muscles of the leg, 16=pes anserinus (cut). The dotted circle indicates a typical location and appearance of a focal cartilage defect. Created by late Pau Golanó, adopted with permission of the University of Barcelona.

Alignment and Kinematics

The major macroscopically visible motion of the knee is a flexion and extension movement. However, the knee kinematics are far more complex than those of a simple hinge. The medial tibia plateau is concave whereas the lateral tibia plateau is slightly convex. The medial femoral condyle has a more continuous radius whereas the lateral condyle is best described by two separate radii. The lateral meniscus can move more than twice the distance of the medial meniscus in the anteroposterior direction due to a much looser attachment to the joint capsule. During flexion, the femur consequently rolls back on the lateral tibia plateau whereas the medial femoral condyle mostly hinges. This rollback also externally rotates the femur during flexion during the first 15 degrees of flexion. The externally rotated femur allows better engagement of the patella. Also, it allows for deeper flexion since the posterior edge of the tibia is moved forward, preventing bony impingement during flexion over 90 degrees (13).

In the normal knee, the mechanical axis passes roughly through the center of the knee joint, which indicates that load is distributed optimally between the medial and lateral condyles, see Figure 4. The mechanical axis is a line drawn from the center of the head of the hip (caput femoris) and the center of the ankle (talus). The anatomical axis – which follows the diaphysis of the long bones – of the femur naturally deviates from the mechanical axis based on the configuration of the joint line, see Figure 4. The femur is, on average, in 6 degrees of valgus relative to the mechanical axis. The joint line of the knee is, on average, in 3 degrees of varus relative to the mechanical axis. Many people however have a leg axis that deviates from these normal values (14). Many men for example have a varus leg axis, where the mechanical axis passes more medial to the center of the knee joint. Such deviations have an impact on the load distribution in the knee, with a varus knee for instance contributing to more load on the medial compartment (13).

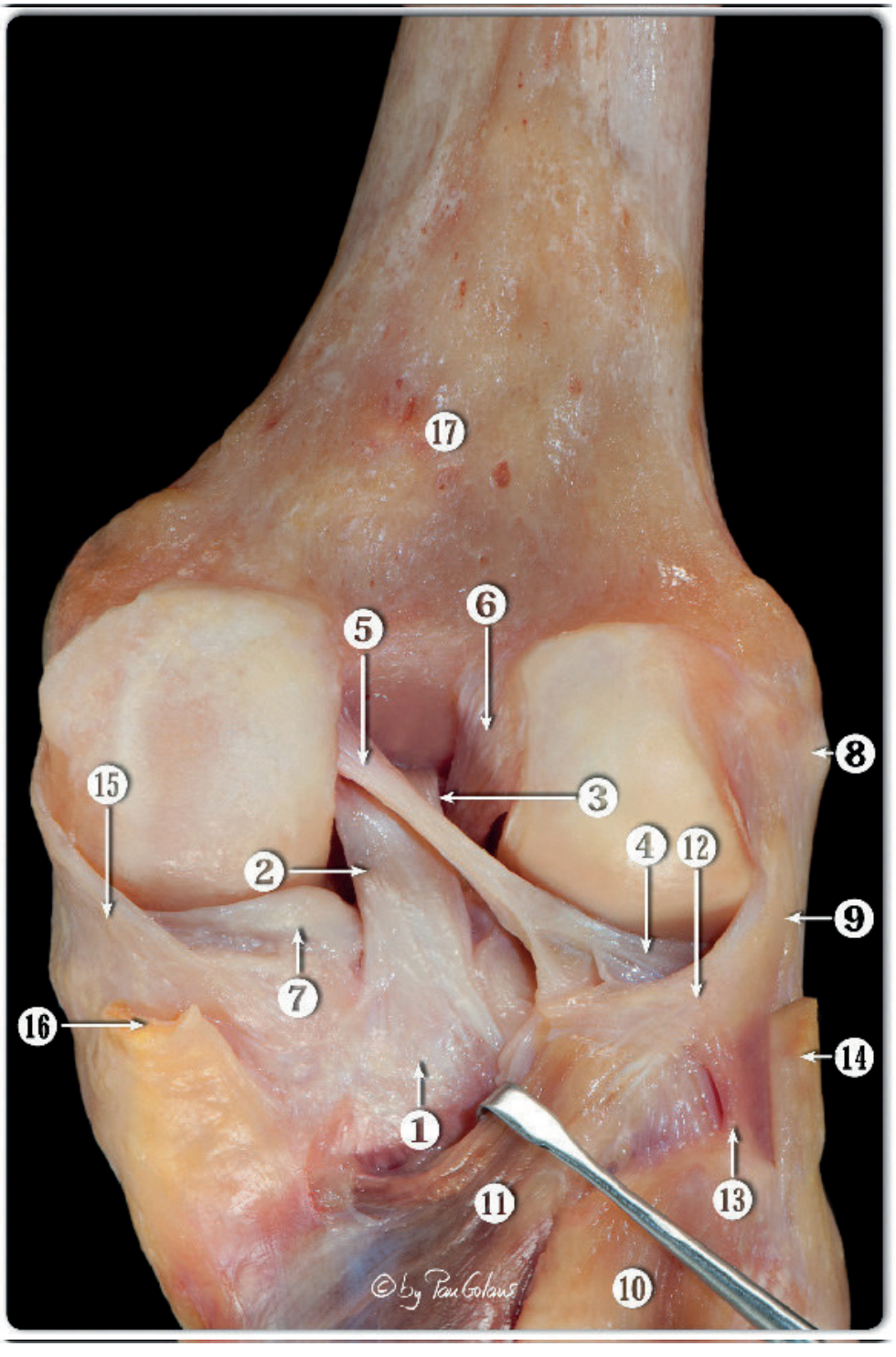
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Figure 3: Osteoarticular dissection showing the posterior structures of the right knee joint after removing the joint capsule. 1=tibial insertion of the PCL, 2=posteromedial bundle of the PCL, 3=anterolateral bundle of the PCL, 4=lateral meniscus, 5=posterior meniscofemoral ligament or the ligament of Wrisberg, 6=femoral insertion of the anterior cruciate ligament, 7=medial meniscus, 8=lateral epicondyle, 9=lateral collateral ligament, 10=soleus fibular insertion muscle, 11=popliteus muscle, 12=popliteus tendon, 13=popliteo meniscal ligament (arcuate ligament), 14=biceps femoris tendon (cut), 15=medial collateral ligament, 16=semimembranosus tendon (cut), 17=popliteal surface. PCL=posterior cruciate ligament. Created by late Pau Golanó, adopted with permission of the University of Barcelona.

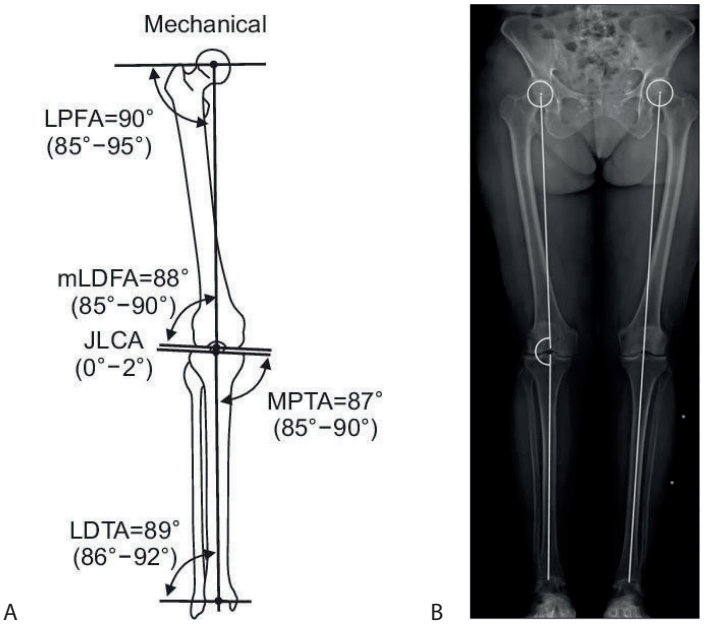

Figure 4. A: Joint orientation angles described by Paley, measured relative to the anatomic axes of the femur and tibia. The numbers indicate normal values with range of variation shown in parentheses. Normal values of coronal lower limb mechanical angles that are used for the evaluation of radiographs: LDFA: lateral distal femoral angle; mLDFA: mechanical lateral distal femoral angle; MPTA medial proximal tibial angle, LDTA lateral distal tibial angle; B: plain anteroposterior radiograph of the lower extremities showing the mechanical axis, a line drawn from the femoral head to the ankle joint, passing through the middle part of the knee.

Patellofemoral alignment, the manner in which the patella tracks in the femoral groove (trochlea) during normal gait, affects the function and load distribution in the patella. Many variables contribute to the alignment and thus stability of the patellofemoral joint, including the bony anatomy, ligaments and neuromuscular control. In the normal knee during full extension, the patella is located proximal to the trochlear groove and starts to engage when the knee is flexed about 20-30 degrees. Hence, the patella relies purely on ligaments such as the medial patellofemoral ligament (MPFL) for its stability in full

extension. Also, the muscular tone of the vastus medialis muscle contributes to the stability. During progressive flexion, the bony anatomy of the patella and trochlear groove increase its share in the stability (13). An abnormally long patellar tendon resulting in an abnormally high patellar position, an MPFL injury, an increased Tibial Tuberosity to Trochlear Groove (TT-TG) distance, or bony dysplasia's of the patella and trochlea, can increase the likelihood of patellar dislocation (15). Patellar dislocations show a 95% concomitant incidence of cartilage damage (16).

Cartilage Defects

Cartilage defects are defined as a focal area of damage to the articular cartilage of the joint. The etiology of cartilage defects varies from acute to chronic and they are classified according to the International Cartilage Repair Society (ICRS) grading system.

Etiology of Cartilage Defects

Cartilage defects either have an acute traumatic origin or a non-traumatic origin (17, 18).

Traumatic cartilage defects are frequently found after direct knee traumas or after sports injuries, such as a pivotal twisting injury (17, 18). Hence, ACL ruptures are often accompanied by cartilage defects (19). Patella dislocations often lead to cartilage defects due to the combination of high shear and compressive forces during the dislocation or due to high compressive force that arise in the dislocated position (16). Patients usually remember the exact moment and mechanism in case of a traumatic etiology. The acute traumatic cartilage defects are often found with well delineated defect edges with surrounding healthy cartilage.

However, there are also non-traumatic cartilage defects. Patients usually cannot pinpoint a specific moment which led to the initiation of symptoms. There are many hypotheses how non-traumatic cartilage defects develop, and it is often a point of discussion whether or not such defect can be considered the first expression of osteoarthritis (OA) (17, 20, 21). Non-traumatic cartilage defects could be caused by a traumatic chondral defect that originated decades ago. Since cartilage has no innervation, a pure chondral lesion might go unnoticed for many years, after which patients forget about the (minor) trauma. Such asymptomatic defect might be large enough to have a pothole effect (discussed later in this section), leading to progressive compartmental degeneration and impairing the joint homeostasis. Cartilage defects only become painful when the highly innervated subchondral bone gets involved or when the synovium becomes inflamed. Another etiology could be the microtrauma induced by obesity, instability, tibiofemoral malalignment or patellar maltracking (22). For instance, in ACL-deficient knees, it has been shown that the increased anteroposterior instability leads to meniscus degeneration (23). It is well known that a damaged meniscus leads to cartilage damage (24). It is also conceivable

that the increased instability directly negatively influences the cartilage. Varus/valgus malalignment leads to overload of medial and lateral compartment respectively and cartilage damage consequently (25). The same holds true for patella maltracking, which leads to patellofemoral cartilage damage (26).

The rarer etiologies of cartilage defects include pathologies in which there is no known direct correlation with an acute trauma or habitus of the patients, such as osteochondritis dissecans (OCD), spontaneous osteonecrosis of the knee (SONK) and subchondral insufficiency fractures (SIF) (27, 28). OCD is a pathologic osteochondral lesion affecting young individuals with an open physis (10-15 years old, i.e. juvenile OCD) and adults, often < 50 years old. OCD is rare in patients who are <10 or ≥ 50 years old. It is believed that adult presentation of OCD is likely an unresolved juvenile OCD (27, 28). The most prevalent location (~ 70%) is the posterior-central aspect of the medial femoral condyle. The etiology of OCD is not completely understood (27, 28). It is often described as an idiopathic disease, however, multiple etiopathogenetic hypotheses are being investigated, including local ischemia, aberrant endochondral ossification of the secondary subarticular physis, repetitive microtrauma, developed of a smaller notch, (29) and genetic predisposition (27, 28). Treatment depends on skeletal maturity and lesion stability. Skeletal immature patients with stable lesions might benefit from activity modification and weight-bearing restrictions. Patients with unstable lesions require surgery (27). Long-term follow-up studies have shown that if the fragment is removed, progression towards OA occurs in 70 to 100% of cases (30, 31). Treatment of the defect with grafting techniques decreases the OA development to 0% (30).

Epidemiology of Cartilage Defects

The incidence of symptomatic cartilage defect is estimated at 56 / 100,000, based on large database studies (32).

The true incidence and prevalence of all cartilage defects is unknown and probably will remain unknown due to the high prevalence of asymptomatic lesions in the general population. Based on earlier large arthroscopy studies, it has been observed that focal cartilage defects in the knee are prevalent in 19-67% of patients with painful knees (32). A systematic review on the prevalence of cartilage defects found in arthroscopy and magnetic resonance imaging (MRI) studies revealed a prevalence of full-thickness cartilage defects of 36% among athletes, of which 14% were asymptomatic (33). A prevalence study using MRI on random but older subjects (n=325) was performed by Ding et al. (34). The average age was 45 years, 17% of the subjects had radiographic signs of OA and 100% had cartilage abnormalities on the MRI. These numbers indicate a very high prevalence of cartilage defects in middle-aged people.

Grading and Sizing of Cartilage Defects

The ICRS grading system is a widely used classification tool that allows physicians and researchers to objectively evaluate the condition of cartilage damage based on the visual appearance of the cartilage during arthroscopy.

The ICRS grading system classifies cartilage lesions into four main grades (17, 35), see Figure 6:

- Grade 0: Normal cartilage
- Grade 1: Nearly normal cartilage. Superficial lesions. Soft indentation (A) and/or superficial fissures and cracks (B)
- Grade 2: Abnormal. Lesions extending down to <50% of cartilage depth.
- Grade 3: Severely abnormal. Cartilage defects extending down >50% of cartilage depth (A) as well as down to calcified layer (B) and down to but not through the subchondral bone (C). Blisters are included in this Grade (D). Grade 3 are full-thickness cartilage defects
- Grade 4: The cartilage has full-thickness lesions that extend through the entire cartilage layer, reaching the subchondral bone.

Next to the depth of the cartilage defect it is important to note the location and size of the cartilage defect. Most cartilage defects are found on the medial femoral condyle or in the patellofemoral joint (17, 18), as the majority of load is typically propagated through the medial compartment. The location of the defect is important as it has been shown that defects on the medial femoral condyle have the best outcomes after cartilage repair and patellofemoral and tibial defects can have less favorable outcomes (36). The size of the cartilage defect can be underestimated on MRI and should ideally be assessed after thorough debridement of the cartilage defect edges during arthroscopy. It is usually described as surface area in cm^2 (18).

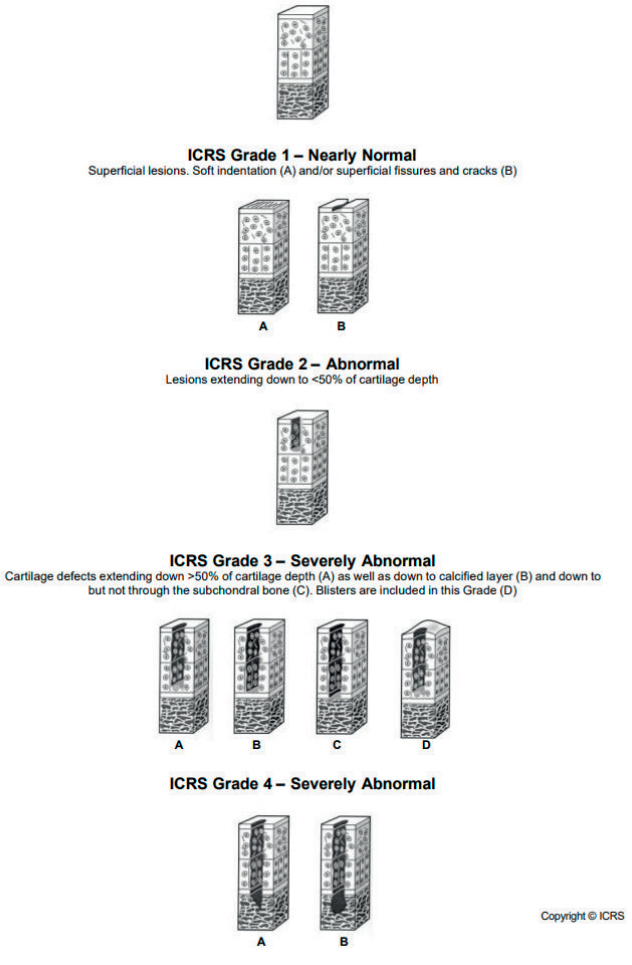

Figure 6. The International Cartilage Repair Society grading system.

Symptoms of Cartilage Defects

Typical symptoms of cartilage defects are pain, decreased knee function due to pain, swelling, stiffness or clicking sensations in the knee. Symptoms strongly overlap with those found in OA. In fact, recent evidence showed that patients with a focal articular cartilage defect can exhibit as severe symptoms as patients with end-stage OA scheduled for TKA (37). However, most of cartilage defects in the general population probably remain asymptomatic.

To date, the reasons for cartilage defects to become symptomatic remain not fully elucidated. It is commonly accepted that the subchondral bone is involved in pain generation and perception. One hypothesis for onset of pain is the presence of fluctuating intraosse-

ous fluid pressures. When the cartilage is damaged, an opening to the subchondral bone is created. During ambulation, the fluid pressure builds up from the synovial fluid leading to high intraosseous pressure, thus sensitizing the highly innervated subchondral bone (38, 39). Another hypothesis for the onset of pain is the development of subchondral bone edema (40). There are studies that show a high correlation between bone marrow edema on MRI and pain (41). From a pathological perspective, a direct relation between pain and bone marrow edema can be explained. Histological analysis of bone marrow edema pattern reveals a complex blend of various pathological changes, comprising bone marrow necrosis, fibrosis, microfractures, increased bone remodeling, and fibrovascular ingrowth (42). It has been shown that during bone remodeling, osteoclasts can penetrate the osteochondral junction, which promotes ingrowth of new blood vessels, accompanied by sensory nerves in the deep layers of articular cartilage (43). It is thus conceivable that bone marrow edema surrounding a cartilage defect is associated with increased innervation in the subchondral bone.

Joint homeostasis and the Development of Osteoarthritis

Joint Homeostasis

The term "homeostasis" was first introduced by the American physiologist Walter Bradford Cannon in his 1926 book "Physiological Regulation of Normal States: Some Tentative Postulates Concerning Biological Homeostatics." Cannon derived the term from the Greek words "homeo," meaning "similar" or "same," and "stasis," meaning "standing" or "static." Homeostasis, therefore, refers to the ability of an organism or a system to maintain a stable and balanced internal environment despite external changes or fluctuations (44).

Although the term homeostasis is often applied to the whole human body, the term 'joint homeostasis' has only been around for roughly two decades (45). 'Joint homeostasis' describes our increasing understanding of the delicate balance between anabolism and catabolism, pro-inflammatory and anti-inflammatory state of the joint, and how internal and external factors influence that balance (46).

The progression from a cartilage or meniscus injury to OA may perhaps start as a (micro-) trauma, but the actual degeneration of the joint towards an advanced stage of OA is also a cell-mediated process. The same holds true for cartilage defects involving varus malalignment, where the medial compartment degenerates, but eventually the lateral side follows as a result of the catabolic micro-environment. It has been postulated that prior to the classical clinical and radiographical signs of OA, the joint homeostasis has been in a catabolic state for an extended period of time, which is referred to as 'pre-OA' state (47). Animal studies confirm such catabolic joint state. Namely, pro-inflammatory cytokines are found prior to radiographic or histological signs of OA in animal models in which OA

is induced by ACL transection (46, 48-50). It has also been shown that pro-inflammatory cytokines are secreted by the intra-articular fat pad (Hoffa's fat pad) in patients with OA or cartilage defects, indicating locoregional production of these cytokines (46). When there is a prolonged pro-inflammatory joint state, the tissues within the joint will eventually degrade (51). It has also been shown that in such a (pre-)OA environment, cartilage repair is hampered (52). For example, cartilage defects were induced in a goat model and repair treatment was performed either early or late (directly versus 10 weeks after trauma). Results showed that late treatment led to worse outcomes due to an altered joint micro-environment (45). Previous clinical work of our group has shown that factors such as an increased age or longer duration of symptoms, and previous surgeries within the same knee, especially meniscectomy and ACL reconstruction, are correlated to less favorable outcomes after microfracture (53). It has been hypothesized that these factors negatively influence the joint homeostasis, apart from the obvious mechanical reasons.

There is currently no single or set of biomarkers known which is able to accurately measure the state of joint homeostasis or more importantly: to determine in what stage of degeneration the knee is (47). Namely, the joint homeostasis is anabolic in the acute phase, directly after a cartilage defect (54), in contrast to the catabolic state with older defects or in the presence of radiographic signs of OA (45, 55). It is also still unclear whether the joint homeostasis remains balanced for a long time after the initial injury and then suddenly rapidly deteriorates, or that it is a more gradual decline which then surpasses a certain cellular threshold. It is also conceivable that factors, such as age, play an important role in the speed of this transition. For example, young patients with diagnosed cartilage defects can remain asymptomatic for many years (19), while cartilage defects progress rapidly in older patients and therefore form a major risk factor for arthroplasty (56).

The term 'joint homeostasis' therefore remains somewhat conceptual as we do not have a simple diagnostic tool to measure it. Joint homeostasis is an umbrella term to describe whether the tissues that make up a joint are in a healthy or degenerative state. However, based on fundamental and clinical evidence, we must acknowledge that the joint works as an organ and the general health of a patient and focal injuries to one compartment can lead to a total joint disease through an impaired joint homeostasis, again, next to the underlying mechanical alterations.

Progression of Cartilage Defects

Progression of a cartilage defect is most likely based on mechanical and biological processes and a combination of both. If there is a hole in a loaded articulating surface, one can imagine that the rims of the hole experience stress when loaded. This leads to degeneration of the rims of the cartilage defect, resulting in enlargement of the defect. This phenomenon is known as the 'pothole-effect'. Guettler et al. (2004) conducted a hu-

man cadaver knee study and used electronic pressure sensors in the medial and lateral compartment (57). Different size osteochondral defects were generated and pressure on the rim of the defect was measured. Defects ≥ 10 mm showed increased rim stress concentrations. Lacy et al. (2016) conducted a biomechanical study on cadaveric human knees using different defect sizes and applied various loads to simulate different patient body mass indices (BMIs) (58). They showed that there was an increased load at the base of the defect for a simulated BMI ≥ 30, defects ≥ 2 cm^2 (16 mm diameter) and rim cartilage thickness < 2 mm. Moreover, a finite element modelling study evaluating simulated defects between 0.5 and 8 mm found that excessive strains in the superficial collagen layer are caused by inward bulging of cartilage into the defect area, an effect that increases non-linearly with defect size (59). Hence, cartilage defects tend to progress faster due to mechanical reasons such as rim overloading and excessive strains on the superficial tissue layer.

There also seems to be a role for joint homeostasis as described previously.

The previously mentioned study by Ding et al. showed that a higher body mass index, female sex, increasing age, larger tibial bone area and presence of osteophytes were all correlated with increased cartilage loss over 2 years of time (34). The average age in this study was 45 years. A recent study by Everhart et al. (2019) using data from the OA initiative showed that in individuals aged 45 to 79 years, the presence of a large (>2cm^2) cartilage defects was a major determinant in predicting the need for future knee arthroplasty, even in the absence of OA changes (56). Interestingly, Shelbourne et al.(2003) compared patients who received an ACL reconstruction with and without an untreated ICRS grade 3 or 4 cartilage defect, and found statistically different, but clinically irrelevant clinical outcome differences, and no difference in radiographic follow-up of 8 years (19). Subjects in the latter study were on average 26 years old. Based on these studies, age could thus be a contributor to the progression of cartilage defects. Perhaps the young patients are in a more anabolic state, producing tissues that are able to withstand the mechanical overload longer, i.e., the threshold for ECM disruption might be higher or the regeneration of tissues is faster and thus more able to resist the next impact. *Vice versa,* older individuals might have more vulnerable 'chondroporotic' cartilage (analogous to osteoporosis) with a lower threshold to withstand mechanical forces.

It can be concluded that large, high grade ICRS cartilage defects in older patients with a high BMI, have a higher chance for progression of the defect based on mechanical factors and disrupted joint homeostasis. Ultimately, a cartilage defect can lead to early OA (EOA) and OA (20, 21, 47). This transition process is still subject to extensive research. Moreover, the repair of a ligament rupture, meniscal tear or cartilage defect is intended to reconstruct native anatomy and improve the symptoms of such injury, but in the absence of a

good and affordable measurement-control system such as biomarkers and/or affordable imaging such as MRI, it remains poorly understood if and how such surgeries influence the progression towards OA.

Osteoarthritis

Osteoarthritis is the most common, progressive, multifactorial joint disease and is characterized by chronic joint pain and functional disability (60). Knee OA accounts for almost four fifths of the burden of OA worldwide and incidence rates increase with obesity and advancing age (61).

The worldwide prevalence of OA is around 9% between age 40-49 and increases up to 35% in patients aged 70-79 years (62). The incidence of OA is projected to rise by 75% between the year 2020 and 2050 due to changing demographics (62). Typically, women are more commonly affected by knee OA than men (62).

The etiology of knee OA is multifactorial and includes predisposing factors such as genetics, age and obesity (63), but mechanical overloading also contributes significantly to the onset of the disease, such as malalignment, instability, traumatic injuries and obesity (64).

OA has classically been described as the loss of articular cartilage and hence joint space narrowing, the latter which is a distinctive feature on conventional plain radiographs. However, it has become widely accepted that OA is a total joint disease that does not only involve cartilage degeneration, but also degeneration of the meniscus and ligaments, changes to the subchondral bone, alterations in Hoffa's fat pad, the synovium and synovial fluid (46). Many molecular signaling pathways such as the Wnt (wingless-related integration site), β-catenin, and TGF (transforming growth factor)- β superfamily are involved in the pathological process but these are outside the scope of this thesis (65).

Knee OA is typically classified using plain anteroposterior radiographs by the Kellgren-Lawrence (KL) classification (66), see Figure 5:

- grade 0 (none): definite absence of radiographically visible changes indicative of OA
- grade 1 (doubtful): doubtful joint space narrowing and possible osteophytic lipping
- grade 2 (minimal): definite osteophytes and possible joint space narrowing
- grade 3 (moderate): moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends
- grade 4 (severe): large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends

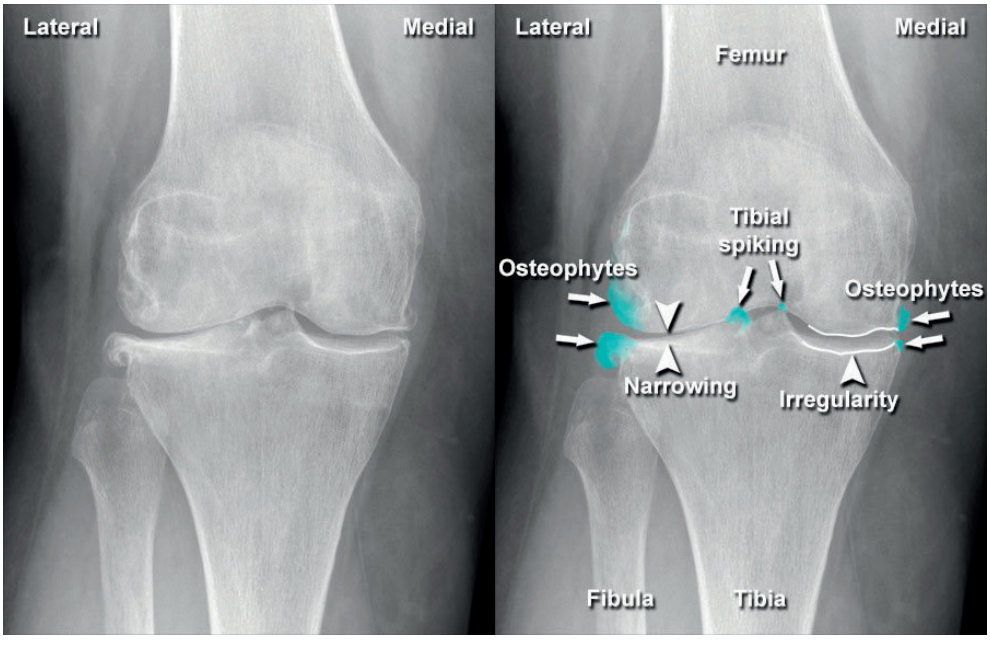

Figure 5. Left: plain anteroposterior radiograph of a right knee with OA; right: same radiograph showing the hallmark features of OA.

EOA is an increasingly used term in the orthopedic field. Based on the work of Luyten et al. (2012 and 2018) (20, 21), EOA is defined as KL grade 0-1 combined with symptoms such as pain and functional impairment. With time and if left untreated, EOA can progress to OA with its typical findings on plain radiographs (47). However, a true objective diagnostic tool to capture EOA is not yet available at this time (47). Efforts are being made to find biomarkers that can determine and monitor the disease process (47). And in fact, EOA is probably highly prevalent. The treatment of end-stage symptomatic knee OA can be straightforward and involves replacing the joint with a UKA or TKA, depending on the extent and localization of the disease and patient characteristics (13). The treatment of EOA is far more complex, depends on patient and disease characteristics, and involves a multitude of approaches such as physiotherapy, lifestyle changes, weight loss and joint preserving surgeries such as cartilage repair (67). The latter is part of this thesis and is discussed throughout the other chapters.

Treatment of Cartilage Defects

Conservative Treatments and Prerequisites of Surgical Cartilage Repair

Conservative measures do not directly treat the cartilage defect but might improve the symptoms. Conservative interventions may include weight management, physiotherapy, and modifying physical activity (68).

The prerequisites for successful cartilage repair, such as alignment correction, ligament reconstruction and meniscus repair are at least as important as repair of the cartilage itself. For instance, unloading the medial compartment after cartilage repair by the means of concomitant high tibial osteotomy (HTO) when indicated has been proven to provide better outcomes than without (69). A brief overview of the currently advised prerequisites is listed in Table 1.

▪ Stable knee with normal alignment (< 5° varus or valgus malalignment: consider an additional osteotomy)
▪ Malalignment >5° at the expense of the defect compartment; consider an additional osteotomy
▪ Unstable knee (e.g., anterior cruciate ligament): consider an additional ligament reconstruction
▪ Aim for BMI <30
▪ Age ≤50 years
▪ Meniscus >50% intact
▪ No signs of osteoarthritis
▪ No (septic) arthritis
▪ Diagnose and correct the predisposing factors of patella maltracking such as patella alta, baja, patellofemoral instability or an increased TT-TG/TT-PCL.
▪ No inflammatory disease of the joint (rheumatoid arthritis, gout, psoriatic arthritis)
▪ Smoking (relative)

Table 1. Abbreviations; BMI: Body Mass Index; TT-TG: tibial-tuberosity to trochlear groove distance; TT-PCL: tubercle-posterior cruciate ligament (TT-PCL). Obtained from the 2019 Dutch Consensus Statement concerning cartilage defect repair in the knee

The Ideal Surgical Cartilage Repair Treatment

Simply said, a cartilage defect repair treatment should relieve a patient's pain, improve the function of the knee and, ideally, postpone or prevent OA development. In other words: clinical outcomes measured using patient reported outcomes (PROMs) should show statistically significant and clinically relevant improvements compared to preoperative levels or should be restored to age-related normative healthy values as closely as possible (70). In order to achieve this, cartilage repair should restore peak mechanical stress to pre-injury levels and restore joint homeostasis to a balanced state.

When considering joint preservation, the relief of symptoms should at least have sufficient durability, able to postpone the first TKA and subsequent potential postponement of the first revision arthroplasty later in life in a substantial fraction of patients. Durability

also includes that the treatment should not interfere with subsequent treatments. It has been shown for instance that some cartilage treatments may negatively affect subsequent treatments (71-74). Related to the desire for joint preservation is that the ideal cartilage repair treatment should prevent the pothole effect (57, 59). An ideal cartilage repair treatment should also delay or halt the development or progression of OA changes on conventional and magnetic resonance imaging and cellular level.

Lastly, from a surgical, patient and socioeconomic perspective, an ideal cartilage repair treatment should be a simple single-step, minimally invasive surgery, with a short rehabilitation duration.

Currently Used Cartilage Repair Treatments

Treatments can be grouped into three groups, namely bone marrow stimulation, cell-based and bone-based techniques. The most commonly used in these groups are respectively microfracture, the latest generations of autologous chondrocyte implantation(ACI), and osteochondral auto- and allografting (17).

Microfracture, popularized by Steadman in the late 1980s, is the most popular treatment within the bone marrow stimulation category of treatments (17). Other treatments include abrasion arthroplasty, nanofracture and historically Pridie drilling (17). After debriding the defect, the subchondral bone is either abrased, penetrated using an arthroscopic awl or drilled to induce bleeding, release of bone marrow derived growth factors and stem cells. After formation of a blood clot in the defect site, the ultimate goal of microfracture is the differentiation of mesenchymal stem cells into chondrocytes (chondrogenesis), which then produce extracellular matrix to fill the defect site. Microfracture is cheap, technically easy and has a high availability. However, many studies have shown that the resulting defect filling is often only of fibrocartilaginous nature (17).

The second group of treatments is the cell-based group. ACI was developed in de 1990s (17). The first generation ACI constituted of arthroscopically harvesting healthy chondrocytes from a non-weightbearing area, culturing them *in vitro* and placing them back in a second open surgery, using a periosteal flap (ACI-p) as membrane to enclose the cells. Next generations included coverage using a collagen flap (ACI-c), matrix associated chondrocyte implantation (MACI) and the latest generation using spheroids (spherical aggregates of ex vivo expanded human autologous chondrocytes and their self-synthesized extracellular matrix) of chondrocytes. The general idea in all these generations is to enhance the expansion and differentiation of chondrocytes by doing this *in vitro*. The ultimate goal after reimplantation is that these chondrocytes produce hyaline or hyaline-like articular cartilage. Drawbacks of ACI include the need of two surgeries and high costs (17).

The third group of treatments is the bone-based techniques. Treatments within this group include osteochondral autografts and allografts (17), and focal knee resurfacing implants (FKRIs) (75). Autografts are osteochondral cylinders, i.e., composed of both the subchondral bone and overlying hyaline cartilage, harvested by a punch from a non-weightbearing area in the patient's knee. One or more cylinders are then implanted into the subchondral bone, refilling the defect with native tissue from another site. Allografting is the same procedure using a fresh graft from a deceased donor (17). FKRIs are composed of non-degradable biomaterials that substitute the complete osteochondral unit (75). Bone-based therapies rely mainly on osseointegration rather than chondrogenesis for their success, as maturation of repair tissue is not needed: the hyaline articular cartilage graft or a biomaterial substitute is used to resurface the joint. Typical drawbacks are limited size options and donor site morbidity for autografts, costs, availability and shelf life for allografts, and the need for planning for FKRIs (17, 75).

Osseointegration

Osseointegration is an important prerequisite for the success of FKRIs, as these implants should be well anchored to the subchondral bone to resurface the joint. Osseointegration, first described by Brånemark and colleagues, was formally defined in a paper by Albrektsson et al. as the direct contact, visible at the light microscope level, between living bone and an implant (76). Histologically, osseointegration is characterized as the direct anchorage of an implant through the formation of bony tissue around the implant, precluding the growth of fibrous tissue at the bone–implant interface. In recognition of the limitations of these histology-based definitions for clinical practice, a biomechanics-based alternative has been proposed: "A process whereby clinically asymptomatic rigid fixation of alloplastic materials is achieved, and maintained, in bone during functional loading" (76).

Terminology used within osseointegration research, and this thesis as well:

- The bone-to-implant contact (BIC) area remains the gold standard for evaluating osseointegration, particularly in preclinical research. The BIC precisely measures the direct contact between the implant surface and adjacent bone tissue on microscopic stained slices, expressed as a percentage of the total implant surface engaged in direct contact with bone (76).
- Osteoinduction. This term means that primitive, undifferentiated and pluripotent cells are somehow stimulated to develop into the bone-forming cell lineage. One proposed definition is the process by which osteogenesis is induced (76).
- Osteoconduction refers to the process wherein bone development occurs on a particular surface. An osteoconductive surface is characterized by its ability to facilitate bone growth on its surface or within its pores, channels, or conduits. Wilson-Hench

Lesion type	Location and ICRS grade	Treatment option in defects <2 cm^2	Treatment option in defects ≥2 cm^2
Chondral	**Femoral condyles and trochlea ICRS-grade 3-4**	• Microfracture • OAT • Nettoyage and debridement	• ACI o First generation: ACI-P o Second generation: ACI-C o Third generation: M-ACI o Fourth generation: Spherox® • Defects <4 cm^2: OAT
Osteochondral	**Femoral condyles and trochlea ICRS-grade 4**	OAT with backfilling or biodegradable osteochondral scaffolds • Nettoyage and debridement • Fresh allograft	• ACI o First generation: ACI-P o Second generation: ACI-C o Third generation: M-ACI o Fourth generation: Spherox® + bone graft or synthetic implant • Fresh allograft
(Osteo) chondral	**Patella ICRS-grade 3-4**	• Nettoyage and debridement • ACI	• ACI o First generation: ACI-P o Second generation: ACI-C o Third generation: M-ACI o Fourth generation: Spherox® + bone graft

Note 1 Combined procedures have a narrow indication area and are best performed in a center of expertise.

Note 2 If previous surgical treatment of an (osteo)chondral defect has failed, referral to a center of expertise is indicated

Note 3 Adequate follow-up treatment as described in the patient/practitioner app and physiotherapy center with ICRS training.

Note 4 Central registration in the Dutch version of the ICRS database

Table 2. Abbreviations: ICRS: International Cartilage Repair Society; OAT: Osteochondral Autologous Transplantation; ACI: Autologous Chondrocyte Implantation; BMI: Body Mass Index; TT-TG: tibial-tuberosity to trochlear groove distance; TT-PCL: tubercle-posterior cruciate ligament (TT-PCL). Obtained from the 2019 Dutch Consensus Statement concerning cartilage defect repair in the knee.

has proposed that osteoconduction involves guiding bone in a manner that conforms to the surface of a material (76).

Dutch Consensus Statement for Surgical Cartilage Repair of (Osteo)chondral Defects in the Knee

Literature concerning cartilage repair is mainly based on low level evidence and the patients included in the current literature do not always resemble the typical patient seeking help for a cartilage defect (77). The choice for a certain cartilage defect treatment becomes even more difficult due to the many different treatments available. Hence, there are many guidelines and treatment algorithms available based on available literature, but also on expert opinion, consensus meetings and the availability and reimbursement of treatments. The treatments are mainly categorized based on lesion size and depth. In the Netherlands, the expert cartilage clinics collaborated under the flag of the national orthopedic association (Nederlandse Orthopaedische Vereniging [NOV]) to formulate the Dutch consensus statement for surgical cartilage repair of (osteo)chondral defects in the knee. Table 2 summarizes the Dutch consensus.

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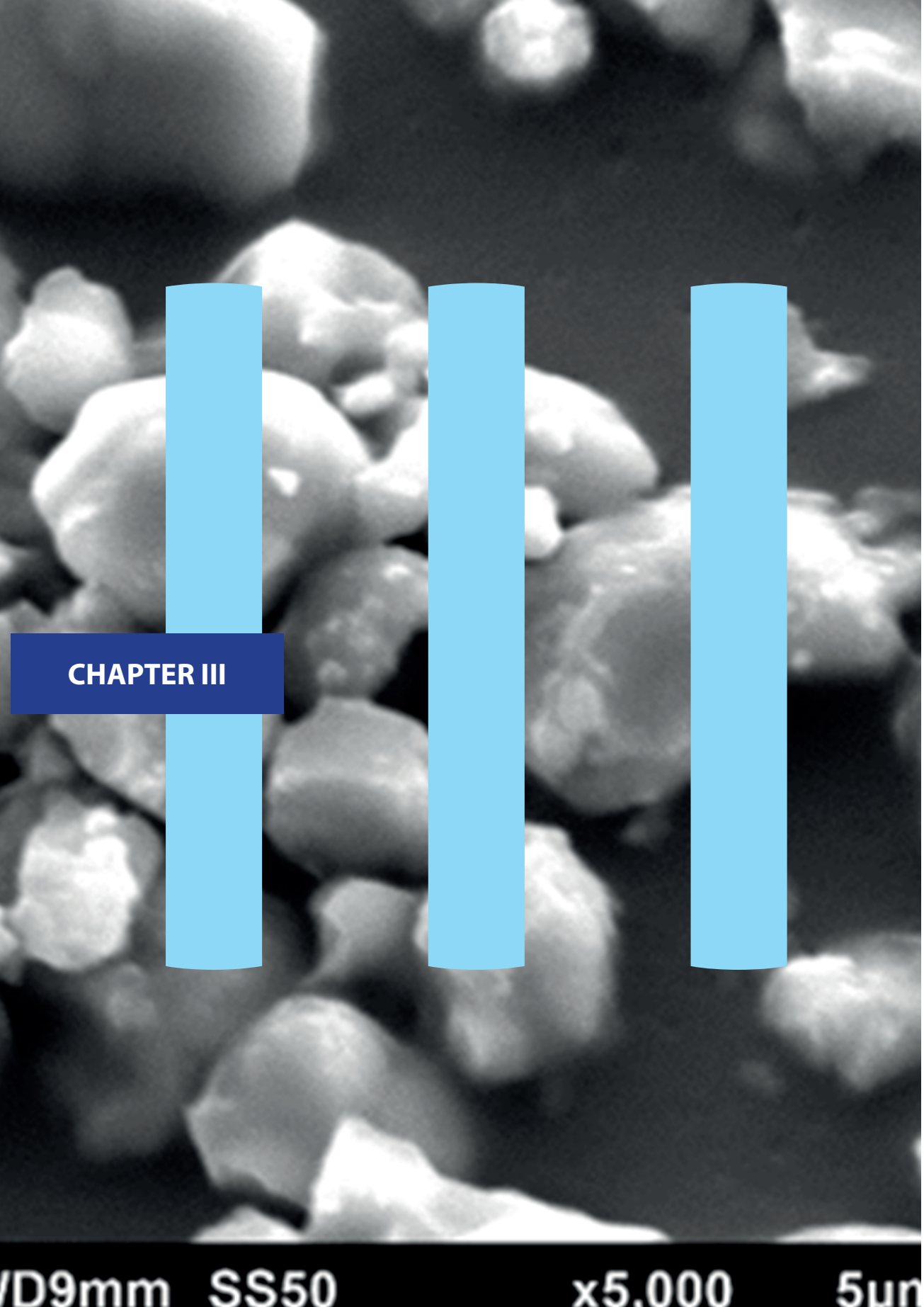
CHAPTER III
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The Modified Hedgehog Technique to Repair Pure Chondral Shear-off Lesions in the Paediatric Knee.

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Abstract

*Objective***.** The paediatric knee is prone to pure chondral shear-off lesions due to the developing osteochondral unit. Refixation of the chondral fragment is commonly done using metalwork or absorbable biomaterials. Both fixation methods come with biomaterial-related drawbacks. Earlier work on chondral allografts for cartilage repair in adults has shown successful osteochondral integration when the chondral allograft is treated with multiple incisions and then glued to the subchondral bone using fibrin glue. This is commonly referred to as the ''hedgehog technique''. This study investigates the feasibility of a modification of the hedgehog technique in autologous cartilage to repair shear-off lesions in children. *Design***.** Three consecutive patients (aged 11, 12 and 14 years) with shear-off chondral fragments of 2, 5 and 8 cm^2 were treated using this modified hedgehog technique. The calcified side of the chondral fragments were multiply incised and trimmed obliquely for an interlocking fit in the defect site. Fibrin glue and, if indicated sutures, were applied to fix the fragment to the defect. In one patient, an anterior cruciate ligament (ACL) repair was also performed. Patients were evaluated clinically and by MRI up to 12 months postoperatively. *Results.* Twelve months after surgery all patients reported no pain and showed complete return to sport and full range of motion. MRI showed no signs of fragment loosening. *Conclusions.* The modified hedgehog technique is a feasible treatment option to repair pure chondral shear-off lesions in the paediatric knee. This was the first time this technique was used in autografting.

Introduction

Traumatic osteochondral fractures and osteochondritis dissecans (OCD) are amongst the most commonly encountered orthopaedic pathologies in paediatric and adolescent knees. (1-3) Fortunately, osteochondral fragments can be surgically reattached yielding good results. (4, 5) This technique relies mainly on osseointegration between autologous bone-to-bone tissue, similar to the osteochondral autograft transfer system (OATS) technique (6). The matter becomes more complicated when the fragment consists solely of cartilage with no, or minimal attached bone. Regrettably, damaged cartilage has limited spontaneous self-repair, even in these young individuals (1), and if left untreated cartilage defects can propagate and eventually lead to osteoarthritis (7). Pure chondral defects in young individuals can result from shear-off trauma (5) or from type 3 OCD lesions (8). Particularly children are prone to shear-forces due to their developing osteochondral unit (3). These shear-forces can lead to delamination of cartilage from the subchondral bone leaving the developing calcified cartilage layer attached to the loose fragment (3). It is not completely understood if, and how, the reparative capacities between grade 3 OCD lesions and shear-off traumas differ (5), but often the same therapies are used (4, 5, 8). Therapies for shear-off traumas range from fragment removal and debridement to resurfacing using OATS or allografting, up to ideally restoring the native joint surface by refixation of the chondral fragment (5). The idea of transplanting pure chondral tissue onto the subchondral bone is not new but has been considered ineffective on the notion that cartilage is passive and indifferent (9). Refixation techniques often employ metal pins or screws for initial stability, or more recently, degradable biomaterials to overcome the need for additional removal procedures (4, 5, 8). For instance, darts were used for the refixation of pure chondral fragments which resulted in reasonable results (10). These darts are composed of stiff and slow degrading poly-L-lactide acid (PLLA), however, which can lead to erosion of the opposing cartilage, prolonged inflammation, and detrimental effects to the adjacent bone and cartilage as a result of its acidic degradation products (11-13). Histologic evidence of osteochondral integration with these techniques is also lacking (4, 5, 8, 10).

Bardos *et al.* performed a preclinical study with 9-month old pigs (14) and a clinical study in adults (9) in which a method is described for the treatment of cartilage defects using pure chondral allografts. As opposed to previously described methods, this technique does not require additional fixation biomaterials. Multiple incisions were made in the deep and intermediate zone of a chondral allograft on the side facing the subchondral bone (14). The intact superficial layer was confirmed histologically (14). Theoretically, incising the deep cartilage zones greatly increases the integrational surface, provides mobility for the chondrocytes and allows easier access for bone marrow-derived stem cells (14). Sparing the superficial zones preserves the tensile strength of the collagen fibres

and the reservoir of progenitor cells (14). The typical appearance of the osseous side after this modification has led to the name "hedgehog technique" (14). Finally, the allograft was secured in the defect site by sutures and sealed using fibrin glue (14). Both studies showed satisfying results (9, 14), even when compared to autologous chondrocyte implantation (ACI) (14).

We modified the hedgehog technique to reattach shear-off chondral fragments in 3 paediatric cases. A critical step of this modification is the creation of oblique shoulders by trimming the edges of both the shear-off fragment and the defect site. The major prerequisite for this step is that the fragment is larger than the defect site. It is well-known that cartilage swells due to increased water uptake when the integrity of its collagen network and bone plate is disrupted (15). As a proof of concept, first an *ex vivo* experiment was set up to investigate if this swelling would be sufficient to allow for modification. Subsequently, the modified hedgehog technique was applied in 3 children. These patients were followed for 1 year for clinical evaluation and magnetic resonance imaging (MRI).

Methods

Ex vivo Swelling Tests

Fresh osteochondral resections of 3 patients undergoing total knee arthroplasty (Local ethical approval 2017-0183), aged 41, 52 and 64 years, were collected. Seven pure chondral cylindrical explants measuring 6 mm in diameter were punched out from Outerbridge 0-1 areas on the medial femoral condyle. Seven similar-sized chondral explants were obtained from 3 young sheep (age: 20 months) in order to compensate for potential loss of osmotically active proteoglycans in the human osteoarthritic cartilage (16). NaCl 11.6 µL 5M was dissolved per mL Dulbecco's Modified Eagle's medium to mimic the synovial fluid osmolarity of 400 mmol/kg (17). Explants were placed in this hypertonic fluid at 37°C directly after harvest. High-quality photographs (Nikon D5600, Nikon micro lens 105mm. fixed distance) were obtained at 5 min, 3 and 24 hours. Synedra view software (Synedra GmbH, Austria) was calibrated and subsequently used to analyse the surface area of the explants over time. Two observers did the measurements. The swelling was assessed using the paired samples T-Test SPSS 23 (IBM Analytics, NY, USA).

Subjects

This study was conducted in accordance with the World Medical Association Declaration of Helsinki. All participants and their caretakers provided oral and written informed consent. The local ethics committee approved the study. Demographics of the subjects are shown in table 1.

Case 1

An 11 year old boy presented to our clinic complaining of pain in his right knee 3 days after sustaining an injury whilst playing soccer. Physical examination revealed effusion of his right knee and an extension deficit of 10-20 degrees. Tests for ligamentous or meniscal injury were negative. Conventional radiographs of the knee did not show any abnormalities. MRI showed an interruption in the cartilage covering the medial side of the medial femoral condyle (**Fig. 1**). The missing shear-off fragment was situated on the ventral side of the knee between the medial femoral condyle and the tibial plateau. Further findings included bone bruise of the medial femoral condyle and extensive effusion. No abnormalities of the menisci and ligamentous structures were found.

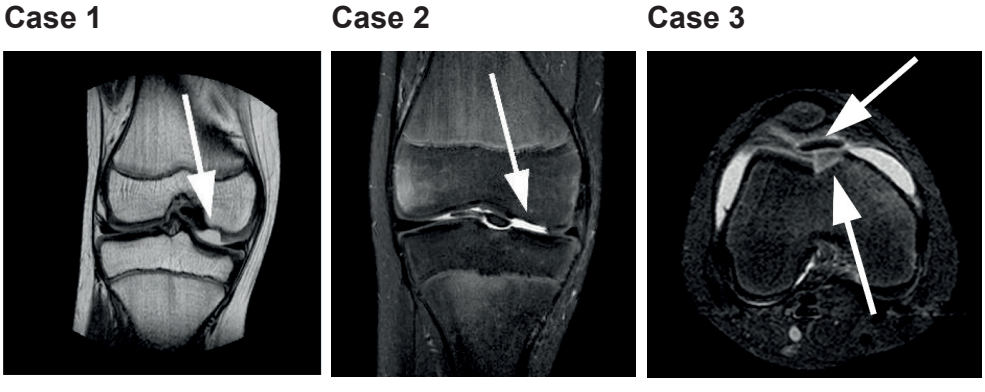
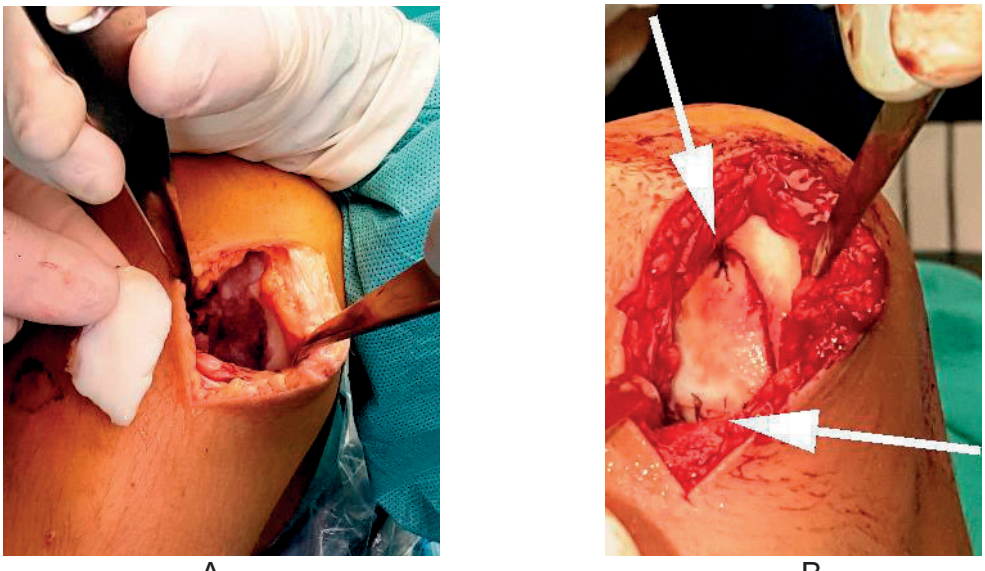


Figure 1. Preoperative Magnetic Resonance Imaging. Case 1: depicted as a proton density weighted coronal section showing a cartilage defect (2 cm^2, white arrow) in the medial femoral condyle; Case 2: depicted as spectral presaturation with inversion recovery coronal section showing a chondral defect on the medial femoral condyle (8 cm^2, white arrow); and Case 3: depicted as a short tau inversion recovery axial section showing a cartilage defect (5 cm^2, lower white arrow) in the junction between the medial facet of the trochlea and the medial femoral condyle. Note the shear-off fragment (upper white arrow) that is larger in diameter than the defect site.

Case 2

A 14 year old boy was referred to our clinic after suffering from a soccer trauma. He was complaining of pain and instability of his right knee. Physical examination revealed joint effusion and a positive anterior drawer and pivot shift test. The MRI showed an anterior cruciate ligament (ACL) rupture and a large chondral defect of his right medial femoral condyle (**Fig. 1**). The shear-off fragment (**Fig. 2**) was situated in the infrapatellar recesses.



A B

Figure 2. Large Shear-off Chondral Defect of Case 2. A: intra-operative image of case 2 showing the large (8 cm^2) shear-off chondral defect with the corresponding fragment; and B: The situation after reattachment of the chondral fragment with sutures (white arrows). Note the slightly depressed position of the fragment.

Case 3

A 12 year old boy presented to our clinic complaining of swelling and locking of his right knee after suffering a rotational trauma whilst playing soccer. Physical examination showed joint effusion, an extension deficit of 5 degrees and no clues for ligamentous injuries. MRI revealed a chondral lesion of the junction between the medial facet of the trochlea and the medial femoral condyle (**Fig. 1**). No abnormalities of the patella, menisci, cruciate or ligamentous structures were noted.

	Case 1	*Case 2*	*Case 3*
Age (years)	11.0	14.4	12.0
Sex	M	M	M
Height (cm)	155	175	158
Weight (kg)	35	52	41
Defect location	MFC	MFC	MFC+T
Defect size (cm^2)	2	8	5
Time between trauma and surgery (months)	1	3	4

Table 1. Demographics of the 3 cases. M: male; MFC: Medial femoral condyle; T: Trochlea.

Surgical technique

All surgeries were performed by the senior author (PE). During the arthroscopy the defect site was inspected and the chondral fragment was identified. Via an arthrotomy the fragment was pulled out of the joint and placed in Ringers lactate solution to prevent deterioration whilst waiting further processing (**Fig. 4a-b**) (18). The defect site was then abraded using a small surgical curette, removing fibrous tissue. The bone was abraded until bleeding of the bone was effected. Then, the defect rim was trimmed using a small surgical knife (No. 15 blade) to create oblique 60-80 degree shoulders (**Fig. 3**). In order to further contribute to a stable interlocking of the shear-off fragment, chisels were used to create sharp 60-80 degree angles at the cartilage-bone transition of the defect (**Fig. 3**).

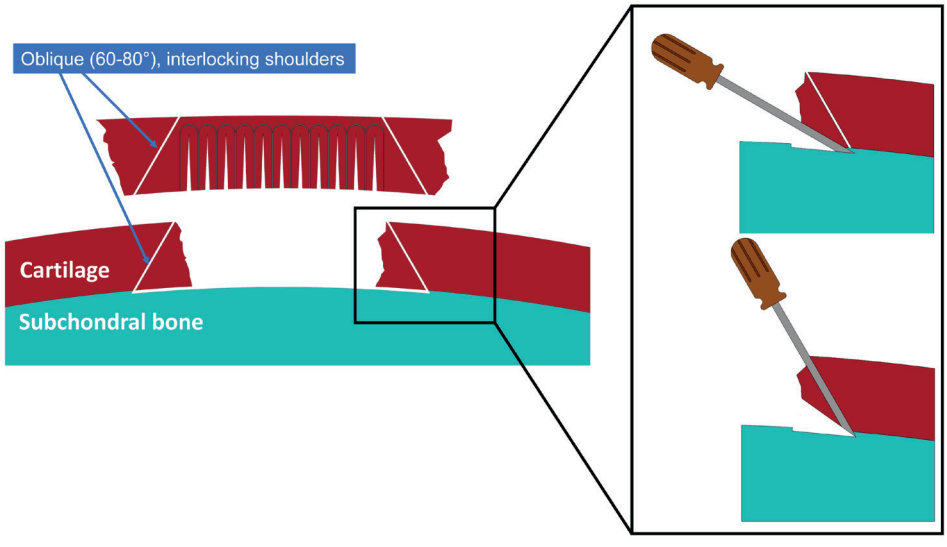


Figure 3. Schematic overview of surgical technique. Swelling of the shear-off fragment allows trimming of the edges in an approximately 60-80 degree angle of both the edges of the fragment as well as the defect, creating an interlocking match. The fibrous tissue is removed from the subchondral bone using a curette exposing vital, slightly bleeding subchondral bone, ultimately placing the fragment flush or slightly recessed to the surrounding cartilage; Magnification: For a proper press-fit fixation of the fragment, it is important to create the sharp angle in the bottom parts using a small chisel.

The shear-off fragment was then processed. First, the edges were trimmed in order to create a 60-80 degree angle and to fit the fragment in the defect site (**Fig. 3**). Macroscopically and upon palpation the osseous side of the fragment appeared hard which is in line with the presence of the calcified layer after shear-off trauma in children (3). Therefore, instead of using an automated device (9, 14), multiple incisions were meticulously performed freehand spaced approximately 1 mm apart (**Fig. 4c-d**). Subsequently, the de-

brided defect site was filled with fibrin glue (Tissucol®, Baxter, The Netherlands) (**Fig. 4e**), and the fragment was placed back to its original position (**Fig. 4f**). Fibrin glue was also applied between the adjacent cartilage and the fragment. Removal of fibrous tissue on both the calcified part of the shear-off fragment as well as the subchondral bone of the defect led to a flush or slightly recessed press-fit position of the graft (**Fig. 2b**). In case 2 biodegradable Vicryl 5.0 sutures were used to secure the processed fragment in its position (**Fig. 2b**). The fresh construct was tested by several flexion- extension iterations before closing the wound. For case 2, the modified hedgehog autografting was performed directly after the ACL reconstruction.

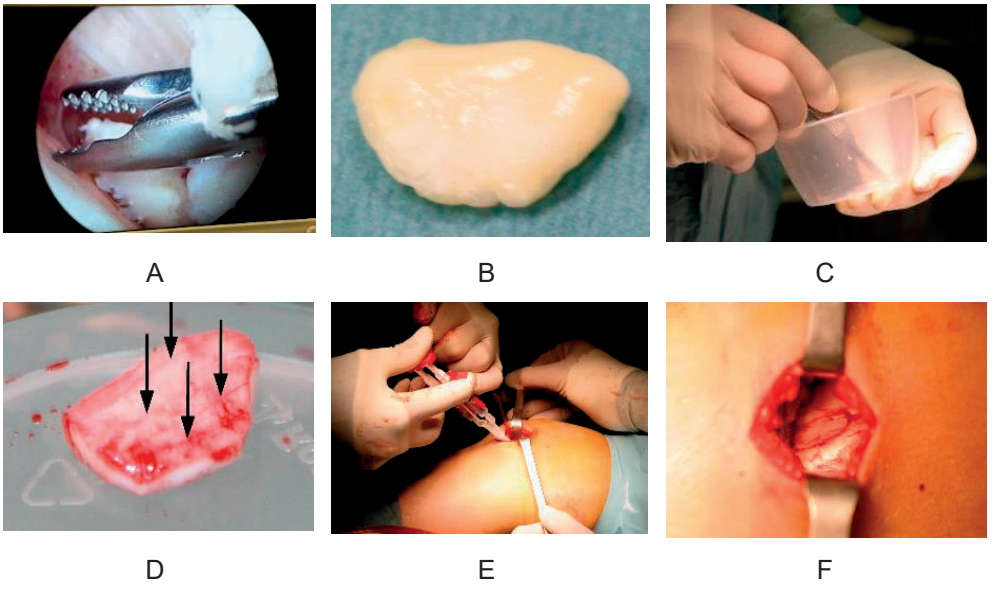


Figure 4. Intraoperative steps of the modified hedgehog autografting technique using case 3 as example. A & B: Arthroscopic identification of shear-off fragment, which is removed out of the knee joint; C: processing of the fragment using a small surgical knife freehand and a plastic tray to support handling; D: calcified side of fragment after the edge has been trimmed and multiple incisions (arrows) are made; E: application of fibrin glue in the abraded defect site and between the shear-off fragment and adjacent cartilage; and F: the chondral fragment after it was placed back to its original position.

Rehabilitation protocol

Early-stage rehabilitation was initiated by 2 weeks non-weight bearing with the knee fully extended in an adjustable hinged brace, followed by incremental steps of 25%, 50% and 100% weightbearing and simultaneously 30 and 60 degrees flexion followed by full range of motion in a 8 week period. This protocol was based on the fast tibiofemoral rehabilitation of the ACI protocol by Hambly *et al (19)*. Next, from 8 to 12 weeks patients

were allowed for gradual increases in training load and volume under physiotherapist supervision. After 12 weeks patients were allowed to gradually increase sports activities.

Magnetic Resonance Imaging

MRI pulse sequence protocols were applied for the 1.0 T dedicated peripheral MRI system (OrthOne™, ONI INC., Wilmington, MA, USA). Subjects were seated with their knee extended and centered in the circumferential extremity coil. Fast spin echo proton density weighted (PDW) T1, and Short Tau Inversion Recovery (STIR) T1 sequences were obtained. MR images were obtained approximately 3 and 12 months postoperatively and evaluated by an experienced musculoskeletal radiologist (DL). The images were examined for fragment delamination and morphology, displacement of the fragment and for interface gaps. Both the signal intensity of the cartilage and the subchondral bone were evaluated.

Clinical Assessment

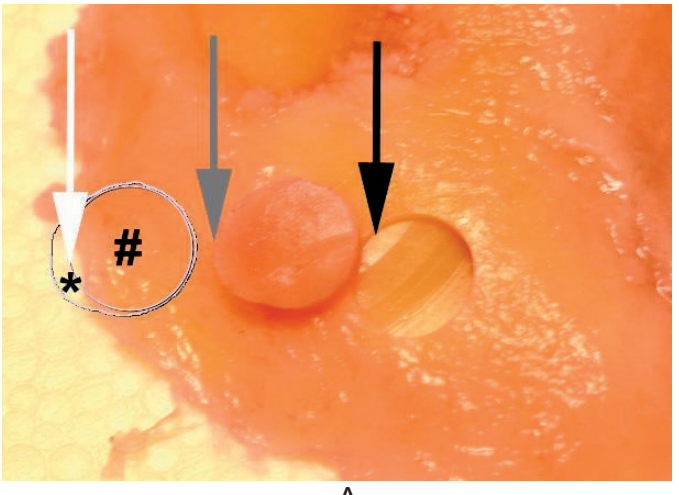
All patients were seen after 2 weeks for wound inspection and at 3 and 12 months postoperatively. Patients were asked for pain, activities of daily living, sports activity and the presence of any restriction. A standard physical examination was performed at the outpatient clinic.

Results

Ex Vivo Swelling test

After 24 hours, human cartilage explants increased 7 ± 7 % (mean ± SD) in surface area ($p = 0.06$), whereas the caprine cartilage increased 54 ± 34 % (mean ± SD) in surface area ($p = 0.03$). Most of the swelling took place in the first 3 hours (4% and 48% respectively) after which it plateaued (**Fig. 5**). Inter-observer agreement was excellent with a Cronbach-alpha intraclass correlation of 0.897. For the smallest cartilage fragment of 2 cm^2, 1 mm trimming translates to 26%, which was considered achievable in the 3 paediatric cases.

Figure 5: Ex Vivo Experiment on Cartilage Swelling



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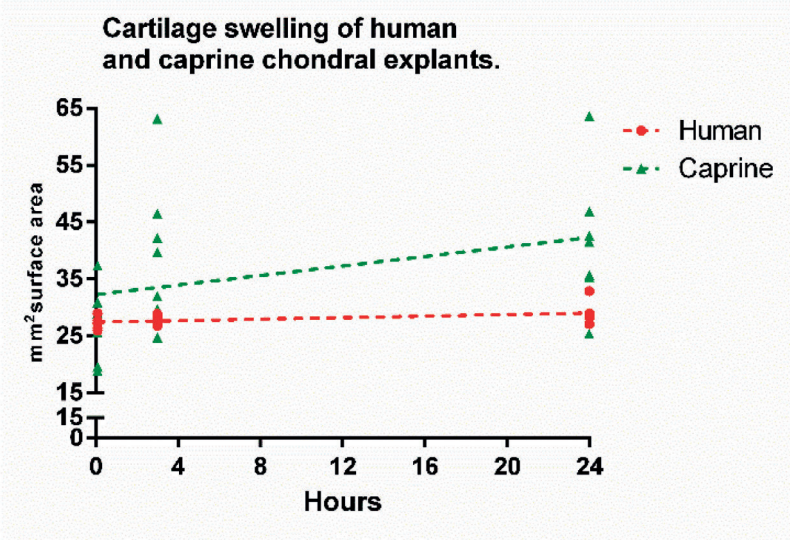

B

Figure 5. A: Human chondral cartilage explant 24 hours after harvest placed next to its original position. Note, right to left, the smaller diameter of the defect site (black arrow), compared with the corresponding larger diameter of the fragment (grey arrow) and overlying surface area measurements (white arrow). (#): 29.0 mm^2; (*): 32.48 mm^2; and B: Average increase in surface area of human and caprine explants 5 minutes and 3 and 24 hours after harvest, being kept in 400 mmol/g 37 °C Dulbecco's Modified Eagle's medium. Individual consecutive human (red beads) and caprine (green triangles) measurements are depicted. The average increase in surface area after 24 hours is depicted per species in the dotted lines. Note that most of the swelling takes place in the first few hours (individual points).

Surgery

No intra- or postoperative complications were observed. The articulating and calcified cartilage layer could easily be identified on all fragments by visual inspection and probing. None of the loose fragments showed any macroscopic evidence of residual bone. All fragments had a good fit in the defect site after trimming with no gaps observed between fragment and adjacent cartilage.

Magnetic Resonance Imaging

None of the subjects showed fragment delamination, displacement, large interface gaps, osteophytes or subchondral cysts one year postoperatively (**Fig. 6**). At 3 months postoperatively in case 2 and 3 MR images demonstrated normal cartilage signal intensity of the implanted shear-off fragment. Case 1 showed mild hyperintense signal of the fragment compared to the surrounding cartilage, persistent after 12 months. Case 2 and 3 demonstrated a mild irregular cartilage fragment surface at 3 months follow-up. At 12 months follow-up case 3 showed a smooth fragment surface, the mild irregularity of the cartilage persisted in case 2. Case 2 demonstrated mild depression of the fragment of about 1 mm at 3 and 12 months follow-up. Oedematous changes in the subchondral bone were visible at the preoperative and first postoperative MRI in all 3 cases, which decreased and completely normalized after 12 months. A small sclerotic band was visible in the subchondral bone in all cases at the first postoperative MRI, and only case 1 demonstrated this mild sclerosis after 1 year. The thickness of the cartilage did not change over time.

Clinical Assessment

Two weeks after surgery all wounds were healed and all patients had stopped using painkillers. At the clinical assessment at 3 months both patient 1 and 3 had regained full range of motion (>130 degrees of flexion) and were playing sports under supervision while patient 2 was on schedule within the ACL protocol. At 3 months patient 1 and 3, reported no pain and gradual return to sports. Complete return to sports was achieved after 6 months for patient 1 and 3 and 12 months in patient 2.

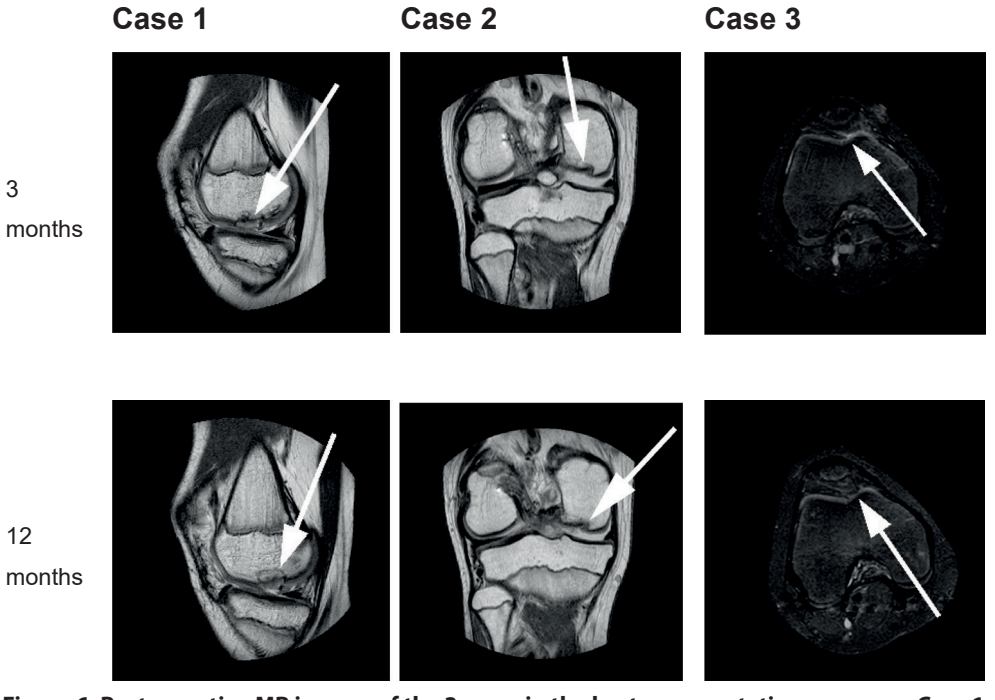

Figure 6. Postoperative MR images of the 3 cases in the best representative sequence. Case 1 is depicted as a proton density weighted sagittal section showing good osteochondral integrity at 3 and 12 months (white arrows); Case 2 is depicted as proton density weighted coronal section showing good osteochondral integrity at 3 and 12 months. Note the pre-existent calcified depression (white arrows) at 3 months which gradually improved at 12 months; Case 3 is a short tau inversion recovery axial section showing no abnormalities at 3 and 12 months (white arrows are the former defect location).

Discussion

The present study describes 3 paediatric cases in which the hedgehog technique is modified to reattach shear-off chondral fragments to the subchondral bone of the knee. Thereby, to the best of our knowledge, using this technique for the first time in autografting. After one year, MR images showed no signs of fragment loosening. All patients completely recovered with full return to sports.

Already in the 1980's Kaplonyi *et al.* investigated the use of fibrin adhesives for refixation of (osteo)chondral fragments in both animal and humans (20, 21). In case of pure chondral fragments, the subchondral bone was drilled prior to fixation to allow for a healing response (20). For osteochondral fragments, joint function after 5 years was generally good to excellent and radiographs showed good osseointegration (20). The integration

of pure chondral fragments could not be evaluated however due to the absence of MR images or histology (20). In 2009, Bardos *et al.* reported for the first time on the results of the hedgehog technique using chondral allografts to repair cartilage defects in nine month old pigs (14). The chondral allografts were prepared by removing the cartilage from the subchondral bone using a blade. It was not described if the calcified cartilage layer was included when using this harvesting method (9, 14). Six weeks after the hedgehog technique, histological assessment revealed cell invasion within the incisions of the allograft (14). Eighteen weeks later histological evidence of hyaline cartilage and complete osteochondral integration was found. The former cartilage cuts in the deep zone were no longer visible (14). Results, based on the histological International Cartilage Repair Society score, were comparable to ACI and superior to microfracture (14). Despite the fact that chondrocytes suffer from steric hindrance, cell migration in cartilage has been proven before in the presence of injury or lesions (22, 23). The cell invasion and the following disappearance of the incisions in the study by Bardos *et al.* (14), are therefore indicative for cell mobility and deposition of extracellular matrix following the hedgehog technique. In 2015, the same group conducted the first clinical study of 8 focal chondral lesions in 7 patients, aged 34.3 ± 8.4 years (mean ± SD), and found reasonable results (9). The MRI at 1- and 2-years follow-up showed normal graft intensity and graft thickness in 83% of the cases. Short Form–36 health survey and Lysholm scores increased significantly after 1-year but had dropped at the 2-year mark without clear explanation (9). Long-term follow-up of these patients would be valuable.

Encouraged by the results of Kaplonyi *et al.* on the pro-integrative capabilities of fibrin glue and the histological evidence of osteochondral integration provided by Bardos *et al.*, we further modified the hedgehog technique for shear-off fragments in children. Modification included the use of autografts instead of allografts, the creation of oblique interlocking edges, the addition of using fibrin glue in between the cartilage and bone and performing the incisions freehand. Instead of drilling into the subchondral bone, as described by Kaplonyi *et al.*, we abraded the subchondral bone to expose vital, bleeding bone. If, and how, the process of integration of allografts as described by Bardos *et al.* differs from the integration of shear-off autografts in this study is not known nor is the role of potentially including the calcified cartilage layer in our cases known. Including histology in future studies could unravel these unanswered questions. Furthermore, in hedgehog allografting the appropriate graft size can be selected. In contrast, for the shear-off fragments it had to be proven that the fragments swell sufficiently to allow for trimming. It was hypothesized that the degree of swelling of paediatric cartilage would be between that of a young animal and that of osteoarthritic cartilage. The fact that all 3 fragments fitted well after modification confirmed this concept. It remains to be determined if shear-off fragments of adults swell sufficiently to allow for the same modified hedgehog technique.

Alternative methods to reattach chondral tissue have been described yielding varying success (5). These include metal screw fixation or the use of biodegradable fixation materials (10, 24). Although reasonable results can be achieved by metal screws (24), the costs and invasiveness of an additional surgery are important drawbacks. Biodegradable fixation methods such as PLLA darts overcome the need for an additional surgery and showed promise in a small case-series (10). However, PLLA remnants and degradation products can remain present for years (25, 26). These potentially jeopardize osteochondral integration due to its negative effects on chondrogenesis and bone formation (11, 12). Moreover, both metal and PLLA are stiff biomaterials which can erode the opposing articulating tissues (13). The modified Hedgehog technique does not have these biomaterial-related drawbacks.

The present study has several limitations. Firstly, we did not evaluate our clinical assessment by patient reported outcome measures. Although none of the patients reported any restriction and all had regained full functionality, validated questionnaires would have made comparison to other techniques easier. Secondly, therapies like microfracture also yield good results up to 2 years, making the follow-up in the present study rather short (27). We also rationalized that the calcified layer was attached to the loose fragment, but this requires histological confirmation. Lastly, survival of the fragments and its viability after a period in the joint space is still not completely known. In 2007, Hembree *et al.* investigated loose osteochondral fragments 5 days after joint trauma and found impaired chondrocyte viability at the cartilage edges but intact viability in the middle and deep zones, supporting our trimming method (28). In 2016, Pascual-Garrido *et al.* found no differences in viability of chondrocytes derived from loose osteochondritis dissecans fragments and unaffected cartilage (time not mentioned) (29). Loose fragments have even been used as viable cell source for ACI (30). Still, it remains to be investigated what the maximum period is after trauma that such fragment can be considered viable and what the effect was of the time to surgery in current cases.

For decades, cell-based therapies have been considered most promising to regenerate hyaline cartilage. In recent years there is an increasing interest however in reintroducing intact cartilage extracellular matrix to the defect, such as with particulated cartilage (31). In this study the hedgehog technique showed great promise to repair shear-off lesions in children. As such it contradicts the long-standing dogma of cartilage being indifferent and passive during cartilage repair by showing integration of full-thickness cartilage to the subchondral bone. Future researchers are encouraged to include longer follow-up periods and make comparison to established techniques. Ideally, higher Tesla scans should be obtained to allow for dynamic evaluation of bone and cartilage at a highly detailed level and to allow for magnetic resonance observation of cartilage repair tissue (MOCART-) scoring (32).

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Declaration of Conflict of Interests

The Author(s) declare(s) that there is no conflict of interest

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CHAPTER IV

A Systematic Review of Focal Cartilage Defect Treatments in Middle-aged versus Younger Patients.

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Abstract

Background: Focal cartilage defects are often debilitating, possess limited potential for regeneration, are associated with increased risk of osteoarthritis and are predictive for total knee arthroplasty. Cartilage repair studies typically focus on the outcome in young subjects, but a high proportion of treated patients is 40 to 60 years of age, i.e. middle-aged. The reality of current clinical practice is that the ideal patient for cartilage repair is not the typical patient. Specific attention to cartilage repair outcomes in middle-aged patients is warranted.

Purpose: The aim of this study was to systematically review available literature on knee cartilage repair in middle-aged patients and include studies comparing results across different age groups.

Study design: Systematic review.

Methods: A systematic search was performed in EMBASE, MEDLINE, and the Cochrane database. Articles were screened for relevance and appraised for quality.

Results: Twenty-one articles (mean Coleman Methodology Score 64 points) were included. Two out of three bone marrow stimulation (BMS) studies, including the microfracture technique, revealed inferior clinical outcomes in middle-aged patients in comparison to younger patients. Ten cell-based studies were included showing inconsistent comparisons of results across age groups for autologous chondrocyte implantation (ACI). Bone marrow aspirate concentrate (BMAC) showed age-independent results up to 8 years follow-up. A negative effect of middle-age was reported in one study for both ACI and BMS. Four out of five studies on bone-based resurfacing therapies (allografting and focal knee resurfacing implants (FKRIs)) showed age-independent results up to five years. One study in only middle-aged patients reported better clinical outcomes for FKRIs when compared to biological repairs.

Conclusion: Included studies were heterogeneous and had low methodological quality. BMS in middle-aged patients seems to only result in short-term improvements. More research is warranted to elucidate the ameliorating effects of cell-based therapies on aging. Bone-based therapies appear to be relatively insensitive to aging and may potentially result in effective joint preservation. Age sub-analyses in cohort studies, randomized clinical trials, and international registries should generate more evidence for the large, but in cartilage repair literature underrepresented middle-aged population.

Introduction

Focal cartilage defects in the knee can cause considerable pain and disability (1), and can impair quality of life to the same extent as osteoarthritis (OA) patients scheduled for total knee arthroplasty (TKA) (2). Articular cartilage possesses limited repair potential, and therefore cartilage defects frequently progress towards OA (1, 3). Cartilage defects have thus been recognized as a major predictive factor for TKA in patients over 45 years (4).

Currently applied cartilage repair therapies include palliative techniques, i.e. chondroplasty, and a wide variety of regenerative techniques with differing complexities, and various bone-based cartilage resurfacing techniques. Regenerative treatments include the bone-marrow stimulation (BMS) techniques such as abrasion arthroplasty (AA), microfracture (MF) (5-8), and autologous matrix-induced chondrogenesis (AMIC) (1). The cell-based techniques include autologous chondrocyte implantation (ACI), ACI with periosteal flap (ACI-p), collagen flap (ACI-c) or matrix assistance (ACI-m) (9-13), and bone marrow aspirate concentrate (BMAC) (14-16). Biological cartilage resurfacing via osteochondral autograft transplantation (OAT) (17) and osteochondral allograft transplantation (OCA) (12, 18), and metallic and biosynthetic cartilage resurfacing via focal knee resurfacing implants (FKRIs) (19-23) are considered bone-based techniques since they rely on osseointegration rather than chondrogenesis (24). Together, these interventions constitute the spectrum of surgical options currently available for cartilage repair.

Large databases have shown that 52-60% of cartilage surgeries are performed in patients between 40 and 60 years old (6, 7, 25, 26). Although there is no official definition, 40-60 years is generally referred to as middle-aged (27, 28). The indication for each cartilage treatment in published guidelines is typically dictated by the size or location of the defect. Patient age is not typically included in these treatment algorithms (1, 29-33). Available randomized controlled studies evaluating the efficacy of cartilage repair typically include an upper age limit as an inclusion criteria (34). The upper age limit is often set at or around 40 years, i.e. the ideal patient. Therefore, the most commonly treated patient is the least represented in literature and subsequent guidelines.

The ultimate goal of cartilage repair in middle-aged is joint preservation by postponing or eliminating the need for unicompartmental knee arthroplasty (UKA) or TKA. In our opinion, this could be attained by improving pain and functional performance sufficiently while preserving the native anatomy, at least the bone-stock, for potential joint replacement later in life.

When cartilage repair fails, arthroplasty is considered the last resort. Orthopaedic surgeons perceive a treatment gap for the management of middle-aged patients who suffer

from cartilage defects due to the lack of conclusive evidence identifying a superior cartilage treatment (19, 21, 35-37). The application of treatments in practice consequently varies greatly among orthopaedic surgeons, particularly in the middle-aged patient population (38, 39). At the same time, the number of arthroplasty procedures in middle-aged patients has shown an undesirable large increase in the past decade (40). There is a high risk of revision surgery later in life when TKA is opted for at a relatively young age (41). Men receiving a knee replacement in their early 50's have a 35% lifetime risk of revision (41). It is well known that TKA revisions result in inferior outcomes and are associated with high costs (42).

The objective of this study is to systematically review available literature on cartilage repair treatment outcomes in middle-aged, and middle-aged versus younger patients. We hypothesize that all cartilage repair techniques performed in middle-aged patients lead to inferior clinical and imaging outcomes and higher failure rates when compared to younger patients.

Material and Methods

Search Strategy and Study Selection

A systematic literature search was performed in MEDLINE, EMBASE and Cochrane Library databases according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. Level I to IV studies (based on the Oxford Centre for Evidence-Based Medicine) on cartilage repair in middle-aged patients were included. The search was conducted with support of a university librarian in December 2020 using the following terms (including its free terms using title and abstract, MesH terms, abbreviations, single and plural forms): (("Chondral" or "osteochondral" or "cartilage") and ("wounds and injuries" or "damage" or "defect" or "lesion" or "injury" or "trauma" or "wound") and ("knee")) and ("cartilage repair" or "regeneration" or "healing" or "microfracture" or "autologous chondrocyte implantation" or "autologous chondrocyte transplantation" or "matrix assisted chondrocyte implantation" or "autologous matrix-induced chondrogenesis" or "chondrocyte/surgery" or "tissue engineering" or "tissue scaffolds" or "mosaicplasty" or "osteochondral autograft" or "osteochondral allograft" or "resurfacing" or "resurface" or "bone-implant interface" or "implant" or "bone-anchored prosthesis" or "prosthesis and implants" or "prosthesis" or "mosaicplasty" or (("focal" or "local") and "arthroplasty")). This review was registered in the international database PROSPERO under the registry number CRD42020179932.

Results from both databases were uploaded to Endnote X7 (Clarivate Analytics). The combined database was scanned for duplicates and then uploaded to the online systematic review software Rayyan (Qatar Computing Research Institute) (43). This allowed two observers (RJ and PvH) to independently screen titles and abstracts. Article screening and selection was performed using the following inclusion criteria: studies evaluating cartilage repair treatment in patients aged between 35 and 65 years, adding 5 years above and below the typical middle-aged (27, 28) extended the search results. Studies with an age comparison between young and middle-aged patients with a clear age cut-off were also included. Furthermore: a minimum follow-up of two years, a minimum number of 25 patients, human studies, and English language. Exclusion criteria included: comparative studies not including middle-aged patients in the 'older' group (upper age limit ≤50 years), mean patients' age <40 years for non-comparative studies, animal studies, studies which stated osteoarthritic change defined as grade ≥1 on the Kellgren-Lawrence scale, other joints than the knee, and patellofemoral- or tibial defects only. Upon reading the full-text articles assessed for eligibility, snowball sampling was performed allowing the inclusion of relevant referred studies. A consensus meeting was held to sort out potential disagreements.

Data Extraction

Two observers (RJ and PvH) systematically extracted study data, which included patient demographics, study design, in- and exclusion criteria, study groups, previous and concomitant surgeries, defect characteristics, follow-up time and study outcomes.

Assessment of Quality and Bias.

The Coleman Methodology Score (CMS) was used to assess the quality and bias of each study (44). This score evaluates among others the participants' selection criteria, the study design, surgical and rehabilitation protocols, assessments of outcomes, providing each study with a scoring from 0 to 100. Articles were designated as "excellent" (>85), "good" (70 to 84), "fair" (55 to 69) or "poor" (<55).

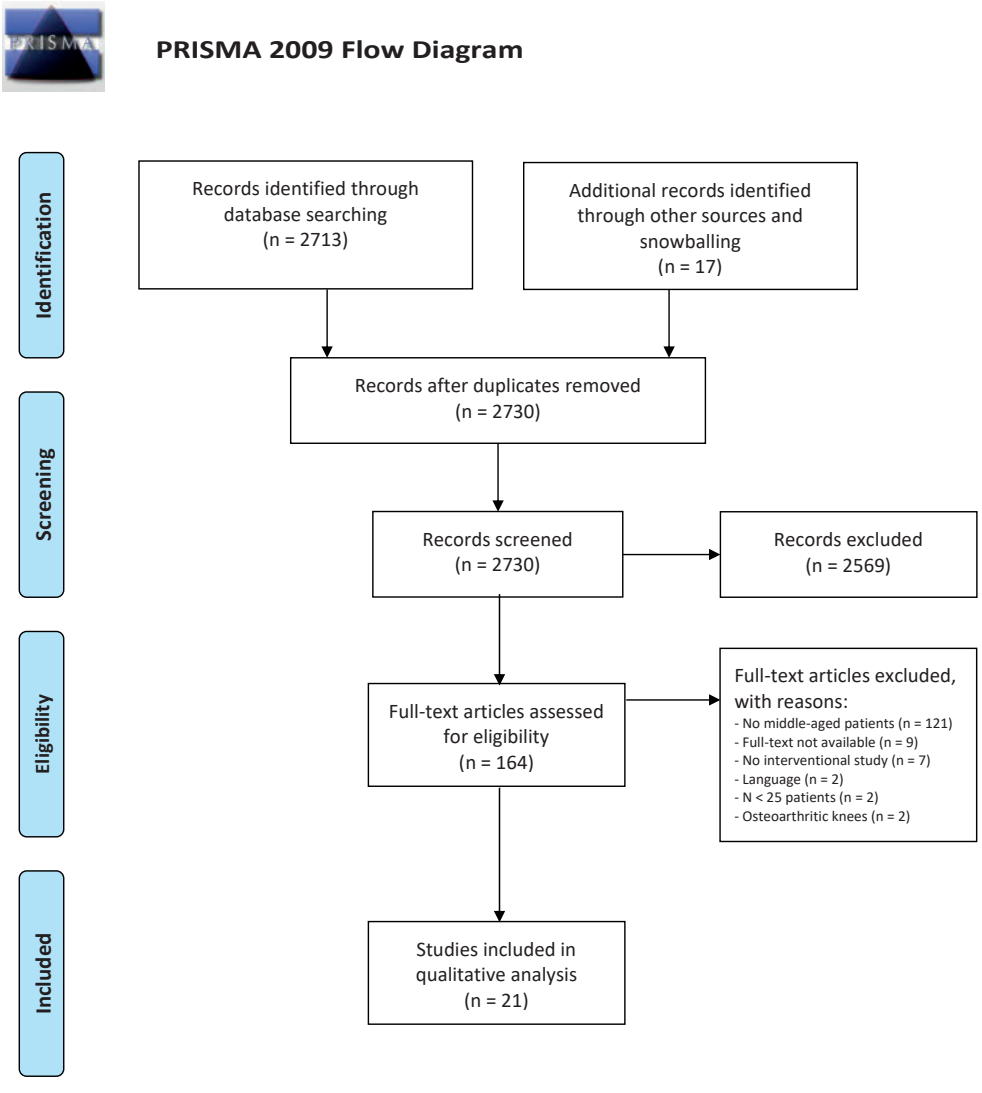

Figure 1: PRISMA flowchart showing the yield of the initial search and exclusion of studies leading to the 19 included studies.

Results

Study Characteristics

The search strategy yielded 21 studies for inclusion as is shown in the PRISMA flow diagram in figure 1. Three studies on BMS (5, 8, 45), nine studies on cell-based techniques (9-15, 46,

47), five bone-based resurfacing studies (18, 22, 23, 48, 49), and four studies comparing different treatments (16, 19, 21, 50) were included. Table 1 shows the study characteristics of all included studies. Level of evidence was IV in 18 studies (5, 8, 9, 11-16, 18, 21-23, 45-49), level III in two studies (19, 50), and one study was level II (10) Various age cut-off values were used to make a comparison between middle-aged and the younger patient population, but 40 years was the most commonly used cut-off. The average CMS was 64, with three studies scoring "poor", 13 "fair" and five "good." Patient characteristics, study setup and outcomes, and a summary of results are provided in the evidence table for each study (Table 2).

Author and year	Study Design	Investigated technique	Age cut-off (y)	Level of Evidence	Coleman Methodology Score (0-100)
Bone marrow stimulation					
Kreuz et al. 2006	Case series	MF	40	IV	64
Sansone et al. 2014	Case series	Abrasion	50	IV	61
Gille et al. 2013	Case series	AMIC	46	IV	53
Cell-based techniques					
Nehrer et al. 2006	Case series	ACI with matrix	30	IV	70
Kirshnan et al. 2006	Case series	ACI collagen	20 / 40*	IV	65
Niemeyer et al. 2010	Case series†	ACI collagen	40	II	69
Brix et al. 2014	Case series	ACI with matrix	40	IV	63
Filardo et al. 2014	Case series	ACI with matrix	40	IV	62
Filardo et al. 2017	Case series	ACI with matrix	40	IV	57
Gobbi et al. 2014	Case series	BMAC	45	IV	54
Gobbi et al. 2017	Case series	BMAC	45	IV	68
Gobbi et al. 2019	Case series	BMAC	45	IV	73
Bone-based techniques					
Levy et al. 2013	Case series	OCA	30	IV	63
Frank et al. 2017	Case series	OCA	40	IV	66
Anderson et al. 2018	Case series	OCA	40	IV	72
Martinez-Carranza et al. 2020	Case series	FKRI	NA	IV	60
Holz et al. 2020	Case series	FKRI	NA	IV	60
Studies including different treatments					
De Windt et al. 2009	Case series	ACI** and MF	30	III	48
Nejadnik et al. 2010	Cohort	BMAC *vs.* ACI periosteum	45	IV	70
Pascual-Garrido et al. 2016	Comparative Cohort††	FKRI *vs.* biologics	NA***	III	63
Nathwani et al. 2017	Case series†††	FKRI *vs.* MF	40	IV	76

Table 1. Overview of included studies. ***MF**: microfracture; **AMIC**: autologous matrix-induced chondrogenesis; **ACI**: autologous chondrocyte implantation; **BMAC**: bone marrow aspirate concentrate; **OCA**: osteochondral allografting; **FKRI**: focal knee resurfacing implant.* †: inclusion according to matched-pair analysis; ††: focal knee resurfacing implant compared to a group biological treatments consisting of MF, OCA and osteochondral autograft, debridement and ACI. †††: including comparison to 4 historical microfracture cohort selected by literature review; *: age cut-offs leading to three groups of patients: <20, 21-40 and >41 years old; **: ACI with either periosteal or collagen flap; ***: comparative study performed in patients between 35 and 65 years.

Author	Study Groups (n)	Mean Age (y)	Additional surgeries
Bone marrow stimulation			
Kreuz et al. (2006)	**Therapy:** All patients underwent MF (70) **Subgroup analysis on age and defect location:** Group 1a: femoral condyles ≤40 years (16) Group 1b: femoral condyles >40 years (16) Group 2a: tibia ≤40 years (4) Group 2b: tibia >40 years (7) Group 3a: patellofemoral ≤40 years (16) Group 3b: patellofemoral > 40 years (11)	**Overall:** 39.5 **Group 1a:** 29.9 **Group 1b:** 47.5 **Group 2a:** 26.2 **Group 2b:** 47.4 **Group 3a:** 34.1 **Group 3b:** 49.4	No previous surgeries reported No concomitant surgeries reported
Sansone et al. (2014)	**Therapy:** All patients underwent bone marrow stimulating abrasion arthroplasty 53) **Subgroup analysis on age:** Patients aged <50 years (25) compared to ≥50 years (28)	**Overall:** 45.9	None (part of the exclusion criteria)
Gille et al. (2013)	**Therapy:** All patients underwent AMIC (57) **Subgroup analysis on age:** Group A: 17 to 32 years (17) Group B: 33 to 46 years (27) Group C: 47 to 65 years (13)	**Overall:** 37.3	**Previous surgeries:** Diagnostic arthroscopies (10), partial meniscectomies (5), shavings (16), drilling or microfracture (4) **Concomitant surgeries:** Patella realignments (2), corrective osteotomies (3), partial meniscectomies (6) and ACL reconstruction (1)
Cell-based techniques			
Nehrer et al. (2006)	**Therapy:** All patients underwent ACI-m (36) **Subgroup analysis on age:** Patients aged <30 years (17) compared to >30 years (19)	**Overall:** 33.0	No previous surgeries reported No concomitant surgeries reported
Krishnan et al. (2006)	**Therapy:** All patients underwent ACI-c (199) **Subgroup analysis on age:** -Group 1: <20 years (28) -Group 2: 21-40 years (137) -Group 3: >41 years (34)	Not provided	Up to three previous surgeries (NOS) No concomitant surgeries reported
Niemeyer et al. (2010)	**Therapy:** All patients underwent ACI-c (74) **Matched-pair analysis:** Patients aged <40 years (37) *vs.* ≥40 (37)	**Patients <40:** 31.1 **Patients ≥40:** 44.8	No previous surgeries reported No concomitant surgeries reported
Brix et al. (2014)	**Therapy:** All patients underwent ACI-m **Subgroup analysis on age:** Patients aged <40 years (22) *vs.* >40 years (5)	**Overall:** 33.4	**Previous surgeries: I**n 75.5% of total group **Concomitant surgeries:** HTO, ACL reconstruction (NOS)

BMI	Defect Characteristics	Measures	Follow-up	Results
Group 1a: 23.3 **Group 1b:** 27.0 **Group 2a:** 24.3 **Group 2b:** 26.0 **Group 3a:** 24.2 **Group 3b:** 28.2	**Size and Outerbridgde classification:** Group 1a: 2.13 cm^2, 3.75 Group 1b: 1.97 cm^2, 3.75 Group 2a: 2.19 cm^2, 3.75 Group 2b: 2.18 cm^2, 3.57 Group 3a: 2.38 cm^2, 3.88 Group 3b: 2.39 cm^2, 3.82	Cincinnati knee score, ICRS scoring system, MRI 18 and 36 months, Cincinnati score	36 months (NOS)	**Cincinnati score:** Group 1a and 3a > group 1b and 3b (P<0.01) Between 18 and 36 months ICRS scores deteriorated in patients >40 years *(P<0.05)* **MRI defect filling:** Patients ≤40 years > patients >40 years *(P<0.05)*
Not provided	**Defect size:** **<50 years:** 7.5% > 4cm^2 **≥50 years:** 28.3% > 4cm^2 (P=0.022) **Defect grade:** All defects grade IV Outerbridge (NOS)	KSS, WOMAC, Survival defined as requiring second surgery	20.1 years	**KSS score:** 84% in patients <50 years vs. 53.6% ≥50 years (*P=0.046*) **Survival:** 89.5% in patients aged <50 years vs. 55.7% in ≥50 years **Arthroplasties:** 1 in patients aged <50 years vs.12 in ≥50 years
Not provided	**Mean defect size:** 3.4 cm^2 **Defect grade:** 35% Outerbridge III and 65% Outerbridge IV	Lysholm, VAS	2 years (NOS)	**Lysholm score:** No statistical differences between the three age groups (*P=0.085)*
Not provided	**Mean defect size:** Range 1.5-8.0 cm^2 (NOS)	Lysholm Cincinnati score, IKDC, form, ICRS grading	36 months (NOS)	**Lysholm and IKDC:** Significant improvement compared to preoperative in patients <30 years vs. no significant improvement in patients >30 years *(P<0.01)*
Not provided	**Mean defect size:** 4.9 $cm^{2,}$	Modified Cincinnati, ICRS grading, Histology	4 years (NOS)	**Cincinnati score:** group 1: 76.7 vs. group 2: 60.8 vs. group 3: 55.0 *(P < 0.001)* **Good/Excellent results:** group 1: 85.7% vs. group 2: 64.2% vs. group 3: 55.9% *(P = 0.04)*
<40 years: 24.5 **≥40 years:** 23.2	**Defect size:** Not significantly different between the groups (NOS). **Defect grade:** ICRS grade III or IV	IKDC, Lysholm, Cincinnati sports activity scale, TAS	24 months (NOS)	**All outcomes:** <40 years = patients ≥40 years *(P<0.05)*
Overall: 24.5	**Mean defect size:** 4.4 cm^2 **Defect grade:** ICRS grade III or IV	Failure, Survival, Lysholm, IKDC	5.0 years	**Lysholm:** Δ 25.8 in patients <40 years *vs.* Δ 5.6 in patients >40 years *(P=0.045)*

Table 2. Evidence table with included studies and corresponding study characteristics and outcomes. (*continued*)

Author	Study Groups (n)	Mean Age (y)	Additional surgeries
Gobbi et al. (2014)	**Therapy:** All patients underwent BMAC (25) **Subgroup analysis on age:** Patients aged <45 years compared to >45 years (multivariate analysis)	**Overall:** 46.5	**Previous surgeries:** Such as HTO are mentioned but no details are provided **Concomitant surgeries:** Knee stabilizing surgeries are mentioned but no details are provided
Filardo et al. (2014)	**Therapy:** All patients underwent ACI-m (142)	**Overall:** 29.2	**Previous surgeries:** In 71/142 patients including: meniscectomy, ACL reconstruction, etc. **Concomitant procedures:** In 54/142 patients including: meniscectomy, ACL reconstruction, etc.
Filardo et al. (2017)	**Therapy:** All patients underwent ACI-m (157) **Subgroup analysis on age:** Patients aged <40 years (122) *vs.* >40 years (35)	**Patients <40:** 25.6 **Patients >40:** 46.0	**Previous surgeries:** Patients aged <40 years 53.3% *vs.* >40 years 71.4% *(NS)* **Concomitant surgeries:** Realignment or ligament reconstruction 50.8% in patients aged <40 years *vs. 42.9% in patients* >40 years 42.9% *(NS)*
Gobbi et al. (2017)	**Therapy:** All patients underwent HA-BMAC (40) **Study groups:** Patients aged >45 years (20) vs patients aged <45 years (20)	**Patients <45:** 36.6 **Patients >45:** 50.0	No previous surgeries reported **Concomitant procedures:** HTO: 9 in >45 vs. 2 in <45; ACL reconstruction: 3 in >45 vs. 1 in the <45; Lateral release (patella): 1 in >45 vs. 3 in <45; Fulkerson: 1 in >45 vs. 5 in the <45; Meniscectomy: 0 in >45 vs. 1 in <45 (no statistics)
Gobbi et al. (2019)	**Therapy:** All patients underwent BMAC (23) **Subgroup analysis on age:** Patients aged ≤45 years compared to >45 years	**Overall:** 48.5	No previous surgeries reported **Concomitant procedures:** HTO (8); ACL reconstruction (3); Lateral release (patella) (2); TTO (3)

BMI	Defect Characteristics	Measures	Follow-up	Results
Overall: 24.4	**Mean defect size:** 8.3 cm^2 **Defect grade:** ICRS grade IV (inclusion criterium)	ROM, VAS, IKDC, KOOS, Lysholm, Tegner, Marx	41.3 months	**Improvements at final follow-up (NOS):** Patients <45 years = patients >45 years (NS)
Overall: 24.0	**Mean defect size:** 2.3 cm^2 **Defect grade:** ICRS grade III or IV	IKDC, EQ, VAS, TAS	7 years (NOS)	**IKDC score:** Improvements of 80% were only observed in patients <40 years and not in patients >40 years (no statistical test)
<40 years: 24.0 **>40 years:** 26.4 *(P<0.0005)*	**Mean defect size:** **<40 years:** 2.7 cm^2 **>40 years:** 3.1 cm^2 **Defect grade:** ICRS grade III or IV	Failure, IKDC, TAS	10 years (NOS)	**IKDC scores:** Patients <40 years < patients >40 year *(P=0.007)*
Not provided	**Mean defect size:** **>45 years:** 8.5 cm^2 **<45 years:** 9.8 cm^2 **Defect grade:** ICRS grade IV	VAS, IKDC, KOOS, Tegner, MRI	**>45 years:** 48.7 months **<45 years:** 52.3 months	**Tegner, KDC, KOOS: N**o significant differences in improvement between the age groups **VAS: I**mproved more in patients >45 years vs. <45 years (*P=0.046)* **Complete/near complete defect filling:** 81% in patients >45 years vs 71% in patients <45 years (no statistics provided) **Integration of graft with adjacent cartilage:** 93.7% in patients >45 years vs 93% in patients <45 years (no statistics provided)
Overall: 24.4	**Mean defect size:** 6.5 cm^2 **Defect grade:** ICRS grade IV (inclusion criterium)	IKDC, KOOS, Tegner, VAS	8 years (Mean)	**IKDC, KOOS, Tegner and VAS pain score:** Patients ≤45 years = patients >45 years (*P >0.05)* Significant negative correlation between age and IKDC, Tegner and KOOS scores.

Table 2. Evidence table with included studies and corresponding study characteristics and outcomes. (*continued*)

Author	Study Groups (n)	Mean Age (y)	Additional surgeries
Bone-based techniques			
Levy et al. (2013)	**Therapy:** All patients underwent Shell OCA **Subgroup analysis on age:** Patients aged <30 years compared to ≥30 years (multivariate analysis)	**Overall:** 32.8 (85% younger than 45)	**Previous surgeries:** Received by the majority of patients, some of them more than one. Chondroplasty was performed in 73.5% and bone marrow stimulation in 40% No concomitant surgeries reported
Frank et al. (2017)	**Therapy:** All patients underwent OCA (170) **Subgroup analysis on age:** Patient aged <40 years (115) *vs.* patients aged ≥ 40 years (55).	**Patients <40:** 27.6 **Patients >40:** 44.9	No previous surgeries reported **Concomitant treatments:** Distal femoral osteotomy <40 years: 8% vs >40 years: 0% *(P=0.028)*. Lateral MAT was significantly more performed in in patients <40 *(P<0.0001)*
Anderson et al. (2018)	**Therapy:** All patients underwent OCA (80) **Subgroup analysis on age:** Patients aged ≤39 years (42) vs. patients ≥40 years (38)	**Patients ≤39:** 27.2 **Patients ≥40:** 52.3	No previous surgeries reported **Concomitant treatment:** Patients aged ≤39 years 33% vs. patients ≥40 years 24% *(P=0.459)* (HTO, Tibial Tubercle Osteotomy, Loose body removal, ACL reconstruction, Meniscectomy, Lateral release or MAT)
Martinez-Carranza (2020)	Therapy**:** All patients received an Episurf customized metal implant (30); 26 on the medial femoral condyle and 4 in the trochlea	47 (SD 8..4)	Concomitant injuries were part of the exclusion criteria
Holz et al.(2020)	Therapy**:** All patients received an Episurf customized metal implant (75); 60 on the medial femoral condyle, 10 in the trochlea and 5 on the lateral condyle	48 (SD 8.3)	**Previous surgeries**: 48/75; 31 MF, 5 debridement, 5 OAT, 4 ACI, 3 Trufit. **Concomitant surgeries:** no details provided
Studies including different treatments			

	BMI	Defect Characteristics	Measures	Follow-up	Results
	Overall: 25.7	No defect characteristics provided	Modified Merle d'Aubigné-Postel scale, IKDC, KSS, Failure	13.5 years (Median)	Graft failure: A 3.5 odds ratio in patients ≥30 years when compared to the reference group (logistic regression analysis)
	<40 years: 25.9 **>40 years:** 28.1 *(P=0.18)*	**Mean defect size:** 4.5 cm^2	Reoperations, Complications, IKDC, KOOS, WOMAC, SF-12.	5.0 years	**Re-surgery rate, time to re-surgery, failure rate:** patients ≥40 years = patients <40 years *(NS)* **KOOS symptoms sub-scores:** Patients ≥40 years > patients <40 years *(P=0.015)*
	≤39 years 26.55 **≥40 years** 28.29 *(P=0.085)*	**Mean defect size:** **≤39 years:** 5.22 cm^2 **≥40 years:** 5.91 cm^2 *(P=0.476)* **Defect grade:** ICRS grade IV	KOOS, IKDC, VR-12	**≤39 years:** 33.8 months **≥40 years:** 44.5 months	**All outcomes:** Patients ≤39 years = patients ≥40 years *(NS)*
	27	**Mean defect size:** 256 mm^2 **Defect grade:** 18/30 were ICRS grade 4	KOOS, EQ5D, VAS	**Mean** 55 months KOOS, EQ5D and VAS 24 months	**Reoperation rate:** 17%, 1/30 patients had conversion to an hemi-arthroplasty **KOOS, EQ5D and VAS**: significant improvements at 24 months > preoperative.
	28	**Mean defect size:** Medial femoral condyle: <3cm^2 Lateral femoral condyle: 3-4 cm^2 Trochlea: >4 cm^2 (*NOS*) **Defect grade:** ICRS III–IVb (by inclusion, *NOS*)	KOOS, VAS, failure	**24 months**	**KOOS and VAS:** all scores improved significantly at 24 months > preoperative. Minimal clinically important difference in KOOS was maintained during follow-up **Failure:** 2 revisions (2.5%) at final follow-up

Table 2. Evidence table with included studies and corresponding study characteristics and outcomes. (*continued*)

Author	Study Groups (n)	Mean Age (y)	Additional surgeries
de Windt et al. (2009)	**Therapy:** ACI (25) and MF (30) **Subgroup analysis on age:** ACI: <30y (11) and >30y (14) MF: <30y (13) and >30y (17)	**Overall:** 35	No previous surgeries reported No concomitant surgeries reported
Nejadnik et al. (2010)	**Therapy:** BMAC (36) vs. ACI (36) **Subgroup analysis on age:** Patients aged <45 years (35) compared to ≥45 years (37)	**BMAC:** 44.0 **ACI:** 42.5	No previous surgeries reported. **Concomitant surgeries:** Patella realignment: 6 in ACI, 5 in BMAC; HTO: 5 in BMAC; Partial meniscectomy: 1 per group; ACL reconstruction: 1 per group
Pascual-Garrido et al. (2016)	**Therapy:** HemiCAP focal femoral condyle resurfacing prosthesis (32) vs. MF (15), OCA (10), OAT (2), debridement (2) or ACI (1)	**Intervention:** 44.5 **Control:** 47.9	**Previous surgeries:** 84% of patients in intervention group had undergone previous surgeries vs. 63% of patients in the control group No concomitant surgeries reported.
Nathwani et al. (2017)	**Therapy:** BioPoly RS Partial Resurfacing Knee Implant vs historical MF cohorts (Saris (2014) mean patient age 32.9 years (72), Saris (2008) mean patient age 33.9 (61), Rotterud (2016) mean patient age 35 years (88), Cole (2011) mean patient age 33 years (9)) **Subgroup analysis on age:** Patients aged ≤40 years *vs.* patients aged >40 years	**Overall:** 42.7 (60,6% >40)	**Previous surgeries: P**erformed in 75.8% of patients.

Table 2. Evidence table with included studies and corresponding study characteristics and outcomes. Abbreviations: ***ACI****: Autologous Chondrocyte Implantation;* ***ACI-c****: Autologous Chondrocyte Implantation with collagen flap;* ***ACI-m****: Autologous Chondrocyte Implantation with matrix assistance;* ***ACL****: Anterior Cruciate Ligament;* ***AMIC****: Autologous Matrix Induced Chondrogenesis;* ***BMAC****: Bone Marrow Aspirate Concentrate;* ***BMI****: Body Mass Index;* ***EQ****: EuroQol;* ***EQ5D****: EuroQol Five Dimensions Health Questionnaire;* ***FKRI****: focal knee resurfacing implant;* ***HA-BMAC****: BMAC with hyaluronic scaffold;* ***HTO****: High Tibial Osteotomy;* ***ICRS****: International Cartilage Repair Society;* ***IKDC****: International Knee Documentation Committee Subjective Knee Form;* ***KOOS****: Knee Injury and Osteoarthritis Outcome Score;* ***KSS****: Knee Society Score;* ***MAT****: Meniscus Allograft Transplant;* ***MF:*** *Microfracture* ***MRI****: Magnetic Resonance Imaging;* ***NOS****: Not Otherwise Specified;* ***OA****: Osteoarthritis;* ***OAT****: Osteochondral Autografting;* ***OCD****: Osteochondritis Dissecans;* ***ROM****: Range of Motion;* ***SF-12****: Short Form 12 questionnaire;* ***SF-36****: Short Form 36 Questionnaire;* ***TAS****: Tegner Activity Score;* ***TTO****: Tibial Tubercle Osteotomy;* ***VAS****: Visual Analogue Score;* ***VR-12****: Veterans RAND 12 Item Health Survey;* ***WOMAC****: Western Ontario and McMaster Universities Osteoarthritis Index.*

BMI	Defect Characteristics	Measures	Follow-up	Results
Not provided	**Mean defect size:** **ACI:** 3.25 cm^2 **MF:** 2.60 cm^2	KOOS	36 months	**KOOS:** patients <30 years > patients >30 years (*P<0.05)*
Not provided	**Mean defect size:** **BMAC:** 4.6 cm^2 **ACI:** 3.6 cm^2 (*P=0.270)*	IKDC, SF 36, Lysholm, TAS	24 months (NOS)	**IKDC: P**atients <45 years = ≥45 years in ACI and BMAC **TAS:** in ACI patients <45 years > ≥45 years; in BMAC patients <45 years = ≥45 years
Intervention: 26.7 **Control:** 30.4 (P=0.03)	**Defect grade:** ICRS grade IV	Need for intervention, WOMAC, SF-12, Satisfaction, Adverse events	**Intervention:** 2.0 years **Control:** 2.6 years (P=0.0002)	**Clinical success:** Intervention: 75% vs. control 53% (*P* <0.001) **WOMAC pain domain: I**ntervention > control (*P*=0.03) **Good/Excellent satisfaction:** Intervention: 91% vs. control: 66% (*P*<0.001)
Overall: 26.7	**Mean defect size:** 2.7 cm^2 **Defect grade:** ICRS grade IV grade II-IV	KOOS, VAS, SF-36, TAS	2 years (NOS)	**KOOS, VAS, SF-36, TAS:** significant increased compared to preoperatively *(P < 0.025)* **KOOS, VAS, SF-36:** Patients ≤40 years = patients >40 years *(P>0.05)* **Quality of life KOOS sub-score:** FKRI > all 4 used MFX studies *(P<0.025)* **Sports KOOS sub-score:** FKRI > to MFX in Saris (2014) and Rotterud (2016) *(P<0.025)* **ADL KOOS sub-score:** FKRI > MFX in Saris (2014) *(P<0.025)*

Bone Marrow Stimulation

Kreuz et al. (2006) (5) showed that MF-treated patients >40 years experienced significant deterioration in International Cartilage Repair Society (ICRS) scores between 18 and 36 months postoperatively, while patients <40 years did not. After 36 months, patients ≤40 years had significantly better defect filling on Magnetic Resonance Imaging (MRI) and significantly better outcomes on the Modified Cincinnati Score (MCS) in comparison to patients >40 years (5).

Sansone et al. (2014) (8) reported 84% "high" (70-100 points) Knee Society Scores (KSS) in AA treated patients <50 years, compared to 53.6% in patients aged ≥50 years. Patients >50 years had significantly more defects > 4cm^2. There were 12 conversions to arthroplasty in patients aged ≥50 years versus one patient in the <50 years subgroup.

Gille et al. (2013) (45) compared three age-groups (17-32, 33-46 and 47-65 years) after AMIC cartilage repair and found no significant differences in outcome across all age-groups.

Cell-based Techniques

Nehrer et al. (2006) (46) reported significant improvements on the Lysholm and International Knee Documentation Committee (IKDC) scores after ACI-m only when patients were aged <30 years; patients ≥30 years showed no significant improvement.

Krishnan et al. (2006) (9) reported significantly higher MCS scores in younger patients when comparing patients aged <20 years, 21-40 years and ≥41 years. Patients aged <20 years reached good/excellent scores in 85.7% of the cases and only 64.2% and 55.9% of patients attained good/excellent scores in the 21-40 and the ≥41 years age categories respectively.

Niemeyer et al.(2010) (10) reported no significant differences after ACI-c in patients aged ≥40 years compared to patients aged <40 years using the IKDC, Lysholm, MCS and Tegner Activity Score (TAS).

Brix et al.(2014) (12) reported significantly better Lysholm score improvement after ACI-m in patients aged <40 years versus ≥40 years: 25.8 ± 20.3 versus 5.6 ± 13.2 points.

Filardo et al. (2014) (11) reported that all of the patients younger than 40 years reached the intended 80% improvement on the IKDC score, none of those aged >40 years reached this level.

Filardo et al. (2017) (13) showed that IKDC scores were significantly lower for patients >40 years compared to patients <40. When scores were standardized to age-normative healthy IKDC values, there were no more significant differences.

Gobbi et al. (2014) (14) performed a multivariate analysis comparing outcomes from patients <45 years to those ≥45 years and reported no significant differences.

Gobbi et al. (2017) (15) performed a study using a hyaluronan-based (HA) scaffold soaked in BMAC (HA-BMAC). There were no significant differences in VAS, IKDC, Knee injury and Osteoarthritis Outcome Score (KOOS), TAS, and MRI between patients aged 45-60 years and patients aged 20-44 years at a mean final follow-up of 41.3 months. The VAS improvement, TAS, and KOOS sport- and recreational activities score at 2 years were significantly higher in the older compared to the younger group. The authors hypothesized that the lower physical demand in older patients was responsible for this finding. Defect filling on MRI was superior in the older compared to the younger patient group, but statistics were not provided and not all patients were evaluated by MRI.

Gobbi et al. (2019) (47) found no significant differences in IKDC, KOOS, TAS and VAS pain score between patients ≤45 years and >45 year after BMAC surgery.

Bone-based Techniques

Levy et al. (2013) (48) performed a logistic regression on OCA treated patients using failure as outcome. Failure was defined as graft revision or conversion to an arthroplasty. After a median of 13.5 years, the odds ratio for failure was 3.5 times higher in patients ≥30 years compared to patients <30 years.

Frank et al. (2017) (49) reported no significant differences in IKDC, KOOS, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and Short Form Health Survey 12 (SF-12) scores after OCA treatment between patients aged <40 years and aged ≥40 years. There were no significant differences in time to reoperation, reoperation rate, and failure rate at final follow-up. The older group had significantly better KOOS symptoms scores compared to the younger group, hypothesized by the authors to be the result of higher expectations in the younger patients.

Anderson et al. (2018) (18) reported no significant differences in KOOS, IKDC, and Veterans RAND-12 scores after OCA treatment between patients aged ≤39 years and patients ≥40 years.

Martinez-Carranza et al. (2020) (22) reported significant improvements in EuroQol Five Dimensions Health Questionnaire (EQ5D), VAS, and KOOS scores in FKRI treated middle-

aged patients two years postoperatively. After a mean follow-up of 55 months, one patient required conversion to a hemi-arthroplasty.

Holz et al. (2020) (23) performed a multicentre multinational study and reported significant improvements in KOOS and VAS scores in FKRI treated middle-aged patients. Two out of 75 patients failed and required implant removal within two years. A sub-analysis found that previous cartilage repair had no negative impact on the outcomes.

Studies Including Different Treatments

De Windt et al. (2009) (50) performed a single variant regression analysis on ACI-p/ACI-c or MF treated patients and showed that those <30 years had significantly higher KOOS improvements compared to those ≥30 years in both groups.

Nejadnik et al. (2010) (16) compared ACI-p to BMAC. A sub-analysis with 45 years as cut-off was performed in both groups using the IKDC and TAS. There was no significant difference in IKDC score between patients aged <45 and ≥45 years in the BMAC group. The TAS was significantly higher in patients <45 years compared to those ≥45 years in the ACI group, but there was no significant difference between age-groups in the BMAC group.

Pascual-Garrido et al. (2016) (19) investigated the use of the first generation metal FKRI and compared it to a group of biologically treated patients. Only middle-aged patients were included in both groups. The biologic group consisted of 15 MF, 10 OCA, two OAT, two debridement and one ACI treated patient. Clinical success was defined as ≥20% improvement on the WOMAC score and was significantly higher in the FKRI treated patients compared to the biologically treated patients: 75% versus 53%. The WOMAC pain- and satisfaction domains were significantly higher in the FKRI group compared to the biologic group.

Nathwani et al. (2017) (21) compared a bilayered polymer and metal FKRI to four historical MF cohorts in middle-aged patients. FKRI patients aged ≤40 years were also compared to those aged >40 years. The KOOS quality of life sub-score was significantly higher for the FKRI group compared to all four referenced MF studies. The KOOS sports- and activities of daily living sub-scores were significantly higher for the FKRI group compared to two MF cohorts. No significant differences in KOOS, VAS, and SF-36 were observed when comparing patients aged ≤40 years versus >40 years.

Discussion

The objective of this study was to systematically review literature on cartilage repair treatment outcomes in middle-aged and middle-aged versus younger patients. Included studies had low quality and heterogeneous methodology. The null hypothesis that all cartilage repair techniques in middle-aged patients lead to inferior clinical outcomes and higher failure rates when compared to younger patients is rejected. Some cell-based, and most bone-based treatments were equally effective in middle-aged patients compared to younger patients. The null hypothesis was confirmed for the conventional BMS techniques MF and AA, which indicates that the effectiveness of these techniques is age dependent.

The BMS studies have shown that applying MF or AA in middle-aged patients results in inferior defect filling, fast deterioration of repair tissue, and higher conversion to arthroplasty rates in comparison to younger patients (5, 8). Even in younger patients, BMS often leads to biomechanically inferior repair tissue (51). Advancing age deteriorates the joint homeostasis and cellular potency, resulting in impaired chondrogenesis and hence lower quality of the repair tissue (3, 52, 53). It has been hypothesized that the treated defect is unable to withstand the detrimental pothole effect (54). In addition, the subchondral bone (SB) starts thickening and forms cysts due to aging, OA, or chronic cartilage defects (55, 56). The standard MF awl causes widespread microarchitecture disturbances in the SB, leading to further sclerosis (57), and cyst formation after MF (58), with good correlation between the severity of SB changes and poor clinical outcomes (59). SB alterations may also explain how BMS jeopardizes consecutive regenerative treatments (48, 60-62). Failure rates of subsequent cartilage repair treatments after failed BMS are up to three times higher (61).

Despite these known disadvantages, the advantages of BMS are its technical ease, low costs, high availability and being a single-stage procedure (63). BMS is the most commonly applied regenerative technique (26). The widespread use of MF in the middle-aged has been reported (6, 7, 25). Salzmann et al. (2011) (38) surveyed German-speaking orthopaedic surgeons and found that over 30% of the respondents did not see age as a limiting factor for MF and only 11.6% uphold 40 years as an upper age limit for MF. Similar numbers were seen in a more recent Turkish survey (39). Gille et al.'s AMIC study showed that supplementing BMS with an overlying matrix can provide good clinical outcomes in middle-aged patients (45). This is consistent with most preclinical BMS studies, which show the mitigating effects of biological stimuli to the detrimental SB disturbances after MF (58).

Cell-based techniques provide biological stimuli without violating the SB. The ACI-c study by Niemeyer et al. (2010) and all three BMAC studies showed age-independent results (10, 14, 15, 47). However, five other ACI studies with longer follow-up periods showed a negative effect of advancing age on clinical outcomes (9, 11-13, 46). ACI relies on the injection of lab-cultured chondrocytes. Chondrocytes yield and potency in middle-aged patients is often diminished compared to younger individuals (3). This may lead to rapid deterioration of the repair tissue. Good clinical outcomes in middle-aged patients were reported for BMAC (14-16, 47), with a follow-up of up to 8 years (47). Results were superior to ACI-p at 2 years postoperatively (16). BMAC contains high concentrations of platelets, which include a significant number of cytokines, chemokines and growth factors that elicit a trophic effect (64). Several studies have shown that the iliac crest yields the highest concentrations of bone marrow derived MSCs (64). BMAC may thus possess a more potent composition than is derived during ACI or MF, as shown in multiple animal studies (64). Substantiated by satisfactory results up to 8 years, postponing arthroplasty with BMAC appears to be possible. In addition to these advantages, BMAC requires only one surgery instead of two when compared to ACI.

Bone-based techniques replace the complete osteochondral unit including the affected SB in a single surgery and provide a mechanically resilient articulating layer. Good clinical outcomes in middle-aged patients with consistent treatment effects across age groups were shown (18, 19, 21-23, 49). Only Levy et al. (2013) showed that OCA-treated patients were subject to age-related differences (48). In contrast to the more recent two OCA studies which both employed the dowel technique (18, 49), 82% of the subjects in the study by Levy et al. were treated by the shell technique. The shell technique requires large SB surface contact and additional internal fixation materials. More surgical trauma is consequently induced, resulting in higher failure rates (51). Bone-based techniques do not require chondrogenesis; animal studies have shown that osteochondral allografts are capable of adequate osseointegration, but a persistent gap between host and donor cartilage typically remains (24). Osseointegration relies on the same physiological process as found in fracture healing (65, 66), which has been shown to suffice up to old age (67). Replacement of the SB may also be an important contributor to pain relief. It is becoming increasingly accepted that the innervated SB is responsible for pain perception in cartilage damage (68). Previous cartilage repair had no effect on FKRI outcomes (23). Rehabilitation after bone-based techniques is generally short (67). A recent OCA cost-effective analysis concluded that OCA is highly cost-effective over a 30 year period and able to eliminate or postpone the need for the first TKA (37). The varying availability of allografts remains an ongoing drawback, particularly limiting its use in Europe (69). FKRIs are readily available and do not suffer from availability issues (19, 21). FKRIs in two included studies were compared to biological-based treatments in middle-aged patients: one study compared FKRI outcomes to a cohort of different biological treatments consist-

ing mainly of MF and OCA (19), while the other study compared the FKRI outcomes to four historical MF cohorts (21). Both studies showed superiority of the FKRIs in comparison to their control group (19, 21). No differences in outcome between patients ≤40 and >40 years for the FKRIs where observed (21). Long-term follow-up of FKRIs is scarce and available long-term evidence from the first generation metal FKRI raised concerns due to high failure rates based on OA progression (70). Novel metal FKRIs with patient-specific surface geometries resulted in very low failure rates up to 55 months (22, 23). Cartilage mimicking polymers such as in the study by Nathwani et al. pose great potential for prevention of OA progression when using FKRIs (21, 71). Failed FKRIs that are explanted result in subchondral bone voids and there are concerns that this may necessitate more extensive revision arthroplasty implants (72).

Limitations

Cartilage repair literature is known for its low quality (73). The average CMS of articles included in this study was 64 points and 86% of the studies were level IV. The methodologic heterogeneity hampered data assimilation for a meta-analysis.

Concomitant- or previous injuries and concomitant procedures were not always or only poorly described, but could have important implications. For instance, unloading the repaired cartilage compartment via a high tibial osteotomy could have potential synergistic effects on cartilage defect repair. These underreported but potential influencing variables make comparison of cartilage defect interventions difficult.

Defect size varied amongst the included studies as a consequence of the size-based treatment algorithms. Sansone et al. showed significantly larger defects in patients >40 years, three studies did not show any significant difference (10, 13, 18), while the remaining studies did not provide statistics on defect size differences. It remains unclear if older patients have larger defects. But if so, this should further discourage microfracture usage in older patients given the consensus that microfracture leads to inferior outcomes in larger defects (74).

Proven OA was out of the scope of this review to prevent clouding of results. However, long-present defects in middle-aged are perhaps already an expression of (early) OA (EOA) (75). OA leads to an impaired joint homeostasis which jeopardizes repair (75). Kim et al. (2020) confirmed that MF could not prevent progression of radiological EOA after MF cartilage repair in middle-aged patients (76). Deterioration of clinical results initiated after 1 year (76). De Windt et al. (2013) conducted a review on cartilage defect repair in EOA patients and concluded that ACI was able to postpone the need for arthroplasty in the short- and midterm (77). Wang et al. showed OCA graft survival rates of 75% after 5 years in EOA middle-aged patients (78).

FKRIs have previously been denoted as partial arthroplasties (79), indicating that their classification as a cartilage repair technique is still under debate. The indications for FKRIs are limited to the treatment of focal cartilage lesions, with radiographic osteoarthritis or severe lesions (ICRS grade 3-4) on the opposing cartilage surface as absolute contraindication (21, 23). UKAs are primarily indicated for diffuse medial or lateral compartment OA, thus both femoral and tibial OA. Due to this important difference, we consider unipolar FKRIs a metallic or biosynthetic cartilage resurfacing technique similar to auto- and allografts, which we consider biological cartilage resurfacing.

We must note that UKAs may also be a viable treatment option for young and active patients suffering from cartilage damage with excellent functional outcomes up to 11 years (80, 81). However, subsequent UKA revision procedures have drawbacks which warrant reservations in its use for the indication of focal cartilage defects. Upon OA disease progression, both biological and metallic or biosynthetic (e.g. FKRIs) resurfacing implants can be converted to UKA/TKA with relative ease (23, 82), while one-third of UKA revisions require additional surgical measures to compensate for loss of bone-stock (83). UKA revisions are associated with high failure rates (83, 84) and exhibit clinical outcomes lower than primary procedures, both similar to TKA revisions (84). To the best of our knowledge, such typical arthroplasty-related drawbacks have not yet been reported for revision of cartilage resurfacing (e.g. FKRI conversions to arthroplasty). For these reasons, we feel that, in line with allografts, FKRIs should be considered as a joint-preserving option ahead of UKA for each individual middle-aged patient suffering from a focal cartilage defect. UKAs are considered a joint-replacement technique disrupting the native knee anatomy and thus outside of the scope of the current review.

The natural decline in knee performance with advancing age may be a confounding factor. The largest natural decrease in normative IKDC data has been reported to occur between the ages of 51-65 years, followed by the decline between 35-50 years (85). Hence, long-term follow-up studies in patients over 40 years overlap with the period of the largest natural decline in knee performance, as was confirmed by Filardo et al. (2017) (13). Without age-normative standardization, no significant differences between middle-aged and younger patients were observed for ACI (10), OCA (49), BMAC (15, 16, 47), and FKRI (10, 14-16, 18, 19, 21, 47, 49), which highlights the age independency of these treatments.

Interpreting the three studies of Gobbi et al. as separate studies might be biasing the results as it is conceivable that the patients included in these studies overlap.

The commonly used threshold age of 40 years to characterize the middle-aged is arbitrary in the context of cartilage repair. Chronological age and biological age are not the same, particularly for a knee with a history of prior injury and intervention. Biomarkers which

differentiate in joint homeostasis are critically needed (86). Until then, orthopaedic surgeons should be aware of the findings in this study when treating middle-aged patients.

With these limitations in mind, it becomes apparent that the present study is significantly hampered. More frequent age sub-analysis in cohort studies and comparative randomized controlled trials in middle-aged patients are warranted. Correction to age-normative data seems imperative when analysing patients on the verge of natural decline. Furthermore, international registries collecting all patient-, defect- and surgical characteristics combined with biomarkers could aid in bias-free identification of successful treatments in middle-aged patients and the prognostic variables to aid in treatment selection.

Conclusion

There is a paucity in available evidence for cartilage repair in middle-aged patients and drawing definite conclusions is severely limited by inadequate methodology and reporting in the included studies. Importantly, no prospective randomized study comparing different therapies such as BMS versus cell- or bone-based therapies specifically in middle-aged patients has been performed to date. MF is still the most frequently performed cartilage repair technique in middle-aged patients. The included studies suggest that conventional BMS leads to inferior outcomes in middle-aged patients compared to younger patients. Literature shows various effects of age categorization on clinical outcomes for cell-based techniques, particularly in the long-term. Bone-based techniques can be considered as relatively insensitive to aging. This review indicates that not all cartilage treatments are affected equally by age and that joint preservation in middle-aged patients is possible. However, further methodically-sound research is warranted for all cartilage repair techniques to elucidate their joint preservation potential.

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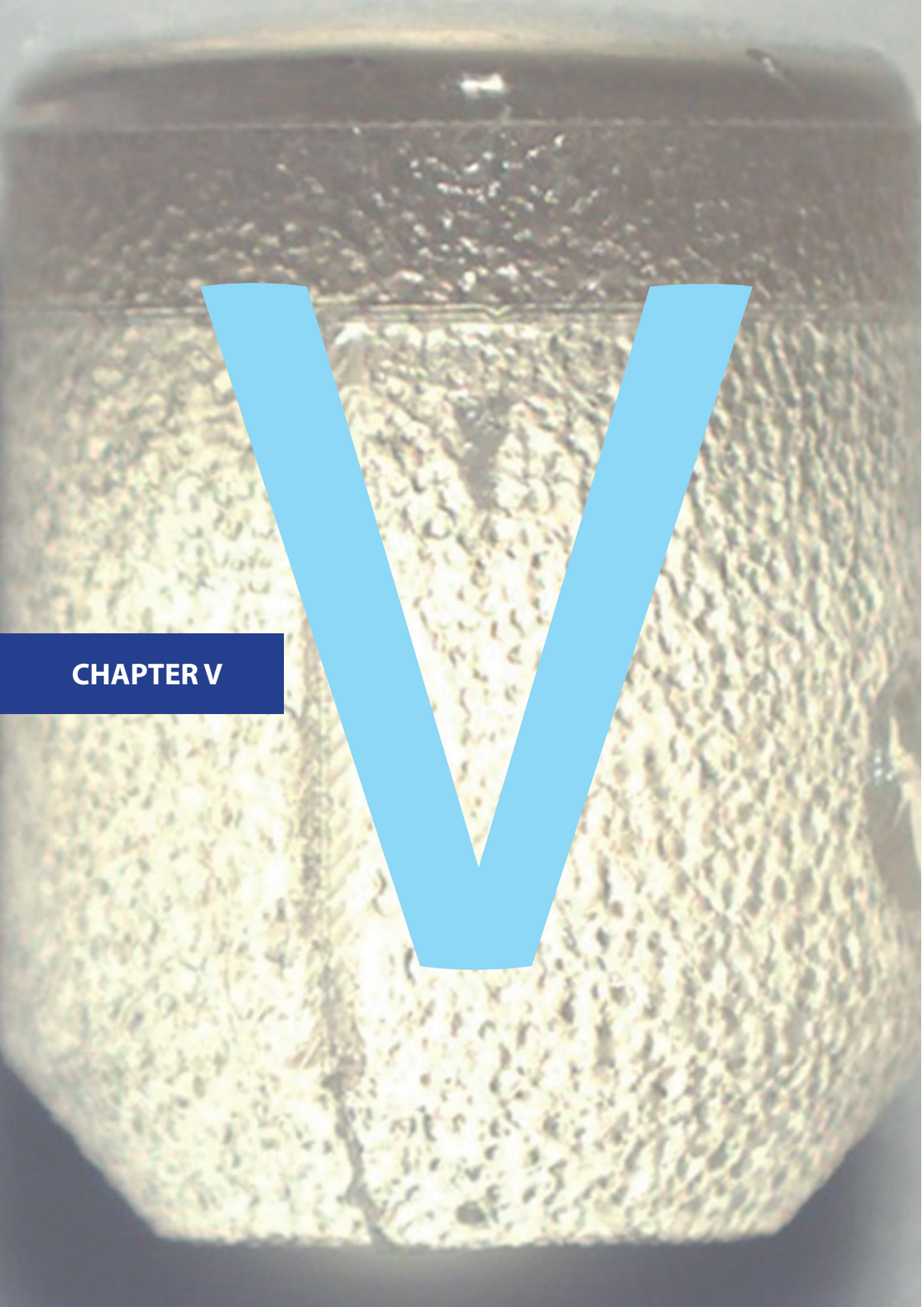

CHAPTER V

Cartilage Repair Strategies in the Knee according to Dutch Orthopedic Surgeons: a survey study

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Abstract

Background

This study surveyed Dutch orthopedic surgeons on the management of cartilage defects in the knee and the adherence to the recently updated Dutch knee cartilage repair consensus statement (DCS).

Methods

A web-based survey was sent to 192 Dutch knee specialists.

Results

The response rate was 60%. Microfracture, debridement and osteochondral autografts is performed by the majority, 93%, 70% and 27% of respondents respectively. Complex techniques are used by <7 %. Microfracture is mainly considered in defects 1-2 cm^2 (by >80%) but also in 2-3 cm^2 (by >40%). Concomitant procedures, e.g. malalignment corrections, are performed by 89%. Twenty-one percent of surgeons treat patients aged 40-60 years. Microfracture, debridement and autologous chondrocyte implantation are not considered to be highly affected by age >40 years by any of the respondents (0-3%). Moreover, for the middle-aged there is a large spread in treatments considered. In case of loose bodies, the majority (84%) only performs refixation in the presence of attached bone.

Conclusion

Small cartilage defects in ideal patients may be well treated by general orthopedic surgeons. The matter becomes complicated in older patients, or in case of larger defects or malalignment. The current study reveals some knowledge gaps for these more complex patients. Referral to tertiary centers might be indicated, as is stated by the DCS, and this centralization should enhance knee joint preservation. Since the data from present study is subjective, registration of all separate cartilage repair cases should fuel objective analysis of clinical practice and adherence to the DCS in the future.

Introduction

Articular cartilage defects in the knee occur frequently and may cause considerable pain and disability (1-3). Cartilage regeneration or repair techniques may be indicated when cartilage defects become symptomatic.

Current techniques used in clinical practice include marrow stimulating repair techniques such as microfracture (MF) and its augmentations, regenerative techniques such as autologous chondrocyte implantation (ACI) and regenerative osteochondral scaffolds, and bone-based repair techniques – i.e. depending on osseointegration – such as osteochondral grafting using autografts or allograft transplantations (OAT and OCA) and focal knee resurfacing implants (FKRIs) (4). MF augmentations include interventions such as autologous matrix-induced chondrogenesis. Regenerative osteochondral scaffolds include treatments such as Trufit™ (Smith and Nephew), MaioRegen (Finceramica) amd Agili-C™ (CartiHeal). Non-degradable bone-based FKRIs include HemiCAP® (Arthrosurface), Episealer® (Episurf) and BioPoly® RS Femoral Condyle (BioPoly).

In an attempt to provide guidance within the complex field of cartilage regeneration and repair, several international cartilage experts have composed guidelines or 'treatment algorithms'. The Dutch Orthopedic Society (Nederlandse Orthopedie Vereniging - NOV) cartilage repair consensus statement for (osteo)chondral surgical repair (abbreviated Dutch Consensus Statement; DCS) were first published in 2011 and were updated in 2019 (table 1) (5). Although the Netherlands is known for its excellent healthcare quality and registration (6), there is no separate registration of cartilage repair, i.e. there are no cartilage specific procedural terminology (CPT) billing codes. There is consequently no information about the perception and adherence to the DCS.

The objective of this survey study was to provide insight in the applied cartilage repair techniques and adherence to the DCS in the Netherlands. In addition, this study emphasized on the patient age-related considerations by orthopedic surgeons. With relatively little literature available related to the treatment of the middle-aged population, insights by orthopedic surgeons concerning their patient age-related considerations in cartilage repair aids in understanding this knowledge gap (3).

Evaluate the starting situation	Defect type	Localisation and ICRS grade	Methods for defects <2 cm^2	Methods for defects ≥2 cm^2
• Stable knee with normal alignment (< 5 ° varus or valgus malalignment: consider an additional osteotomy) • Malalignment >5 ° at the expense of the defect compartment; consider an additional osteotomy • Unstable knee (e.g. anterior cruciate ligament): consider an additional ligament reconstruction • Aim for BMI <30 • Age ≤50 years • Meniscus >50% intact • No signs of osteoarthritis • No (septic) arthritis	**Chondral**	**Femoral condyles and trochlea ICRS-grade 3/4**	• Microfracture • OAT • Nettoyage and debridement	• ACI o First generation: ACI-P o Second generation: ACI-C o Third generation: M-ACI o Fourth generation: Spherox® • Defects <4 cm^2: OAT
	Osteochondral	**Femoral condyles and trochlea ICRS-grade 4**	OAT with backfilling or biodegradable osteochondral scaffolds • Nettoyage and debridement • Fresh allograft	• ACI o First generation: ACI-P o Second generation: ACI-C o Third generation: M-ACI o Fourth generation: Spherox® + bone graft or synthetic implant • Fresh allograft
Diagnose and correct the predisposing factors of patella maltracking such as patella alta, baja, patellofemoral instability or an increased TT-TG/TT-PCL.	**(Osteo) chondral**	**Patella ICRS-grade 3/4**	• Nettoyage and debridement • ACI	• ACI o First generation: ACI-P o Second generation: ACI-C o Third generation: M-ACI o Fourth generation: Spherox® + bone graft

Note 1 Combined procedures have a narrow indication area and are best performed in a center of expertise.
Note 2 If previous surgical treatment of an (osteo)chondral defect has failed, referral to a center of expertise is indicated
Note 3 Adequate follow-up treatment as described in the patient/practitioner app and physiotherapy center with ICRS training.
Note 4 Central registration in the Dutch version of the ICRS database

Table 1. The 2019 Dutch Concensus Statement concerning cartilage defect repair in the knee. Abbreviations: ICRS: International Cartilage Repair Society; OAT: Osteochondral Autologous Transplantation; ACI: Autologous Chondrocyte Implantation; BMI: Body Mass Index; TT-TG: tibial-tuberosity to trochlear groove distance; TT-PCL: tubercle-posterior cruciate ligament (TT-PCL).

Methods

Participants

The survey recipients consisted of members of the Dutch Knee Society (DKS), which is part of the Dutch Orthopedic Society (Nederlandse Orthopeden Vereniging – NOV) and the Dutch Association for Arthroscopy (Nederlandse Vereniging voor Arthroscopie - NVA), totaling 192 orthopedic knee surgeons.

Questions

Three specialized cartilage orthopedic surgeons (JC, RC, PE) and two residents in training (RJ, PvW) prepared questions for this survey. Questions were critically analyzed during one general meeting and three digital meetings until consensus was reached. The survey consisted of a total of 19 questions related to the treatment of cartilage defects. Questions were written in Dutch. For the purpose of the current international publication, answers were translated into English by a native English-speaking author (AW), as shown in Appendix I.

The survey consisted of 12 general questions, including questions related to the surgeon's experience, characteristics of typically treated patients, defect type, utilization of available therapies, and application of concomitant treatments. The general questions were followed by seven in-depth questions related to the treatment choice for different defect characteristics using the International Cartilage Repair and Joint Preservation Society (ICRS) scoring, the strategy for patients in different age categories and treatment preference for rare defects and loose cartilage bodies. In addition, a qualitative assessment was performed to assess specific rehabilitation protocols. The adherence of orthopedic surgeons to existing guidelines was evaluated using the general and in-depth questions such as: cut-off points for age and body mass index (BMI), treatment choice for a given size and depth of defect, the indication and application of additional surgical techniques, and the utilization of rehabilitation protocols.

Survey Distribution

The web-based survey was created in SurveyMonkey® (San Mateo, CA, USA). Orthopedic surgeons were invited by e-mail to participate in the survey. To increase the response rate, two subsequent follow-up e-mails were sent after 3- and 6-weeks. Using IP-based duplicate protection, orthopedic surgeons were prevented from completing the questionnaire twice. This study was performed according to Best Practices for Survey Research Reports (7).

Results

Participants and General Questions

The response rate was 60% and 75% of respondents (n=115) completed the survey, resulting in an overall completion rate of 44%. Respondent demographics are shown in table 2.

Ninety-nine per cent of the respondents perform cartilage repair on the medial femoral condyle, 93% on the lateral femoral condyle, 34% on the trochlea, 16% on the patella and 13% on the tibia plateau, see figure 1. When asked which surgical techniques surgeons utilize, 95% of the respondents indicated they use MF, 71% use debridement, 2% use osteochondral autografts, 6% use ACI and degradable FKRIs, 6% use MF augmentations, 2% use fresh frozen osteochondral allografts and 1% use non-degradable FKRIs, see figure 2.

When faced with ICRS grade I/II defects, most respondents opt for debridement regardless of the defect size, see figure 3a. For ICRS grade III/IV defects up to 3 cm^2, debridement and MF are both popular techniques, while the DCS maintain 2 cm^2 as upper limit. Most surgeons indicate that they treat defects of 3-4 cm^2 using osteochondral autografts and defects larger than 4 cm^2 with ACI, see figure 3b. When indicated, 89% of surgeons perform concomitant surgeries; 70% perform meniscal augmentations, 64% perform ligamental reconstructions, 57% perform correction of the leg axis and six per cent answered other, see figure 4.

An upper BMI (kg/m^2) limit of 30 has been adopted by 46% of the respondents in accordance with the DCS. BMI was set at unlimited by 24%, at 35 by 21%, at 40 by 5% and at 25 by 4%.Seventy-two per cent of the respondents treat patient who smoke and 28% indicated they do not treat smoking patients.

The median angle at which the respondents performed leg axis correction is 6 degrees, the mode 5 degrees, of which the latter is in accordance with the DCS, see figure 7. The majority (57%) of respondents prescribes a specific rehabilitation protocol for cartilage repair with varying strategies but none of them mentioned specifically to employ an ICRS trained physiotherapist as dictated by the DCS, see table 3. When indicated, 95% of respondents indicated to refer to a tertiary center.

In-depth Questions

When the respondents were asked if they would fixate a loose body, eighty-four per cent responded that they fixate an osteochondral loose body, 9% fixate a chondral loose body and 7% would not attempt any fixation, see figure 6. Twelve per cent of the respondents

treat ICRS grade 5 lesions (deeper than 6.5 mm). Eighty-four per cent of the respondents treats single lesions, 35% treats multiple lesions and eight per cent treats kissing lesions. Sub-analysis revealed that 75% of respondents who work in a tertiary cartilage expert clinic would treat multiple and kissing lesions, whereas 28% of surgeons in a non-expert clinic would do this.

Patient age-related considerations

The vast majority of the respondents (96%) treat patients aged 18-30 years and 67% treats patients 30-40 years. Fifty per cent treats patients under the age of 18 years, 21% treats patients 40-60 years and one per cent treats patients older than 60 years, see figure 7.

When stratifying the different treatments by age group, most surgeons indicated to consider microfracture, debridement, MF augmentations, osteochondral autografts and -allografts, ACI, in the categories ≤10 up to 40 years of age. The ultimate treatment choice thus appears to be dictated by defect characteristics in accordance with the treatment algorithms for this age group. For the age groups 40-50 and 50-60 years there was a large spread in treatments considered, see figure 11. On a 5-point scale, debridement and MF augmentations are considered (58% of respondents) least affected by being middle-aged (40-65). Common treatments (debridement, MF) are not considered highly affected by middle-age by any of the respondents (0-3% of respondents). Nine per cent of respondents considered allografts and biodegradable osteochondral scaffolds to be highly affected by age.

Respondents	N (%)
Members of Associations	192
Respondents	115 (60%)
Completed survey	85 (44%)
Orthopedic surgeon	107 (93%)
Resident in training	8 (7%)
Experience	
Years of experience	
<1 year	8 (7%)
1-5 years	23 (20%)
>5 years	84 (73%)

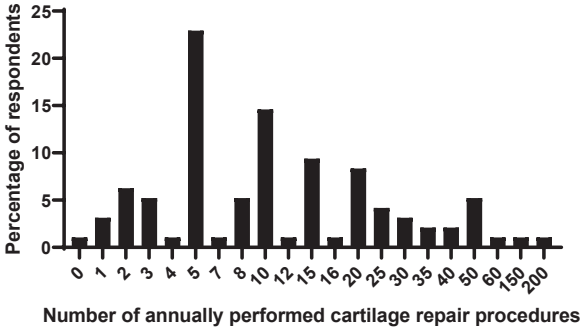


Table 2. Characteristics and experience of respondents to the national cartilage repair survey. SD: standard deviation. Note in the histogram that the numbers on the X-axis are not linear.

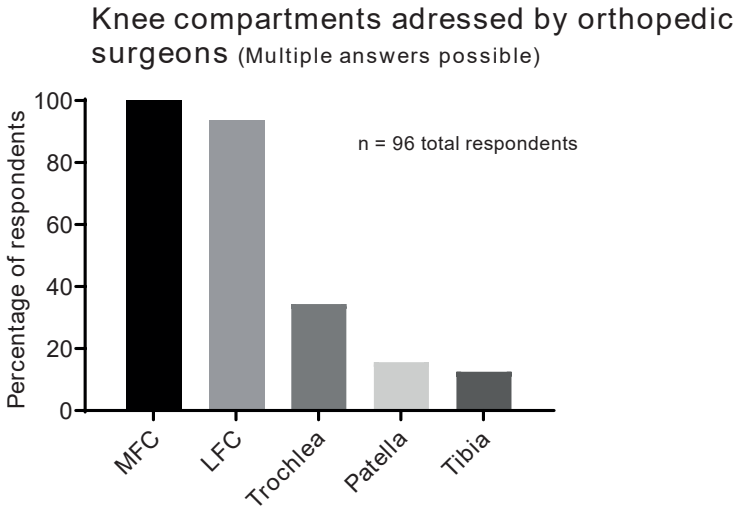


Figure 1. Typical knee compartments addressed according to respondents. The question leading up to these results was '*I apply cartilage repair to the following compartments of the knee:*' Abbreviations: MFC: medial femoral condyle; LFC lateral femoral condyle.

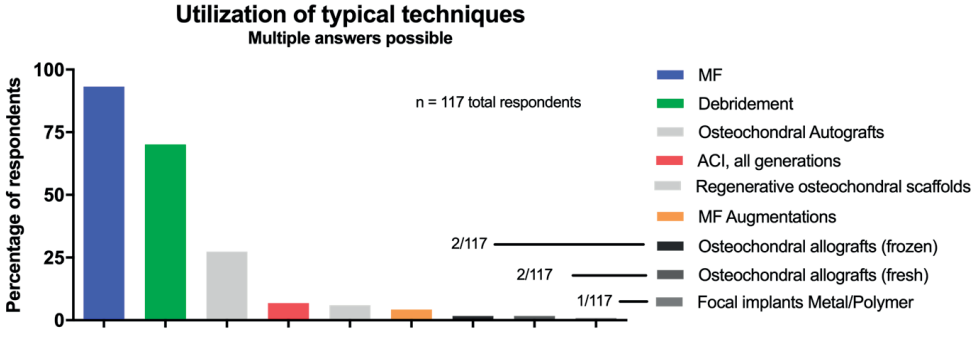
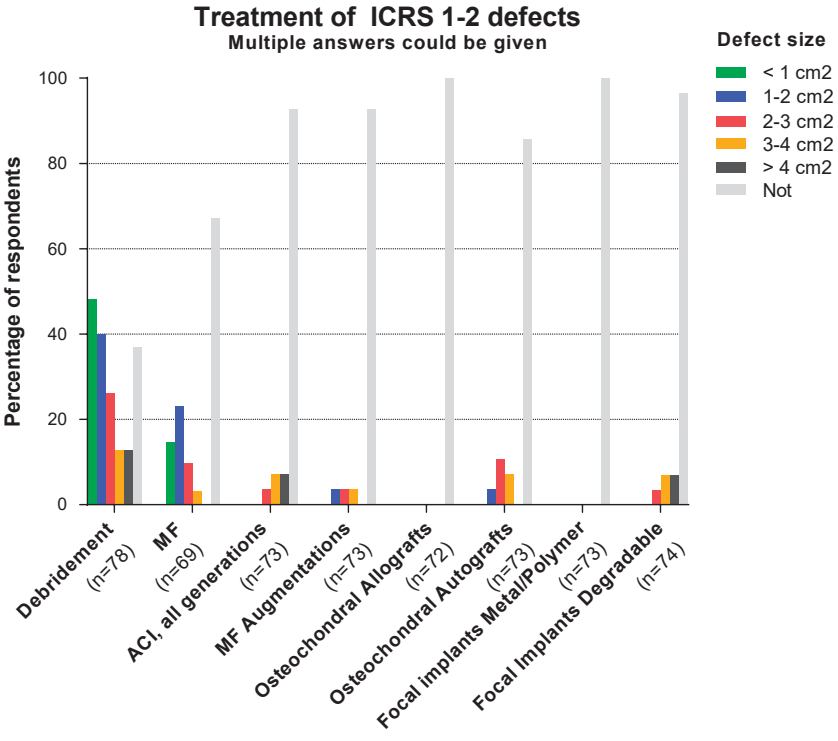
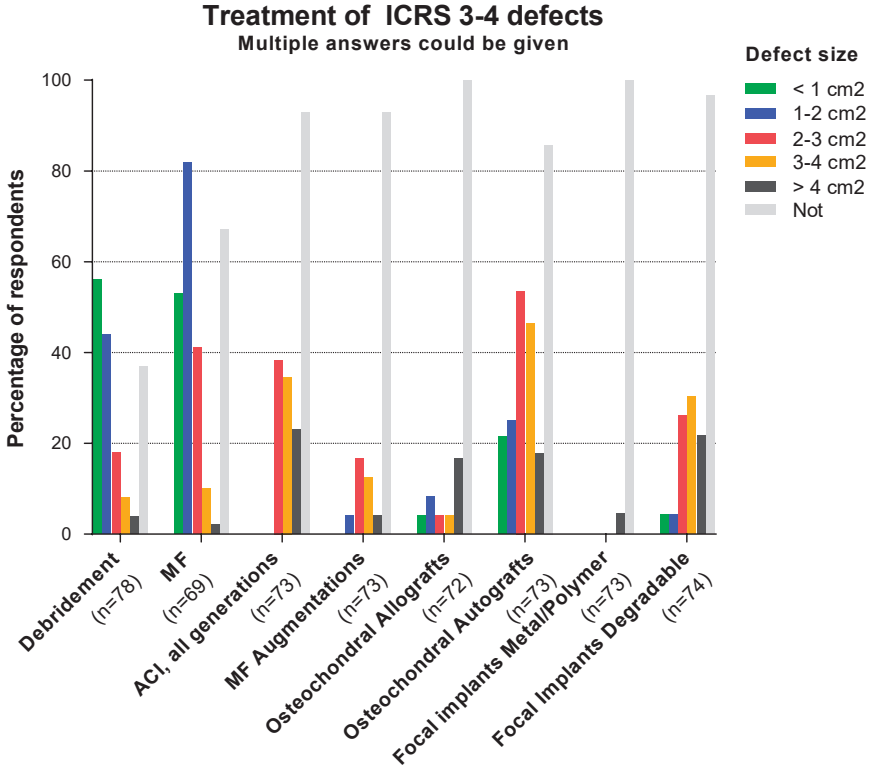


Figure 2. Utilization of typical cartilage repair techniques. The question leading up to these results was A: '*I use the following techniques for cartilage repair of symptomatic cartilage defects*' Abbreviations: MF: Microfracture; ACI: autologous chondrocyte implantation; Regenerative osteochondral scaffolds include treatments such as Trufit™ (Smith and Nephew), MaioRegen (Finceramica), Agili-C™ (CartiHeal); MF Augmentations: MF augmentations such as autologous matrix-induced chondrogenesis. Focal implants Metal/Polymer includes treatments such as HemiCAP® (Arthrosurface), Episealer® (Episurf) and BioPoly® RS Femoral Condyle (BioPoly).


Treatment of ICRS 1-2 defects
Multiple answers could be given
Defect size
< 1 cm2
1-2 cm2
2-3 cm2
3-4 cm2
> 4 cm2
Not
Percentage of respondents
100
80
60
40
20
0
Debridement (n=78)
MF (n=69)
ACI, all generations (n=73)
MF Augmentations (n=73)
Osteochondral Allografts (n=72)
Osteochondral Autografts (n=73)
Focal implants Metal/Polymer (n=73)
Focal Implants Degradable (n=74)

A


Treatment of ICRS 3-4 defects
Multiple answers could be given
Defect size
< 1 cm2
1-2 cm2
2-3 cm2
3-4 cm2
> 4 cm2
Not
Percentage of respondents
100
80
60
40
20
0
Debridement (n=78)
MF (n=69)
ACI, all generations (n=73)
MF Augmentations (n=73)
Osteochondral Allografts (n=72)
Osteochondral Autografts (n=73)
Focal implants Metal/Polymer (n=73)
Focal Implants Degradable (n=74)

B

Figure 3. Use of different cartilage defect repair techniques for defects with ICRS 1/2 or 3/4 depths and different sizes. The question leading up to these results was A: *'I would treat symptomatic, ICRS grade 1/2, cartilage defects with a maximum size of, with the following techniques'* ; and B: *'I would treat symptomatic, ICRS grade 3/4, cartilage defects with a maximum size of, with the following techniques'*. Abbreviations: MF: Microfracture; ACI: autologous chondrocyte implantation; Regenerative osteochondral scaffolds include treatments such as Trufit™ (Smith and Nephew), MaioRegen (Finceramica), Agili-C™ (CartiHeal); MF Augmentations: MF augmentations such as autologous matrix-induced chondrogenesis. Focal implants Metal/Polymer includes treatments such as HemiCAP® (Arthrosurface), Episealer® (Episurf) and BioPoly® RS Femoral Condyle (BioPoly).

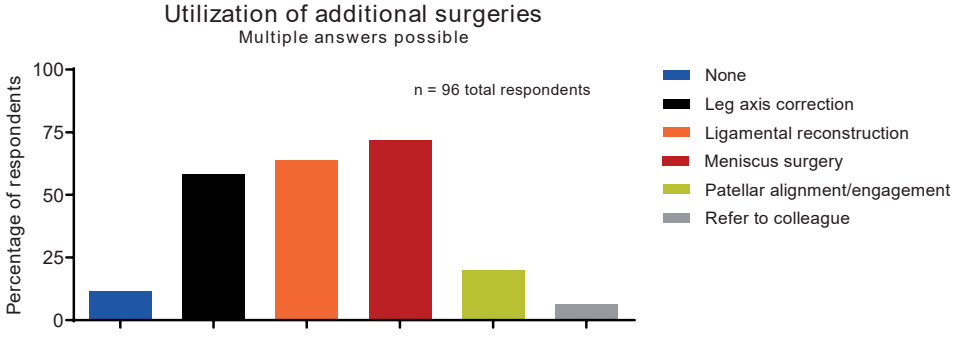


Figure 4. Utilization of additional surgeries. The question leading up to these results was *'In patients with a symptomatic cartilage defect I apply the following techniques in addition to cartilage repair:'.* Abbreviations: Ligamental reconstruction: includes cruciate and or collateral ligaments; Meniscus surgery: includes all meniscus surgeries such as suturing, regenerative procedures, allografts and biomaterial implants.

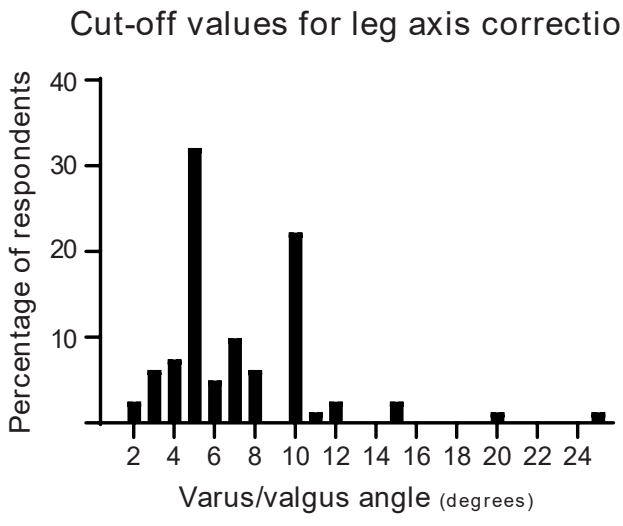


Figure 5. Histogram with the cut-off values of varus/valgus angle (degrees) indicated for leg-axis correction. The question leading up to these results was *'Starting from how many degrees (varus/valgus)*

Respondents having a specific rehabilitation protocol and its qualitative assessment.	
No	Yes
43%	57%
	'Six weeks 50% load bearing, frequent cycling on home trainer'
	'Dependent on defect location'
	'Progressive loading'
	'Six weeks unloaded, then six weeks unloader brace'
	'Physiotherapy'
	'I adapt my rehabilitation protocol to defect location and size and patient specifics'
	'Brace'
	'For MF on the medial or lateral femoral condyle: six weeks onloaded, then progressive loading up to three months. For MF in the patellofemoral joint, I consider a brace for range of motion restriction'
	'Phased'
	'Six weeks 10% loading'
	'Six weeks permissive weightbearing under supervision of a physiotherapy'
	'Six weeks 50% weightbearing, also depending on defect location, maximum flexion 90 degrees'
	'For MF six weeks unloading'
	'Physiotherapy'
	'Depending on defect location. On loaded parts of the femoral condyle 50% loading during the first six weeks'
	'No brace, six weeks partially loading with a physiotherapist, 4 months no peak or pivoting movements.'
	'Two weeks brace and passive range of motion, 6 weeks onloaded'
	'Onloaded'

43%	*'Cyclic exercises, six weeks unload, then progressive loading'*
	'Six weeks unload, then six weeks progressive loading supervised by physiotherapist. Refrain from loaded roll/glide movements for three months. Playing sports after six weeks'
	'Six weeks crutches: 4 weeks unloaded, then progressive loading'
	'Using an app'
	'unloaded/partially loaded for six weeks without restrictions in range of motion. Then functional loading. Sports only after 12 months'
	'MF protocol'
	'Depending on defect location'
	'Unloaded, physiotherapy, brace depending on location'
	'Six weeks hinge brace, partially loaded, then progressive loading'
	'Six weeks unloaded, then six weeks progressive loading'
	'Six weeks onloaded'
	'Condyle protocol, patellofemoral protocol, combined protocol'
	'Six weeks unloaded and 90 degrees range of motion restriction'
	'Partially loading and sometimes corrective brace'
	'Six weeks unloaded, crutches, physiotherapy'
	'Six weeks unloaded, restriction range of motion depending on location'
	'Six weeks 50% loading and then progressive loading'

Table 3. The question leading up to these results was: *'My clinic has a specific cartilage repair rehabilitation protocol'*. Respondents were asked to answer yes or no and elaborate on the protocol if they answered yes.

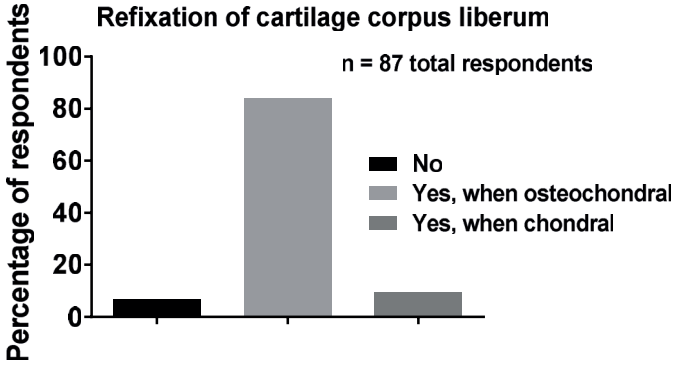


Figure 6. Refixation strategies when encountering a loose cartilage body, i.e., corpus liberum. The question leading up to these results was *'In case of a cartilage corpus liberum I attempt refixation:'*

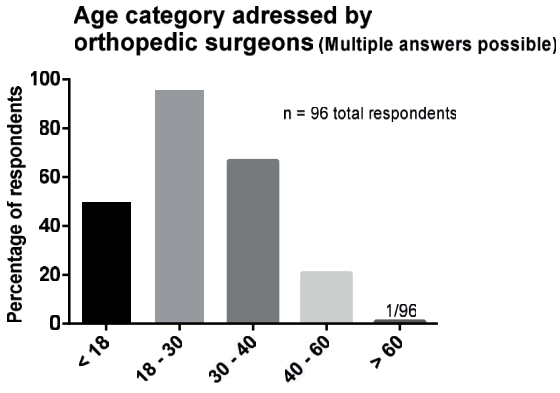


Figure 7. Typical age of patients on which cartilage repair is performed by the respondents. The question leading up to these results was '*In general I treat cartilage defects within the following age categories:*'.

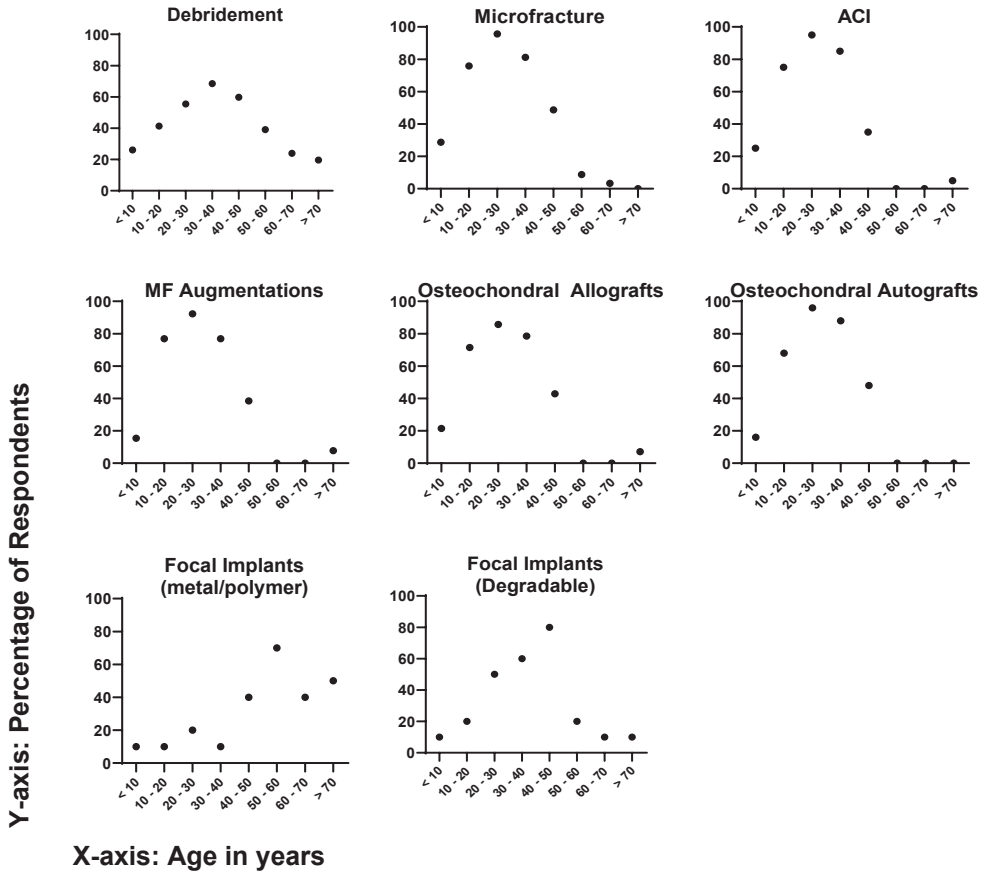


Figure *8* Techniques that were considered by the respondents for different patients' age. The question leading up to these results was 'I would consider the following techniques for the following age categories, irrespective of ICRS grade:'. **Abbreviations:** MF: Microfracture; ACI: autologous chondrocyte implantation; Regenerative osteochondral scaffolds include treatments such as Trufit™ (Smith and Nephew), MaioRegen (Finceramica), Agili-C™ (CartiHeal); MF Augmentations: MF augmentations such as autologous matrix-induced chondrogenesis. Focal implants Metal/Polymer includes treatments such as HemiCAP® (Arthrosurface), Episealer® (Episurf) and BioPoly® RS Femoral Condyle (BioPoly

Discussion

This was the first national survey on cartilage repair in the Netherlands. The response rate of 60% was considered to be adequate, given the typical response rate of 37-51% for e-mail-based surveys, (8, 9) Such response rate highlights the widely accepted challenge in addressing cartilage defects.

Debridement and MF were the treatments employed by most surgeons and most surgeons indicate to treat the medial femoral condyle with a single lesion. This is in accordance with the incidence of cartilage defects as reported from large epidemiologic database studies (10-14). For symptomatic ICRS I/II defects, no surgical intervention, debridement and to a lesser extend MF, were most considered as treatment when correspondents were asked. Debridement and MF were also most considered for ICRS III/IV defects up to 3 cm^2. Similar trends were previously found in a German and Turkish survey (15, 16). However, due to the mounting evidence of its ineffectiveness (17), both the 2011 as well as the 2019 DCS discouraged the use of MF in defects larger than 2 cm^2. Hence Dutch orthopedic surgeons seem to deviate from the DCS on this point.

In accordance with the DCS, the BMI limit for cartilage repair was set at 30 kg/m2 by 72% of the respondents. Although it has been well established that a BMI of 30 or larger is correlated with inferior outcomes after cartilage repair procedures (18). the prevalence of patients with such a BMI and the growth of this group is significant (19).

Concomitant surgeries like meniscal repair and leg axis corrections were performed by a large majority of respondents. This is in accordance with recent German registry data (20). Although the question related was asked from a symptomatic cartilage defect perspective, this survey did not scrutinize if cartilage repair could also have been concomitant to an anterior cruciate ligament (ACL) repair for instance. Such situation is conceivable when an unexpected cartilage defect is encountered during arthroscopy. The incidence of severe cartilage defects with ACL injuries for instance was previously found to be 16-46% (21). On the other hand, there is also an increasing notion that combined treatments might decrease the risk for reoperation and improve outcomes (22, 23). With a leg axis corrections considered at 5-6 degrees malalignment, most correspondents follow the DCS. Similar cut-offs were found in a previous West European study (16). Some experts in the field however, have advocated to correct malalignment in the mechanical axis from 2 degrees or more to unload the treated compartment and enhance repair (22, 24). In a recent German database study the cut-off for varus axis correction was 3 degrees (23). Emphasized by the obesity pandemic, it is of great relevance to further clarify the indication and cut-off values for alignment corrections or overcorrections since unloading is potentially beneficial for the repair (22, 25). In addition, the large spread in the cut-off

values indicated by the respondents further confirms that there is yet no consensus for when to perform a corrective osteotomy in cartilage repair.

In general, respondents indicated a low employment of more complex techniques such as ACI, MF augmentations, allografts and FKRIs. At the same time, when specifically asked, these very treatments were indicated by the respondents for larger defects with higher ICRS scores, or, in older patients. This discrepancy suggests that there is a lack of availability of treatments. In addition, the previous debate in the Netherlands concerning the cost-effectiveness and hence reimbursement of ACI is possibly related to this (26). Similar issues were also previously reported by Elmali et al (15). Other explanations include the availability of allografts in Europe which is – contrary to the US – hampered due to regulatory issues (27). But perhaps the best explanation for this is the centralization of cartilage repair in the Netherlands, highlighted by a 95% referral rate on indication. Indeed, due to regulation in the Netherlands, more complex techniques such as ACI or combined surgeries are only allowed to be performed in expertise centers. Hence, the orthopedic surgeon working in the periphery may only use debridement, MF and small osteochondral autografts.

Only half of the respondents would treat patellofemoral defects, which is a surprising finding given the fact that over 1/3 of patients present with patellofemoral defects (28). Perhaps surgeons are discouraged by the inferior outcomes in this compartment (29). At the same time, respondents also indicated to treat tibial defects, which are considered expert level treatments with only limited evidence and often inferior outcomes (30). Hence, the latest DCS discouraged the surgical treatment of tibial defects. In line with this is the 8% of respondents treating kissing lesions which is also discouraged. Perhaps the fact that 28% of respondents seeing patients with multiple or kissing lesions did not work in a tertiary center contributed to these unadvised treatment indications.

Loose cartilage bodies were only fixed by the majority of the respondents if there was residual bone present, i.e. osteochondral shells. This is a critically important finding since a recent study indicated that pure chondral loose bodies could in fact serve as a functional autograft, even without the need for anchoring biomaterials (31). Moreover, the patients' own cartilage could potentially also serve as chondrocyte or chondron source for ACI and the novel minced cartilage repair options (32).

Patient Age-related considerations

Cartilage defects have been shown to be a major risk factor for osteoarthritis (OA) (3, 4). One of the great challenges in the orthopedic community is to prevent or delay the onset of knee OA and thus prevent or delay total knee arthroplasty (TKA) (3). Particularly middle-aged patients – i.e. undergoing TKA in their 50s – have a high risk for revision

surgery later in life (33). Unfortunately, the fastest growing age-group undergoing cartilage repair or TKAs are the middle-aged patients (3). Postponing TKA by means of long-lasting cartilage repair has therefore become a pressing topic. Not coincidentally, the International Cartilage Repair Society changed their society name by including 'joint preservation' in 2018.

The middle-aged patient is underrepresented in most of the studies investigating cartilage repair (3, 34). It is not surprising therefore that in present survey there was a large spread in the results of respondents choosing treatments for older patients. For example, roughly 60% of respondents would consider MF as treatment in patients over 40 years of age. Importantly, when asked, most respondents did not see being middle-aged as a negative variable in cartilage repair. In fact, almost none of the respondents indicated that they believed any of the treatments to be highly affected by advancing age. Previous studies however, have shown a negative effect by age on MF outcomes (3), and the detrimental effects of failed MF on consecutive treatments (3). A recent systematic review concluded that more complex therapies such as cell-based therapies (ACI, bone marrow aspirate therapies), allografts or FKRIs have greater potential in older individuals (3).

With the aging population, it is also becoming increasingly important to evaluate the outcome of various cartilage repair treatments for different patient age categories. A major drawback in such age categorized research is that chronological age and biological age are obviously not the same. Biomarkers which differentiate in joint homeostasis are critically needed as they potentially can determine the 'joint age' rather than only relying on chronological age (35). Combining biomarker data with a non-biased international registries could aid in understanding the prognostic factors of each treatment on individual level and age.

Limitations

The major limitation of present study are its subjective outcomes, which is inherent to the nature of a survey study (36). In the absence of CPT codes to register individual cases and different repair techniques, we are unable to compare current results to objective epidemiologic values. The results of the present study should therefore be interpreted as Dutch orthopedic surgeons describing how they would treat a given patient and defect, not as a completely objective measure of how to they actually treat their patients. Nevertheless, with the assumption of relatively similar demographics, the results of present studies can be compared to large database studies (11-14) and other survey studies (15, 16). A nationwide registry system, analogous to or combined with the Dutch Arthroplasty Register, for different cartilage defects and prognostic factors could provide objective data, rather than relying merely on subjective data.

Since we restricted the inclusion to members of orthopedic knee associations we only included surgeons with affinity for knee surgery. The very low volume orthopedic surgeon operating in a small peripheral hospital may therefore not be included. However, only a small number of respondents indicated to work in an expertise center and the results of the non-experts could consequently be extrapolated to the general orthopedic surgeon. Perhaps the knowledge gaps in present study would be even more profound in those who are not a member of a knee association.

Conclusion

In the absence of a nationwide cartilage repair registry, this survey gives an impression of cartilage repair in the Netherlands. The present survey study showed that cartilage defects are treated by experts and many by non-experts. Both groups revealed a relative adherence to (inter)national guidelines. Small (<2cm^2) and simple cartilage defects in the absence of additional injuries or malalignment may therefore be treated by general orthopedic surgeons if they follow the latest national recommendations. However, several knowledge gaps for specific defect and patient characteristics were shown, indicating that not everyone is fully aware of the latest insights. Caution should be exercised concerning the opportunistic use of MF, treating rare defects such as defects > 2 cm^2 or those in the presence of loose viable cartilage bodies. Particularly patients with suboptimal characteristics such as an increased age (>40 years), high BMI or malalignment should be considered for referral. This survey indicated that the recently introduced centralization of cartilage care is widely adopted in the Netherlands, which potentially aids in better knowhow and availability of advanced treatments, consequently better outcomes, and perhaps, joint preservation. Future research should focus more on dominant demographics such as older patients with typical comorbidities. This study should encourage orthopedic surgeons to engage in (inter-) national cartilage registries. Combining these registries with the Dutch Arthroplasty Register could aid in understanding the conversions to arthroplasties. Structural support from both the government and industry is necessary to enable the proper registration of all cartilage surgeries and products.

Acknowledgement

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APPENDIX I – Survey questions

General

1. You are:
   a. Orthopedic surgeon
   b. Resident
2. I use the following techniques for cartilage repair of symptomatic cartilage defects: (multiple answers possible)
   a. Debridement / nettoyage
   b. Microfracture
   c. Autologous Chondrocyte Implantation (all generations)
   d. Microfracture augmentations (such as Autologous Matrix-Induced Chondrogenesis [AMIC])
   e. Osteochondral Allografts
   f. Mosaicplasty (Osteochondral Autograft Transfer System [OATS])
   g. Non-degradable cartilage implants (Metal/Polymer)
   h. Biodegradable osteochondral scaffolds (such as Trufit™, MaioRegen, Agili-C™)
3. My experience with the treatment of cartilage defects is:
   a. None
   b. 0-1 year
   c. 1-5 year
   d. >5 years
4. Approximately how many cartilage defects have you treated in the past year? (open)
5. In general I treat cartilage defects within the following age categories: (multiple answers possible)
   a. < 18 years
   b. 18-30 years
   c. 30-40 years
   d. 40-60 years
   e. >60 years
6. I carry out cartilage repair in patients who smoke:
   a. Yes
   b. No
7. When treating cartilage defects, I apply an upper limit for BMI (kg/m2) of:
   a. ≤25
   b. <30
   c. <35
   d. <40
   e. No limit

8. I apply cartilage repair to the following compartments of the knee: (multiple answers possible)
    a. Medial femoral condyle
    b. Lateral femoral condyle
    c. Trochlea
    d. Patella
    e. Tibia plateau
9. Within the same knee I would address: (multiple answers possible)
    a. Single lesions
    b. Multiple lesions
    c. Kissing lesions
10. In patients with a symptomatic cartilage defect I apply the following techniques in addition to cartilage repair: (multiple answers possible)
    a. Surgical correction leg axis (tibiofemoral & patellofemoral)
    b. Ligamental correction (cruciate, medial patellofemoral ligament, collaterals)
    c. Meniscus surgery (nettoyage, sutures, allograft implants)
    d. Other (pleaseelaborate)
11. Starting from how many degrees (varus/valgus) would you carry out a surgical correction of the leg axis?: (open)
12. I refer patients to one of the specialized centres:
    a. Yes
    b. No
    c. I work in such a centre myself

Specific

13. I would treat symptomatic, ICRS grade 1/2, cartilage defects with a maximum size of, with the following techniques: (multiple answers possible)

	< 1 cm^2	1-2 cm^2	2-3 cm^2	3-4 cm^2	> 4 cm^2	not
Debridement / nettoyage						
Microfracture						
Autologous Chondrocyte Implantation (all generations)						
Microfracture augmentations (such as Autologous Matrix-Induced Chondrogenesis [AMIC])						
Osteochondral Allografts						
Mosaicplasty (Osteochondral Autograft Transfer System [OATS])						
Non-degradable cartilage implants (Metal/Polymer)						
biodegradable osteochondral scaffolds (e.g. Trufit™, MaioRegen, Agili-C™)						

14. I would treat symptomatic, ICRS grade 3/4, cartilage defects with a maximum size of, with the following techniques: (multiple answers possible)

	< 1 cm^2	1-2 cm^2	2-3 cm^2	3-4 cm^2	> 4 cm^2	not
Debridement / nettoyage						
Microfracture						
Autologous Chondrocyte Implantation (all generations)						
Microfracture augmentations (such as Autologous Matrix-Induced Chondrogenesis [AMIC])						
Osteochondral Allografts						
Mosaicplasty (Osteochondral Autograft Transfer System [OATS])						
Non-degradable cartilage implants (Metal/Polymer)						
Biodegradable osteochondral scaffolds (as Trufit™, Maio-Regen, Agili-C™)						

15. I would treat deep cartilage defects myself (ICRS grade 5/deeper than 6.5 mm):
    - Š. Yes
    - ‹. No
16. In case of a cartilage corpus liberum I attempt refixation: (multiple answers possible)
    - Œ. No
    - . Yes in case of an osteochondral corpus liberum
    - Ž. Yes in case of a chondral corpus liberum
17. I would consider the following techniques for the following age categories, irrespective of ICRS grade): (multiple answers possible)

	< 10 years of age	10-20 years of age	20-30 years of age	30-40 years of age	40-50 years of age	60-70 years of age	> 70 years of age
Debridement / nettoyage							
Microfracture							
Autologous Chondrocyte Implantation (all generations)							
Microfracture augmentations (such as Autologous Matrix-Induced Chondrogenesis [AMIC])							
Osteochondral Allografts							
Mosaicplasty (Osteochondral Autograft Transfer System [OATS])							
Non-degradable cartilage implants (Metal/Polymer)							
Biodegradable osteochondral scaffolds (such as Trufit™, MaioRegen, Agili-C™)							

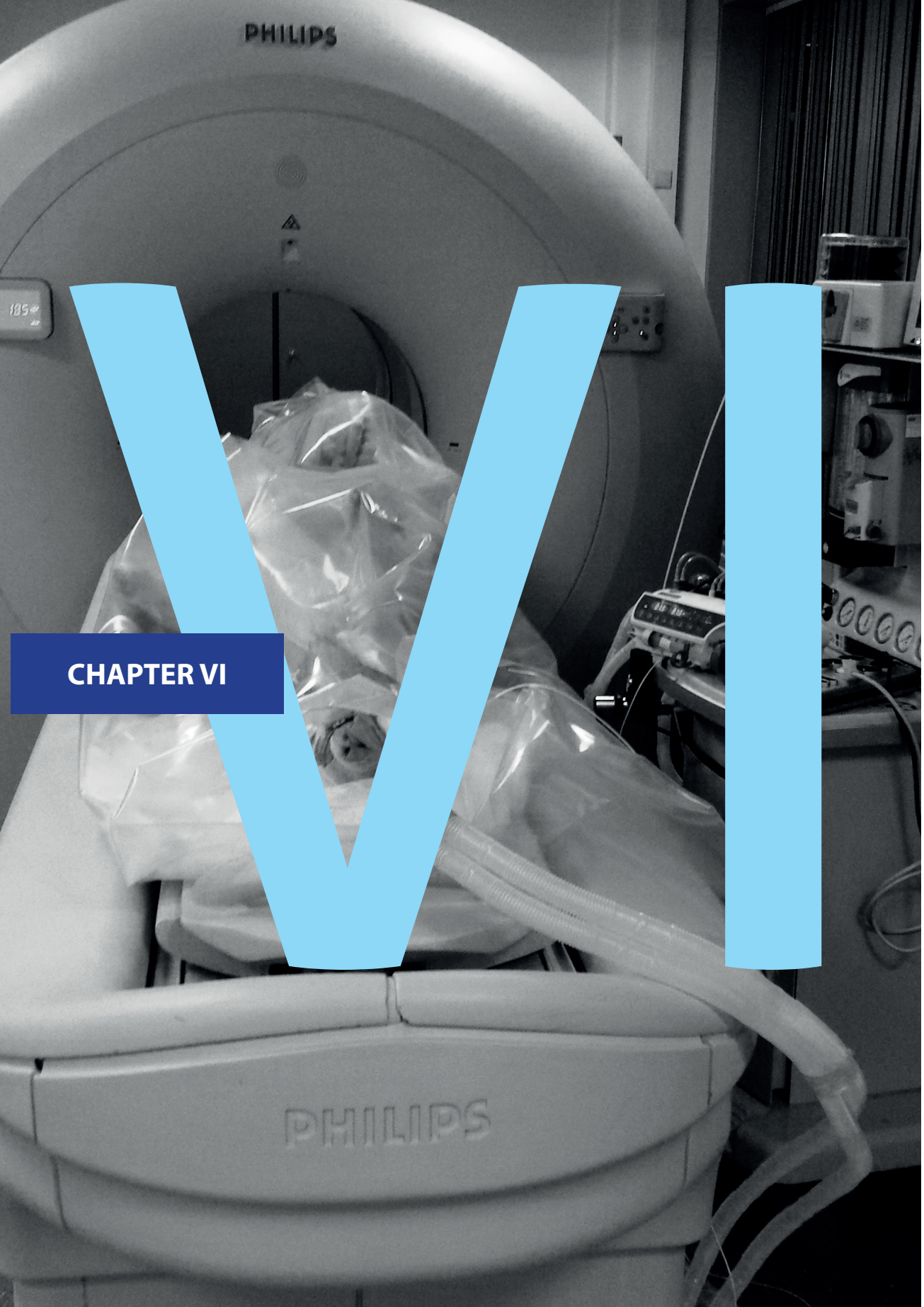
18. I consider the effect of middle age (40-65 year) on the success rate on the following techniques to be:

	Low		Average		High
	1	2	3	4	5
Debridement / nettoyage					
Microfracture					
Autologous Chondrocyte Implantation (all generations)					
Microfracture augmentations (such as Autologous Matrix-Induced Chondrogenesis [AMIC])					
Osteochondral Allografts					
Mosaicplasty (Osteochondral Autograft Transfer System [OATS])					
Non-degradable cartilage implants (Metal/Polymer)					
Biodegradable osteochondral scaffolds (such as Trufit™, MaioRegen, Agili-C™)					

19. My clinic has a specific cartilage repair rehabilitation protocol

. No

. Yes, please elaborate (brace, loading etc)



CHAPTER VI

Polymers in Cartilage Defect Repair of the Knee: Current Status and Future Prospects.

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Abstract

Cartilage defects in the knee are often seen in young and active patients. There is a need for effective joint preserving treatments in patients suffering from cartilage defects, as untreated defects often lead to osteoarthritis. Within the last two decades, tissue engineering based techniques using a wide variety of polymers, cell sources and signaling molecules have been evaluated. We start this review with basic background information on cartilage structure, its intrinsic repair, and an overview of the cartilage repair treatments from a historical perspective. Next, we thoroughly discuss polymer construct components and their current use in commercially available constructs. Finally, we provide an in-depth discussion about construct considerations such as degradation rates, cell sources, mechanical properties, joint homeostasis and non-degradable/hybrid resurfacing techniques. As future prospects in cartilage repair, we foresee developments in three areas: first, further optimization of degradable scaffolds towards more biomimetic grafts and environment. Second, we predict that patient-specific non-degradable resurfacing implants will become increasingly applied and will provide a feasible treatment for older patients or failed regenerative treatments. Third, we foresee an increase of interest in hybrid construct, which combine degradable with non-degradable materials.

1. Introduction

Articular cartilage defects occur in all age groups, but are most often encountered in young athletes as a result of trauma. Symptoms include severe pain, swelling, joint locking, and clicking. Cartilage lesions have been identified as the underlying pathology in as much as 60-67% of exploratory knee arthroscopic procedures (1-3). Most patients with focal cartilage defects are too young and too active for joint replacement therapy. Their high demands would lead too premature failure of the prosthetic components and an increase in revision surgeries (4-6). As cartilage possesses very limited capacity for self-repair and regeneration due to its avascular nature and hypocellularity, cartilage defects result in substantial impairment of quality of life in the short term and are likely to progress to osteoarthritis if left untreated (7-10). Therefore, easy and efficient treatments for focal cartilage defects are indicated.

Multiple surgical techniques have been developed within the last decades to repair isolated focal cartilage defects, aiming to prevent further deterioration of the joint, providing pain relief, and increasing functional outcomes. These techniques can be classified into one of three categories: marrow-stimulating techniques, cell-based regenerative therapies, and osteochondral grafting techniques. Developments in the field of tissue engineering have substantially boosted interest in marrow-stimulating and cell-based regenerative therapies for articular cartilage defects in the last two decades (11).

Marrow-stimulating techniques such as subchondral drilling, abrasion arthroplasty, and the microfracture technique evoke the natural healing response by exposing the bone marrow underneath the cartilage defect, thereby triggering blood inflow and subsequent fibrin clot formation (12, 13). The microfracture technique has become the most popular bone marrow stimulation technique, and involves the creation of several holes in the lesions spaced approximately 3-4 mm apart using an arthroscopic awl. Microfracture typically yields satisfactory results in younger patients in the short-term (14). However, mechanically inferior fibrocartilage is formed, as is typical of the natural healing response, with a decline in clinical outcome over time (15, 16).

The autologous chondrocyte implantation (ACI) technique, as first described by Brittberg et al. in 1994, pioneered cell-based regenerative therapy of articular cartilage (17). ACI is a two-step procedure, consisting of an initial diagnostic arthroscopy procedure in which cartilage is harvested from a low weight-bearing area. From this tissue, chondrocytes are then enzymatically isolated and multiplied in a laboratory for several weeks. During a second procedure, the cultured chondrocytes are injected underneath a periosteal flap, which has been harvested from the proximal tibia to seal off the defect site and confine the cells (18, 19). A drawback of first-generation ACI is that the cultured chondrocytes lack

the capability to fully withstand loading in the knee joint in the absence of a supportive structure, which often results in dedifferentiation into a fibroblast phenotype, with associated loss of collagen type II and proteoglycan production capability (20).

Matrix-assisted autologous chondrocyte implantation (MACI) was introduced as a possible improvement. During the cell culture process, chondrocytes are embedded in three-dimensional scaffolds, which was hypothesized to result in improved extracellular matrix (ECM) production (21, 22).

With the introduction of more mechanically stable scaffolds, one-stage repair techniques that enable steering and modulating the natural healing response regained interest. Autologous matrix-induced chondrogenesis (AMIC) combines microfracture with the implantation of a biological scaffold in a one-step procedure. The three-dimensional (3D) matrix bears load, while its open structure allows for influx of mesenchymal stem cells (MSC's), which ideally differentiate into chondrogenic lineage (23).

Osteochondral Autograft Transfer System (OATS) or mosaicplasty is a resurfacing treatment option in which osteochondral cylinders are harvested from low weight-bearing area and implanted (press-fit) into the defect. This treatment option yields good results, but its application is limited due to donor site availability and different surface curvatures (24, 25). A schematic overview of the described techniques is given in figure 1.

Two-step regenerative procedures such as MACI are costly and invasive (26), but provide assurance that a high density of chondrocytes is attained. Chondrocytes may be injected into a construct directly after harvest and enzymatic digestion (27), or mature allograft chondrocytes may be used (28). Bone marrow-derived MSC's (29) and adipose-derived MSC's (30), which are both able to differentiate into chondrocytes, have also been used as cell source. Since the introduction of AMIC, one-step procedures have been performed using a variety of cell sources. The use of platelet-rich plasma (PRP) and bone marrow concentrate (BMC) has recently been popularized. PRP is a sample of plasma with a two-fold or more increase in platelet concentration above baseline (31). PRP contains several stimulatory signalling molecules such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), fibroblast growth factor (FGF) and epidermal growth factor (EGF), and has been used in combination with synthetic polymers in preclinical studies (32-39). BMC is very similar to PRP and is generated by centrifuging bone marrow aspirate. BMC contains both stimulatory signalling molecules and MSC's (40), which therefore hypothetically would be superior to PRP. Both sources result in the formation of a natural scaffold via clotting.

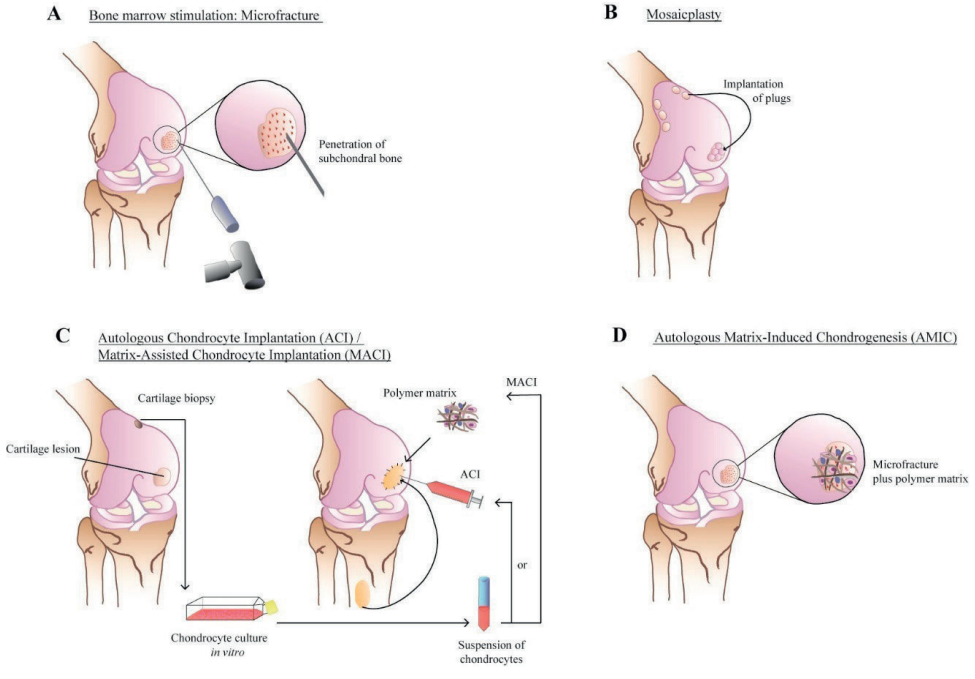

Figure 1. Schematic representation of current regenerative cartilage repair techniques: (a) Microfracture; (b) Mosaicplasty; (c) autologous chondrocyte implantation (ACI) and matrix-assisted chondrocyte implantation (MACI); and (d) Autologous matrix-induced chondrogenesis (AMIC). Reprinted with permission from M.M.J. Caron.

A wide variety of natural and synthetic polymers, in hydrogel or solid matrix form, have been assessed as cell carriers for cartilage repair. Cellular and acellular constructs, two or one-stage procedures, a wide range of cell sources, and the possible addition of biological growth or differentiation factors add to the vast array of constructs that has been assessed clinically and pre-clinically. In this comprehensive narrative review, we briefly discuss cartilage composition and its intrinsic repair mechanism . Next, we provide an overview of individual graft components, which have been clinically used for the repair of focal cartilage defects in the knee, from a chemical perspective. Furthermore, we provide an overview of commercially available constructs and their compositions. We will discuss the considerations which must be kept in mind in the graft design process and contributing factors and we will end with future perspectives in cartilage repair.

2. Cartilage: structure and repair

This section briefly summarizes cartilage biology. There are several papers available providing more in-depth information on this matter such as (41), (42) and (43).

Articular, or hyaline cartilage, possesses unique biomechanical properties due to its composition and structure. Its lubricated surface provides low friction articulation, and the strong ECM in combination with the high water content provides the capability to resist high compressive and shear loads, even when applied cyclically. Cartilage is principally composed of a dense ECM with a sparse distribution of cells; chondrocytes are the sole cell type present in cartilage, accounting for <5% of the total volume. The solid ECM is composed primarily of collagen type II which accounts for 15-22% total volume, and highly hydrophilic proteoglycans (4-7% total volume), and elastin. The high osmotic pressure created by the proteoglycans results in water content of 70-80% (13, 44). Proteoglycan aggregates are composed of a protein backbone, with numerous aggrecan branches connected via link proteins. Aggrecan covalently binds long polysaccharide chains known as glycosaminoglycans (GAG's), with chondroitin sulphate and keratin sulphate being the most abundantly present GAG's in articular cartilage.

Zonal variations in structure and composition provide the ability to withstand complex, combined loads (45, 46). The superficial zone contains dense collagen fibrils oriented parallel to the articular surface, with a relatively high density of ellipsoid-shaped flattened chondrocytes (45, 47, 48). The superficial zone compromises 10-20% of the total thickness (49), and is essential for distributing loads over a larger surface area (50, 51) and therefore protects cells against impact loading (52). The transitional zone provides a functional and anatomic transition towards the deeper zones as collagen fibrils are orientated obliquely. The transitional zone is thought to be responsible for dealing with shear loads at the cartilage surface (53). It compromises 20-60% of the total cartilage thickness depending on the location in the joint (48, 54), and is further characterized by a high proteoglycan content and low density, spherical chondrocytes. The deep or radial zone is characterized by thick and heavily abundant collagen fibrils oriented perpendicular to the articulating surface, high proteoglycan content, and vertically stacked chondrocytes (55). The deep zone provides the greatest resistance to compressive forces due to its composition and structure. The calcified layer anchors the cartilage to the subchondral bone, and is separated from the deep zone by the tidemark region, which is typically considered as the calcification front (56). A schematic representation of articular cartilage structure is given in figure 2.

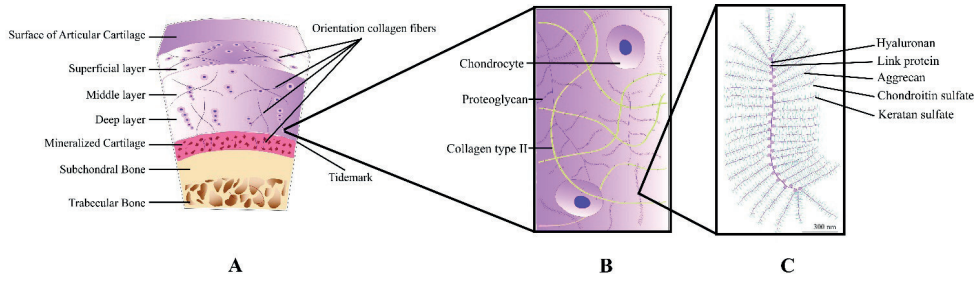

Figure 2. Schematic representation of articular cartilage and its contents: (a) Normal view of cartilage as osteochondral unit with specific zones; (b) Magnification of middle zone and its content; and (c) Representation of typical proteoglycan structure. Reprinted with permission from M.M.J. Caron.

Chondrocytes originate from MSCs and are able to undergo several stages of differentiation. Proliferative chondrocytes are typically only found in the developing stages, mature chondrocytes produce cartilage's distinct ECM, and hypertrophic chondrocytes are typically found in the calcified layer. Terminal differentiation, characterized by hypertrophy followed by apoptosis, does not normally occur in healthy mature cartilage, but may occur in the diseased state.

When cartilage is damaged the resulting chondral (partial thickness) lesions are partly filled with MSC's from the synovial membrane which migrate into the defect. Unfortunately, the filling already starts to degenerate within weeks to months (9, 57). Poor integration of the repair tissue may lead to necrosis of the contiguous surface over time and consequently to increases in defect size (10, 58). Osteochondral (full thickness) lesions partly heal naturally through an inflammatory process fuelled by the subchondral bone marrow. An influx of pluripotent MSCs results in fibroblastic differentiation, with subsequent production of both collagen type I and type II. However, this repair tissue does not integrate well with the adjacent native cartilage, and lacks an orderly structural organization (59), which results in inferior mechanical properties (60). Therefore, it is unable to cope with the severe mechanical demands in the joint and it is doomed to fail in the (mid)long-term.

Chondrocyte differentiation is controlled by a wide variety of cytokines, hormones, and growth factors, which are present in different stages of chondrogenesis and play an essential role in cartilage homeostasis and thus also its repair. These factors include complex proteins such as Insulin-like Growth Factor-1, TGF's, bone morphogenetic proteins, insulin, FGF's, steroids (vitamin D, sex hormones, glucocorticoids), prostaglandins, and interleukins are known to have differential effects in cartilage homeostasis and repair (61).

The review by Mariani et al gives a comprehensive overview of these bioactive molecules (62).

Incorporation of such bioactive molecules is an established method to enhance or modify the function of tissue engineered constructs. The actual methods and abilities of incorporating these bioactive molecules are rapidly expanding within the field of tissue engineering (63, 64). Typically, there are three routes through which these bioactive molecules are incorporated into the construct: (1) bioactive molecules are directly dispersed, adsorbed or immobilized into the construct (65-69); (2) bioactive molecules are incorporated within micro- and nano-particles which in turn can serve as additive for in vitro cell cultures or can be injected into the defect site potentiating *in situ* tissue healing. (70-72); (3) microspheres or nanoparticles (NPs) loaded with bioactive molecules can be incorporated into the constructs to enhance their biofunctionality .

There are several noteworthy techniques with promising features which need to be explained in more depth. Bio-electrospraying and cell electrospinning are relatively new techniques creating a variety of delivery routes for these bioactive molecules. They both rely on the principal of exploiting an electrical field between two charged electrodes. This electrical field draws a liquid jet which either generates droplets or continuous fibres. These techniques are able to produce nanometer-sized droplets and threads, large densities of materials in suspension and process highly viscous liquids (>10 000 mPa s) (73). Although the electrospinning technique has been around for over a century, it has only recently been explored for directly drawing fibers with cell suspensions containing a wide variety of cells, including MSCs (74). Processing cells using electrospinning was first conducted in 2005/06 and is referred to as cell electrospinning (75). Electrospraying is an important method for the production of NPs and has been combined with several cell types including bone marrow MSCs and bioactive molecules such as celecoxib (76, 77).

The use of NPs in cartilage repair is an emerging field with substantial growth in the last decade. The use of NPs in cartilage repair provides several advantages for the bioactive agents such as protection from degradation, reduction of side-effects and control of release. NPs support the release of multiple bioactive molecules simultaneously or sequentially or with a specific release pattern, thereby mimicking the natural tissue healing response (63, 78-81).

There are several other 3D printing techniques which have been tested to incorporate bioactive molecules and cells. These include methods such as fused deposition modelling, pneumatic extrusion printing, stereolithography, extrusion printing gels, inkjet printing and selective laser sintering. Since hydrogels are commonly used in cartilage tissue engineering, inkjet and pneumatic extrusion printers are the most commonly used

techniques in cartilage 3D bioprinting. A recent review provides an excellent overview of the latest studies on these techniques (82).

3. Construct components

Polymers used in cartilage tissue engineering can be divided into natural and synthetic polymers. Commonly used natural polymers in clinical studies for cartilage repair include polysaccharides, GAGs, and different proteins. Clinically, polyesters from the poly(lactic-co-glycolic acid) (PLGA) family are the most commonly used synthetic polymers. The chemical structures of polymers currently used in the clinical setting are depicted in figure 3 and summarized in table 1.

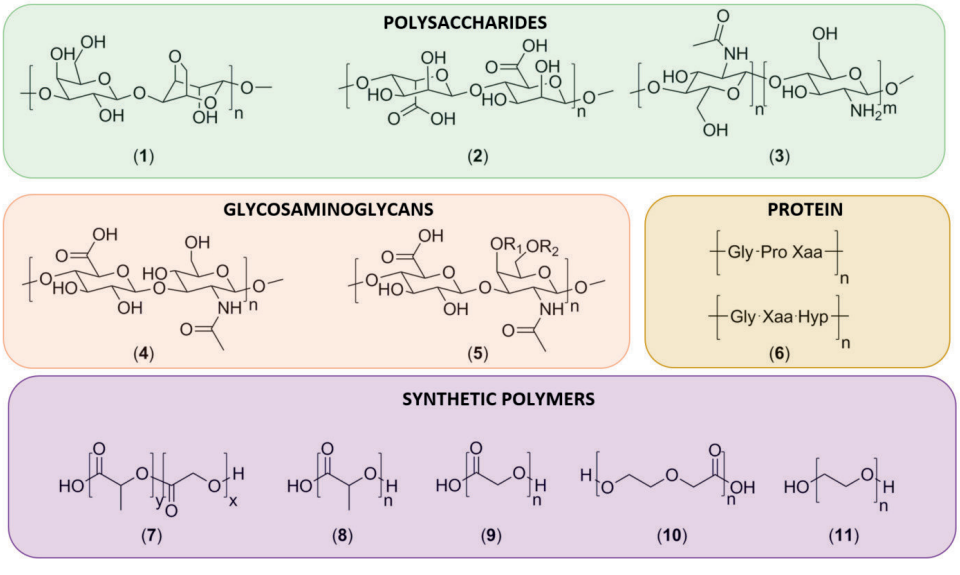

Figure 3. Structures of commonly used (bio)polymers in cartilage repair. Displayed are the natural polymers (1) agarose, (2) alginate, (3) chitosan with partial deacetylation, (4) hyaluronic acid, (5) chondroitin-4-sulfate, where R1 = SO3H; R2 = H or chondroitin-6-sulfate, where R1 = H; R2 = SO3H, (6) collagen, showing two common tripeptide repeats, where Hyp represents L-4-hydroxyproline and X represents any amino acid other than Gly, Pro or Hyp, and is often a basic or acidic amino acid. Synthetic polymers (7) poly(lactic-co-glycolic acid), (8) poly(lactic acid), (9) poly(glycolic acid), (10) polydioxanone and (11) poly(ethylene glycol)

Table 1 gives general properties of polymers used in clinical repair of osteochondral lesions.

Polymer type	Scaffold type	Degradability	Degradation time	Advantages	Disadvantages	References
Natural						
Agarose	Hydrogel (thermal)	Hydrolysis	Slow	Injectable Favorable solution-gel transition temperature	No direct cell adhesion Non-load-bearing	(83-86)
Alginate	Hydrogel (non-covalent cross-links)	Hydrolysis	Slow	Injectable	No direct cell adhesion Non-load-bearing Source dependent variation Difficulty controlling structural uniformity	(83, 87-89)
Chitosan	Hydrogel (non-covalent cross-links) or solid scaffold	Enzymatic, hydrolysis	Slow, dependent on deacetylation degree	Chemically modifiable structure Allows cell interaction	Source dependent variation	(90-101)
Hyaluronic acid	Hydrogel	Enzymatic, hydrolysis	Fast	Natural component in synovial fluid/cartilage, High low friction	Source dependent variation Non-load-bearing	(102-107)
Chondroitin sulfate	Hydrogel	Enzymatic, hydrolysis	Fast	Natural component in synovial fluid/cartilage, low friction	Source dependent variation Non-load-bearing	(108-112)
Collagen	Hydrogel or solid scaffold	Enzymatic	Fast (weeks)	Natural cartilage component, Fully degradable Injectable (in situ gel formation)	Fast degradation, unstable mechanical properties due to degradation	(113-118)
Fibrin	Hydrogel (enzymatically cross-linked)	Enzymatic	Fast (weeks)	Injectable (in situ gel formation)	Sensitive to gel shrinkage Non-load-bearing Fast degradation	(119-126)
Synthetic						
PLGA, PLA, PGA	Solid scaffold	Enzymatic, hydrolysis (bulk degradation)	Tunable (weeks to months)	Monomer ratio determines degradation rate Fully degradable Load-bearing	Inert, acidic degradation products,	(127, 128)
PDS	Solid scaffold	Enzymatic, hydrolysis	Months	Fully degradable Load-bearing	Inert, acidic degradation products	(129-137)

Table 1 gives general properties of polymers used in clinical repair of osteochondral lesions. (*continued*)

Polymer type	Scaffold type	Degradability	Degradation time	Advantages	Disadvantages	References
PEG	Cross-linked hydrogel	Non-degradable polymer; degradable cross-links possible	Non-degradable	Injectable (in situ gel formation)	Inert Non-load-bearing	(138-140) (141)

3.1 Natural polymers

3.1.1 Polysaccharides

Polysaccharides, such as agarose, alginate, and chitosan, show structural similarity to native GAG's, and result in high osmotic pressure and thus high water contents, enabling mechanical force transduction and nutrient and waste exchange. Agarose and alginate are derived from sea algae (83). Gelling properties depend on the concentration used (typically in the range of 1 – 3 % (w/v)) and average molecular weights (respectively ranging from 80 000 – 140 000 kDa and 200 000 – 500 000 kDa) (142).

Agarose is commonly used due to its favourable solution-gel transition temperature at around 37°C. However, agarose does not provide cellular adhesion sites to allow interaction of cells with the encapsulation matrix. This problem has been addressed by incorporating ECM molecules, such as fibronectin, which contain the adhesive tripeptide RGD (arg-gly-asp), as most cells bind to the ECM via RGD motifs (84, 85). The major drawback of agarose alone is its poor biodegradability which leads to a foreign body giant cell reaction, inhibiting repair processes *in vivo (86)*.

Alginate requires cross-linking to attain stable hydrogels using divalent cations. Calcium ions are often used in cartilage tissue repair since this cation is abundant in the joint environment (87). However, the physiological calcium concentration in the joint (up to 4 mM) is higher than the concentration often used in *in vitro* studies (typically 1.8 mM), which in turn leads to an increased crosslinking density, decreased porosity and supressed GAG production *in vivo*. Cross-linked chondrocyte-seeded alginate gels exhibit a compressive modulus and shear modulus of respectively 25 and 30 times lower than native cartilage (88). Like agarose, it provokes a foreign body reaction which limits its clinical use (89).

Chitosan is a polysaccharide structurally similar to chondroitin sulphate and its analogues. It is derived from the natural polymer chitin via partial deacetylation, and thus is widely available (90). The *N*-acetyl-glucosamine groups that can be found in chitosan are also present in articular cartilage and present some specific interaction sites for many growth factors, adhesion proteins and receptors (91). A major advantage of chitosan is

that its physicochemical and biological characteristics can be highly tailored by utilizing the reactivity of glucosamine residues such as acylation, alkylation, carboxymethylation, quaternization and grafting of chitosan with lactic and methacrylic acid (92-96). Chitosan by itself lacks fast gelling properties, which limits use in one-stage procedures as it may migrate and form cartilage-like tissue ectopically (97). Like other naturally derived polysaccharides, chitosan is typically combined with other materials to enhance its properties in cartilage repair. Examples include combinations with polycaprolactone (PCL) (99) and polyoxamers (98) to improve mechanical properties and with polyol salt to improve its gelling properties (100). Chitosan contains an amine group, which allow for chemically modification and provide a positive charge, which promotes cellular adhesion (101).

3.1.2 Glycosaminoglycans

Glycosaminoglycans (GAG's) are a subgroup of polysaccharides which occur in native cartilage.

Hyaluronic acid, or hyaluronan, is a GAG present in native cartilage, providing a highly hydrated environment, thus capable of entrapping and supporting chondrocyte proliferation and differentiation (102). Industrial manufacturing of Hyaluronic acid can be manufactured industrially via two processes: via extraction from animal tissue or via microbial fermentation using bacterial strains (103, 104). Its native properties (high molecular weight and high biocompatibility) make hyaluronic acid an ideal matrix component. However, by itself hyaluronic acid exhibits low intrinsic biomechanical properties. To improve its mechanical performance, hyaluronic acid is often combined with stronger polymers in cartilage repair (105). Hyaluronic acid is commercially available as a product which can be woven or spun to form a scaffold for cell growth (106, 107).

Chondroitin sulphate is a sulphated GAG which is one of the most abundant physiologically present GAGs in the ECM, providing good cell encapsulation and adhesion properties (108). Challenges for its use in tissue engineering include low thermal resistance, fast degradation by chondroitinase, and low mechanical strength. The low mechanical properties can be addressed by constructing a double network structure in which a stronger polymer interpenetrated (109). Chondroitin sulphate used in tissue engineering shows conflicting evidence regarding chondrocyte behaviour and is therefore often combined with other polymers (110-112).

3.1.3 Proteins

Although sixteen types of collagen are known, 80-90 percent of the bodily collagen consists of collagen type I, II and III. Collagen type II makes up the majority of the proteins in articular cartilage. Collagen is composed of a triple-helix structure, which primarily consists of three amino acids: glycine, proline and hydroxyproline in a typical repeating

Gly-Pro-X motif in which X can be any amino acid. The collagen types have different biomechanical properties and differ mainly by the segments that interrupt the triple helix and the way it folds into three-dimensional structures. A publically available chapter gives more in-depth information about collagen (113). As a natural body constituent, collagen fibrils provide a natural adhesion surface for cells and are mainly responsible for mechanotransduction. Chondrocyte behaviour is affected by the type of collagen used in a matrix: chondrocytes are more capable of maintaining their spherical phenotype in type II collagen as compared to type I (114). While the use of collagen type II in cartilage grafts mimics the natural environment most closely, collagen type I is easily isolated based on acetic acid dissolution as an animal by-product and therefore often used in tissue engineering (115-117). Collagen type I has the advantage of spontaneously polymerizing into a stable gel at neutral pH and physiologic temperatures, also making it suitable as injectable hydrogel (118).

Fibrin, a fibrous protein mainly responsible for the formation of blood clots, is formed by fibrinogen monomers. Fibrin hydrogel can be made from animal-derived purified fibrinogen and purified thrombin (119), self-assemble into a polymer network, promote cell attachment, and mimic the natural blood-clotting process (120). It has very low mechanical properties and is therefore often only used as cell-carrier combined with a mechanically stronger polymer such as polyglycolic acid (PGA) or PLGA (120, 121). Supraphysiologic levels of thrombin and fibrinogen are obtained after the fractionation of pooled plasma and this product is also labelled as fibrin glue (122, 123). Commercially available and autologous fibrin contains several bioactive molecules such as thrombin. Fibrin was shown to promote migration and proliferation of human chrondrocytes when used in combination with type I/III collagen MACI through the effect of specific thrombin receptors (protein-coupled protease activated receptor) (143). A drawback of fibrin constructs is their fast degradation by fibrinolysis. However, by adding fibrinolytic inhibitors the degradation rate can be tuned to allow for the production of sufficient ECM (126). Another approach is to denature and modify the fibrinogen and combine this with poly(ethylene glycol) (PEG) diacrylate into a UV light curable hydrogel (124). This natural synthetic hydrogel has advantages in terms of resorption time and has shown promising results in an early clinical trial (125).

3.2 Synthetic polymers

3.2.1. Poly(lactic-co-glycolic) acid, polylactic acid and polyglycolic acid

Poly(lactic-co-glycolic) acid (PLGA) is a synthetic linear copolymer that consists of different ratios of its constituent monomers, lactic acid (LA) and glycolic acid (GA). Due to two existing enantiomeric isomers of LA, PLGA is present in D-, L-, and D,L-isomers. PLGA degrades trough hydrolysis of the ester bonds. PGA is relatively hydrophobic by

nature, degrades rapidly in aqueous solutions and loses its mechanical integrity in between two and four weeks. Polylactic acid (PLA) has one extra methyl group making it more hydrophobic, leading to a slower hydrolysis rate. The ratio of LA to GA consequently determines the specific form of PLGA, providing degradation rate control which results in sustained mechanical integrity ranging from a few weeks up to months and even years (127). The parameters of the PLGA production process further influence the physicochemical characteristics of the end product. For example, poly-condensation of LA and GA at temperatures above 120 °C results in low molecular weight PLGA (128).

3.2.2. Polydioxanone

The poly(ester-ether) polydioxanone (PDS) has been used for a wide variety of applications in medicine, and is particularly known for its application as a monofilament suture. In the past few years, electrospun PDS has gained interest for its excellent biomechanical properties, which are relatively similar to the major molecules of the ECM, in particular collagen and elastin. PDS degrades via bulk erosion into 2-hydroxyethoxyacetic acid, a physiologic metabolite that can be excreted (129). Since organic solvents are needed for nearly all scaffold fabrication methods, PDS' poor solubility has limited its incorporation into commercial products (130-137).

3.2.3. Poly(ethylene glycol)

In contrast to the synthetic polymers mentioned above, poly(ethylene glycol) (PEG) is soluble in water and can be used to form hydrogels when cross-linked. The material is biocompatible and allows the diffusion of nutrients and bioactive molecules into its matrix (138). The diacrylated forms are particularly of interest due to their ability to be gelled into complex defects *in situ* using UV-light (139). One drawback of PEG-based hydrogels is that they are bio-inert and provide no biological signals to the cells (138). This problem has been addressed by incorporating several types of bioactive molecules into a PEG-based scaffold, resulting in the formation of hyaline-like cartilage in *in vitro* (140) and *in vivo*. Recently, PEG was combined with chitosan by crosslinking, also showing hyaline like cartilage *in vivo* (141).

3.3 Polymers used in preclinical settings

Extensive Food and Drug Administration (FDA) master files are available for polymers that are currently used clinically (144). To expedite and lower the costs of regulatory body approval procedures, novel constructs are often based on the same set of polymers. Less commonly used polymers in clinical work, but widely assessed in *in vitro* and *in vivo* preclinical settings are the natural polymers cellulose (145), silk (146, 147), gelatine (148, 149), and the synthetic polymers polyurethane (150-154), PCL (155, 156), polyvinyl acid (PVA) (157-160) and poly(N-isopropylacrylamide) (161, 162).

3.4 Construct Implications

For satisfactory outcomes it is important that tissue engineered construct closely mimic the distinct characteristics of articular cartilage (163, 164). The high water content in cartilage can be reproduced by hydrophilic polysaccharides such as agarose, alginate and chitosan. These polymers form hydrogels thereby mimicking the amorphous ground substance of proteoglycans and GAGs. However, without additional support these hydrogels cannot bear load (84-89, 97, 99). Synthetic polymers such as the PLGA family, provide more mechanical support that mimic the characteristics of collagen fibrils in native cartilage (127, 128). Collagen itself is also used, but lacks its native complex organization as an artificially applied construct component and therefore exhibits inferior mechanical properties (117). More biomimetic constructs, consisting of both natural and synthetic polymers, combining the high osmolarity of polysaccharides with the load-bearing capabilities of synthetic polymers, have logically received increased interest in the last few years (165).

Integration of a tissue engineered construct with adjacent cartilage and bone requires fully or partially degrading scaffolds or cell carriers [54]. The difficulty in creating a tissue engineered construct is tailoring the degradation speed to match the rate at which natural ECM components are produced by newly introduced chondrocytes in order to maintain constant mechanical properties over time [55].

3.5 Construct production technologies

Traditionally, constructs are produced *ex vivo* prior to the surgery and under controlled conditions using one of the following techniques: (1) construct production using porogens in biomaterials such as solvent casting and particulate leaching, gas foaming, freeze-dying and phase separation; (2) the use of woven or non-woven fibers such as fiber bonding or electrospinning; (3) solid free-form or rapid prototyping technologies such as selective laser sintering, stereolithiography, 3D printing and wax printing. Elaborating on these individual techniques is outside the scope of this review and the excellent review by Chan and Leong will provide the reader with more in-depth information (166).

Ideally, cartilage repair is performed using a minimally invasive approach. This can be a mini-arthrotomy or even less invasive by using an arthroscopic approach. Minimally invasive surgery limit trauma to the connective tissue, scarring and subsequently lead to a faster recovery. Hence, efforts have been made to develop these self-assembling injectable constructs (167). Besides the benefits of being a minimally invasive approach, these injectable constructs also do not need *ex vivo* production, hereby reducing it to a one-step surgery. Moreover, these constructs are likely to fill any defect, even those that are irregularly shaped or hard to reach. There are several methods to induce *in-situ* polymerization for injectable constructs such as chemical crosslinking, the use of thermo-

responsive gels and photopolymerization (168). Photopolymerization works through the addition of a photoinitiator into a monomersolution. This photoinitator is consequently converted into radicals by light energy. (169). To be photocurable polymers used in photopolymerization have to be functionalized with photo-reactive groups such as acrylates (170)

Photopolymerization has some benefits compared to the other forms of polymerization. It is for instance able to control the spatial and temporal dimensions of the polymerization process. Moreover, since the light intensity and exposure can be adjusted the depth of gelling can be modified (171).

Similar to chemical crosslinking and thermoresponsive gells, photopolymerization has been used for cell encapsulation and the incorporation of bioactive molecules (81, 172).

4. Commercially available products

Regenerative and resurfacing products that are available for clinical use often share similarities in techniques or polymers that are used. Table 2 summarizes the discussed products below.

4.1 PLA/PLGA-based constructs

BioSeed®-C (BioTissue AG) is a MACI based product which combines a PGA/PLA and PDS based supportive matrix with culture-expanded autologous chondrocytes suspended in fibrin glue (131). In two comparative studies BioSeed®-C did not show clinical superiority over conventional ACI using periostal flap (ACI-p) treatment (135, 136) using patient reported outcome measures (PROM's). However, the radiological outcome was better for the BioSeed®-C treatment, possibly indicating the beneficial effect of a using scaffold (136).

Chondrotissue® (BioTissue AG) is an absorbable non-woven, pure PGA textile treated with hyaluronic acid, and has been used in AMIC procedures in combination with PRP or BMC as cell source (173, 174, 205, 206). Chondrotissue® has been investigated in several case series studies and followed up to 5 years (173-175, 205, 206). The authors describe the results as promising, with case series studies showing hyaline cartilaginous tissue in biopsies in a small number of patients without further specification (175). For objective evaluation, comparative studies for Chondrotissue® are required.

4.2 Collagen-based constructs

NeoCART® (Histogenics Corporation) is a bovine type I collagen based MACI procedure, which is seeded with autologous chondrocytes and subsequently mechanically loaded in a bioreactor to induce cartilage glycoproteins synthesis (176). A FDA phase II trial comparing NeoCART® to microfracture showed significantly better results in all clinical outcome measures in the NeoCART® treated patients (177).

Table 2 gives an overview of the commercially available products, their composition, the procedure type and typical clinical findings.

	Group	Product	Company	Composition
Degradables	PLGA-based	BioSeed®-C	BioTissue, AG	PGA-PLA scaffold reinforced with PDS and seeded with autologous chondrocytes and suspended in fibrin
		Chondrotissue®	BioTissue AG	Non-woven PGA textile treated with hyaluronic acid combined with either PRP or BMC.
	Collagen-based	NeoCart®	Histogenics Corporation	Scaffold using bovine type I collagen seeded with autologous chondrocytes cultured in a bioreactor
		NovoCART® 3D	TETEC® Tissue Engineering Technologies AG	3D collagen-chondroitin sulfate scaffold seeded with autologous chondrocytes.
		CaReS®	Arthro Kinetics	Hydrogel using type I collagen from rat tails seeded with autologous chondrocytes cultured in autologous blood
		Chondro-Gide®	Geistlich Pharma AG, Wolhusen, Switzerland	Collagen type I/III matrix sutured to debrided microfractured defect and supported by fibrin glue
		Maioregen®	Fin-Ceramica Faenza S.p.A., Italy	Threelayered nanostructured scaffold with a top layer consisting of type I collagen, a middle layer of 60% type I collagen and 40% hydroxyapatite and a bottom layer with 60% hydroxyapatie and 40% type I collagen.
	Other natural polymer-based constructs	Hyalograft® C	Anika Therapeutics, Inc.	Hyaluronan (HYAFF-11S), a benzylic ester of hyaluronic acid, scaffold seeded with autologous chondrocytes and fixated using fibrin glue
		Cartipatch®	Tissue Bank of France	Hydrogel using an ultrapurified agarose-alginate suspension (GelForGel) seeded with autologous chondrocytes cultured in monolayer conditiones in autologous serum.
		Chondron™	Sewon Cellontech Co. Ltd	Hydrogel using autologous chondrocytes mixed with fibrin glue (ratio 1:1).
		BST-CarGel®	Piramal Healthcare Ltd	Chitosan mixed with autologous blood.
		GelrinC™	Regentis Biomaterials	PEG-fibrinogen hydrogel applied as liquid formulation and cured in-situ using long wave UV light.

	Procedure	Typical clinical findings	References
	Two-step procedure; MACI	No clinical superiority compared to ACI-p; radiologically better than ACI-p.	(135, 136)
	One-step procedure; AMIC	Promising outcomes from case series with evidence of hyaline cartilaginous tissue; no comparative studies available.	(26, 173-175)
	Two-step procedure; MACI	Good clinical outcomes and superior to microfracture in comparative study.	(176, 177)
	Two-step procedure; MACI	Performed better than ACI-p in high demanding patients, effect was not significant; high rate of graft hypertrophy in case series studies.	(178-180)
	Two-step procedure; MACI	Superior results when compared to microfracture in matched-pair analysis after 3 years	(181, 182)
	One-step procedure; AMIC	No comparative studies available.	(183-185)
	One-step procedure; AMIC	No comparative studies available.	(186, 187)
	Two-step procedure; MACI	Performed better than microfracture after 2 years up to 7 years; faster improvements compared to Chondro-Gide®	(188-191)
	Two-step procedure; MACI	Inferior results compared to mosaicplasty after 2 years in comparative study.	(192, 193)
	Two-step procedure; MACI	No comparative studies available.	(194-196)
	One-step procedure; AMIC	Little evidence; clinically equal to microfracture but radiologically superior in comparative study	(197)
	One-step procedure; AMIC	No comparative studies available.	(125, 198)

Table 2 gives an overview of the commercially available products, their composition, the procedure type and typical clinical findings. (*continued*)

	Group	Product	Company	Composition
Non-degradables	Metals	HemiCAP®	Arthosurface INC.	Titanium cancellous screw with cobalt-chrome articular surface
		Episealer® Condyle Solo	Episurf medical AB	Cobalt-chrome monobloc with titanium-hydroxyapatie coating

NovoCART® 3D (TETEC® Tissue Engineering Technologies AG) is a 3D collagen-chondroitin sulfate scaffold, which is seeded with autologous chondrocytes in MACI procedures. In a comparative study involving 19 high demanding patient, including athletes and soldiers with large defects, NovoCART® 3D and ACI-p failed to bring these patients back to their pre-injury level of activity. NovoCART® 3D did not perform better than ACI-p (178). However, the included patients in this study might not be representative for the normal indication and more comparative studies are indicated. Two case series found that NovoCART® 3D led to graft hypertrophy in up to 25 % of the patients (179, 180).

CaReS® (Arthro Kinetics) is a hydrogel based on rat tail derived type I collagen. This MACI treatment was compared to microfracture in patellofemoral defects in a matched-pair analysis study, and did not show superior PROM results compared to microfracture after 3 years (207). Promising results were obtained in small and large scale case series studies (181, 182). However, some scores only improved significantly after three years (182). More prospective comparative studies are indicated.

Chondro-Gide® (Geistlich Pharma AG, Wolhusen, Switzerland) consists of a bilayer collagen type I/III matrix. Chondro-Gide® was the first described AMIC based treatment, but still only case series studies have investigated the use of this novel treatment in clinical settings (183-185).

Maioregen® (FinCeramica Faenza S.p.A. Faenza, Italia) is a three-layer nanostructured scaffold. The top layer consists of deantigenated type I equine collagen resembling the articular surface. The middle layer consists of type I collagen (60%) and magnesium-enriched hydroxyapatite (40%), creating a tide-mark-like layer. The bottom layer mimics subchondral bone, and is composed of magnesium-enriched hydroxyapatite (60%) and type I collagen (40%). This AMIC treatment was only investigated clinically in two small

Procedure	Typical clinical findings	References
One-step procedure; FKR	No comparative studies available; possible feasible treatment option for failed regenerative treatments.	(199-201)
One-step procedure; FKR	No clinical evidence yet.	(202-204)

case series: in patients with rather large defects (n=20) and in patients with tibial plateau lesions (186, 187).

4.3 Other natural polymer-based constructs

Hyalograft® C autograft (Anika Therapeutics, Inc.) is MACI procedure based on the use of HYAFF-11®, an esterified hyaluronic acid. In a comparative study, Hyalograft®-C and microfracture both showed improved results at two years follow-up. However, after another five years, these initial good results deteriorated in microfracture whereas they remained stable in Hyalograft®-C (189, 190). The same research group compared the same interventions in a study with high demanding professional soccer players. Although the Hyalograft® C treated patients required a longer duration for their return to play, the results were sustainable up to seven years, whereas the microfracture patients again showed deterioration of the results at long-term follow-up (191). Clinical scores improved faster in Hyalograft® C when compared to Chondro-Gide® in a comparative study with older patients (188).

Cartipatch® (Tissue Bank of France) is a MACI hydrogel procedure composed of an ultra-purified agarose-alginate suspension (GelForGel; Tissue Bank of France). Cartipatch® was compared to mosaicplasty in a randomized clinical trial with two year follow-up. Clinical and histological scores were better for mosaicplasty patients, including a subgroup of patients with large defects (192, 193).

Chondron™ (Sewon Cellontech Co. Ltd) is a MACI procedure which uses a hydrogel composed of autologous chondrocytes and fibrin glue in a 1:1 ratio mixture. Chondron™ has been investigated in small and large scale case series studies and showed promising results. However, only one study was conducted using frequently used and established outcome measures (194-196). Therefore more studies are needed, preferably studies comparing this product to other products or accepted treatments.

BST-CarGel® (Piramal Healthcare Ltd, Bio-Orthopaedics Division) is a chitosan-based scaffold used as AMIC treatment. BST-CarGel® was compared to microfracture alone and showed comparable clinical outcomes after 1 year. MRI assessment on the other hand showed significant lesion filling and superior repair tissue in BST-CarGel® (197).

GelrinC™ (Regents Biomaterials) is CE marked PEG-fibrinogen hydrogel AMIC procedure. It is applied as liquid formulation, cured in-situ using long-wave ultraviolet light, and is resorbed over the course of several months. In-vitro as well as in-vivo evidence suggests that GelrinC is gradually resorbed through surface mediated erosion as it is replaced by hyaline-like cartilage tissue (125). More comparative studies are indicated to confirm these promising findings

4.4 Clinical evidence in the pipeline

Several studies are currently taking place to investigate the safety and efficacy of new techniques.

Cartilage Autograft Implantation System (CAIS) (DePuy Mitek) is a biodegradable scaffold consisting of PCL and PGA reinforced with PDS which is implanted in a one-stage procedure. Cartilage is harvested from a non-weight bearing area similar to ACI, but is minced and dispersed into the scaffold. Pilot data from 29 patients showed promising results. Two studies are registered on ClinicalTrials.gov to confirm these findings of which one has recently been completed but not yet published (208, 209).

The INSTRUCT therapy (CellCoTec B.V.) is a similar technique which provides the surgeon with an intra-operative cell processing unit to process the patient's own cartilage and bone marrow, seed the scaffold and implant the scaffold into the defect. One prospective study registered on ClinicalTrials.gov has recently been completed but is not yet published (210).

BioMatrix™ Cartilage Repair Device (CRD) (Arthrex) is a bilayered scaffold with a top layer composed of type I collagen and a subchondral layer composed of ß-Tricalciumphosphate with PLA at the ratio of 80% to 20%. Recently, a five year retrospective, single center non-randomized 37 patient clinical study with MRI and clinical score follow up has been submitted to the American Journal of Sports Medicine. One multi-center study is currently recruiting patients and the estimated completion date is December 2018 (211).

4.5 Resurfacing treatment options: closing the bridge between regenerative treatments and arthroplasties?

Resurfacing implants are an alternative to regenerative techniques for active symptomatic middle-aged patients who are not eligible for total knee arthroplasty (202). Metallic

resurfacing implants provide a new focal articulation and weight bearing surface (212), which may potentially bridge the gap between (failed) regenerative treatments and arthroplasties.

HemiCAP® (Arthosurface INC.) is a resurfacing implant consisting of two components: a titanium cancellous bone screw for subchrondal fixation, and a cobalt-chrome articular component. HemiCAP® is available in several standard sizes, for example the UniCAP® for the femoral condyle is available in 10 different sizes. Early clinical outcomes show promising results (199-201), but lack comparison to other techniques.

Episealer® Condyle Solo (Episurf Medical AB, Stockholm) is a patient-specific cobalt-chromium monobloc resurfacing implant with a titanium-hydroxyapatite double coating for subchondral fixation. Preclinical evidence is promising and a human trial will be completed in 2018 (202-204).

5. Discussion and future prospects

Osteochondral cylinders harvested during mosaicplasty procedures can be considered as the ideal graft, as obviously structural components and environmental factors are already of physiological composition. Not surprisingly, mosaicplasty often outperforms most novel regenerative techniques (192, 193). However, the drawbacks of mosaicplasty are the limited donor site availability and the technical challenge associated with matching the surface congruency. In tissue engineered constructs, the graft's surface contour is attained primarily by the surgeon's intraoperative manipulation and afterwards by re-shaping and remodelling of the ECM due light joint movements during the postoperative immobilization period, which is similar to intrauterine and early childhood development (213, 214). Whereas the complete intrauterine development and cartilage maturation process during early childhood takes approximately 2-3 years, patients and surgeons are demanding full recovery and functionality within a much shorter time scale. We are demanding constructs to be fully weight-bearing and thus integrated with host tissue and optimally constructed from a mechanotransduction perspective within six months, while middle-aged patients possess diminished regenerative capacity. Hence, we are taking on an immense challenge.

Mimicking cartilage's unique mechanical properties (47) poses the largest challenge in the design of a functional long-term stable, cartilage graft. Implants should be able to withstand shear loads at the surface and high compressive loads deeper down towards the subchondral bone relatively soon after implantation. More importantly, grafts should not only be able to withstand normal daily forces, they should enable forces to

be distributed throughout the entire implant as mechanotransduction perhaps plays the most vital role in controlling ECM production and cell differentiation (215-221). This has been extensively demonstrated by Ingber and colleagues (222), who have shown that mechanical stimuli introduced via tensegrity (tensional integrity) appear to be the most primordial cellular control mechanism. Different structural networks have been shown to produce characteristic cellular phenotypes and cell fate transitions during tissue development (223, 224). Additional environmental factors, such as osmolarity, pH, and oxygen concentration are theorized to be lower in the cellular control hierarchy. However, environmental factors in cartilage regenerative therapy should also mimic the physiological cartilaginous environment as closely as possible in order to stimulate growth factors secretion and attain/maintain the chondrocytic phenotype (225-228). Mechanically inferior fibrocartilage may otherwise be formed, as typically occurs in microfracture, or chondrocyte hypertrophy may occur, leading to more solid bone-like tissue formation (15, 16). Ideally, a biomimetic construct is created.

Advances in solid free-form fabrication, e.g. 3D microprinting, resulted in the capability of producing thin polymer layers with different zonal physical structures, thereby increasingly improving the similarities to the osteochondral structure (229). Recent advances include the use of a wide variety of materials and molecules such as calcium polyphosphate and PVA, hydroxyapatite and tricalcium phosphate, calcium phosphate with collagen in binder, PCL and chitosan, and even living cells such as bovine and human chondrocytes (230, 231).

Furthermore, the increasing advancements in nanotechnology has led to the possibility of releasing bioactive molecules in a highly specific spatiotemporal pattern and the incorporation of multiple bioactive molecules, hereby mimicking the native tissue to a greater extent (63, 65-72, 232). For example, in a recent study TGF-β3 was incorporated in printing ink which was used to produce a 3D-printed polyurethane-hyaluronic-acid scaffold. This scaffold provides time-dependent release of bioactive ingredients and allows for the incorporation of self-aggregating MSC's (233) Another recent paper highlights the use of NPs in cartilage repair. In this randomized controlled trial cartilage defects in rabbits were left untreated or were treated by an *in-situ* photo-cross-linkable hydrogel of acrylated hyaluronic acid containing kartogenin-loaded PLGA nano-particles Kartogenin is an organic compound known for its chondrogenic potential. Although only compared to untreated defects, this cell free one-step surgical intervention was able to show hyaline cartilage after 12 weeks with high collagen type II content. (81) The capability to tailor materials layer-by-layer fulfilling biomechanical needs and the control over bioactive molecules orchestrating the repair response may prove to be a feasible combination to a attain a more complete biomimetic graft. In an ideal world, all of these benefits should be available for *in situ* bioprinting during surgery in the operation theatre. Although efforts

have been made towards this concepts, this technical goal remains a major challenge which will have to be tackled in the future of cartilage tissue engineering (234).

Resurfacing the articulation surface with a non-degradable implant is a much simpler approach, which surprisingly has received only marginal interest. There are several important requirements for permanent implants: first, a low friction articulating surface is required, which is typically attained by polishing cobalt chromium to a minimal surface roughness. Secondly, stable integration with subchondral bone is needed, which depends on the surface roughness, hydrophobicity, and material chemical composition of the anchor (235, 236). Third, no voids should remain around the implants, as synovial fluid flow may cause osteolysis of the subchondral bone (237). Fourth, the surface of these permanent implants should be congruent with the adjacent cartilage, and as a final requirement, resurfacing implants should not interfere with future treatment options later in life such as total knee replacement (238). Attaining surface congruency is of critical importance with resurfacing techniques, and therefore an accurate, reproducible surgical technique is required. Patient-specific implants have been introduced in the last decade to improve surface congruency (202-204). The use of metals such as cobalt-chrome in joint resurfacing is not surprising as excellent outcomes have been reported in total knee arthroplasties for decades, but their biomechanical properties are far from similar to the adjacent and surrounding tissue in cartilage repair. The coefficient of friction and stiffness are much higher than the native tissue (239). Custers et al. have shown that metal implants lead to degradation of opposing cartilage with similar severity to untreated defects in goats (212). Addressing the huge difference in mechanical stiffness between currently available metal resurfacing implants and surrounding tissue will likely yield better outcomes for the opposing cartilage, for example by creating a hybrid metal-polymer implant. An example of such a hybrid resurfacing treatment option is BioPoly™ which combines ultra-high molecular weight polyethylene and hyaluronic acid. A multi-centre case series study is currently recruiting patients (240).

With the wide range of commercially available products and the lack of a true golden standard, making an objective comparison is extremely difficult. Most of the currently available literature consists of case series, with very few well-controlled, multi-center trials comparing novel techniques to either microfracture, ACI or mosaicplasty (241). Multiple publications often describe the same patient cohorts, case series are often performed at medical centers involved in the product development process, and both homogeneous and heterogeneous patient characteristics can cloud objective comparison. In general, younger patients (< 30 years of age), with normal body mass index (BMI <30 kg/m^2) and short duration time between the onset of symptoms and treatment tend to have better outcomes (242). Defects caused by early osteoarthritis and avascular necrosis have a worse outcome compared to defects caused by osteochondritis dissecans, trauma or

salvage situations (243). Superior results are also attained in single and smaller lesions compared to complex and larger lesions (244, 245). Defects of the femoral condyle often have better outcomes than other defect sites such as patellofemoral or tibial defects (246). Moreover, previous treatment of the defect increases the likelihood of failure of subsequent cartilage repair (247). These are just a few examples of all the factors which ultimately effect clinical outcome. Efficacy of tissue engineered constructs is evaluated using patient reported outcome measures (PROMs), which may be not be sufficiently distinctive. Evaluation using MRI or longer term follow-up may be needed to capture differences. However, large cost increases in comparison to microfracture or mosaicplasty are difficult to justify if it does not result in significantly improved clinical outcomes.

For the past decades, the field of tissue engineering has mainly focused on the repair of the cartilage defect even though the entire joint homeostasis is involved in cartilage defect repair. It is well known that the individual tissues and fluids communicate via a delicate environment with a balanced metabolism in healthy joints (248). The metabolic homeostasis may change towards an inflammatory catabolic state when sufficiently forceful cartilage damage has occurred (249). Patients often only present themselves to the outpatient clinic when they experience substantial pain and function loss, with joint homeostasis in an advanced catabolic state. For this reason, patients with a long duration between the onset of symptoms and eventual surgical treatment show less improvement (242). Conditioning the joint homeostasis and restoring its equilibrium, or even creating an anabolic state, may hypothetically lead to better outcomes after cartilage defect repair. For instance, growth factors (250) or anti-inflammatory drugs (251) may be administered to the synovial fluid to facilitate this conditioning (250). Furthermore, novel polymer drug delivery systems, such as microspheres and nanoparticles, may provide suitable platforms for the controlled release of such molecules (252).

6. Conclusions

In the next decade, we foresee developments in three joint preserving strategies for cartilage repair: first, further optimization of degradable scaffold towards more biomimetic grafts combined with improved cell signalling and an improved joint homeostasis. Second, improvement of non-degradable resurfacing implants with material properties that resemble those of native tissue more closely. Finally, the development of hybrid constructs, consisting of both degradable and non-degradable components. The age of the considered patient will likely play an important role in selecting on of these three treatment options. Fully degradable biomimetic constructs are preferential for young patients, while resurfacing implants may be the technique of choice for middle-aged patients with limited regenerative potential or for patients with failed regenerative therapy.

The increasing number of available options will help bridge the gap between regenerative strategies and total knee arthroplasty for patients with cartilage defects.

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Conflicts of Interest:

The authors declare no conflict of interest.

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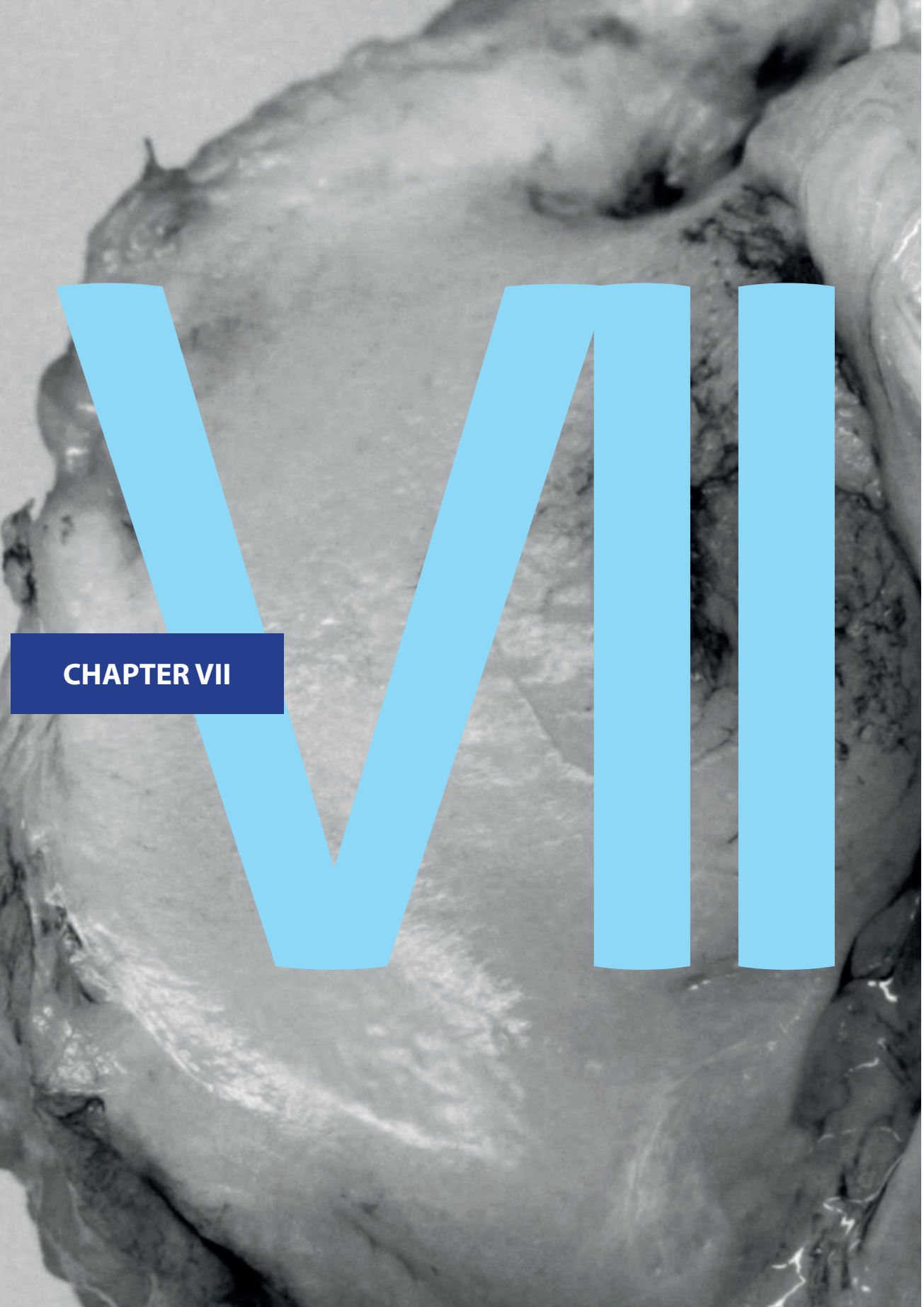

CHAPTER VII

In Vitro and *In Vivo* Study on the Osseointegration of BCP-coated versus Uncoated Non-Degradable Thermoplastic Polyurethane Focal Knee Resurfacing Implants.

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ABSTRACT

Focal knee resurfacing implants (FKRIs) are intended to treat cartilage defects in middle-aged patients. Most FKRIs are metal-based, which hampers follow-up of the joint using magnetic resonance imaging and potentially leads to damage of the opposing cartilage. The purpose of this study was to develop a non-degradable thermoplastic polyurethane (TPU) FKRI and investigate its osseointegration. Different surface roughness modifications and biphasic calcium phosphate (BCP) coating densities were first tested *in vitro* on TPU discs. The *in vivo* osseointegration of BCP-coated TPU implants was subsequently compared to uncoated TPU implants and the titanium bottom layer of metal control implants in a caprine model. Implants were implanted bilaterally in stifle joints and animals were followed for 12 weeks, after which the bone-to-implant contact area (BIC) was assessed. Additionally, 18F-sodium-fluoride (18F-NaF) positron emission tomography PET/CT-scans were obtained at 3 and 12 weeks to visualize the bone metabolism over time. The BIC was significantly higher for the BCP-coated TPU implants compared to the uncoated TPU implants (P=0.03), and did not significantly differ from titanium (P=0.68). Similar 18F-NaF tracer uptake patterns were observed between 3 and 12 weeks for the BCP-coated TPU and titanium implants, but not for the uncoated implants. TPU FKRIs with surface modifications could provide the answer to the drawbacks of metal FKRIs.

1. Introduction

Cartilage defects are highly prevalent in patients over 40 years of age (1), and may lead to severe impairment of quality of life (2). Moreover, cartilage defects are a major independent risk factor for total knee arthroplasty (TKA) (3). A survey amongst orthopaedic surgeons reveals a perceived treatment gap for cartilage defect repair in the middle-aged patient population (i.e. 40-60 years) (4). Cartilage regeneration is not consistently effective in this age-group due to an impaired regenerative potential (5-7). TKA on the other hand, is not primarily indicated due to faster wear of prosthesis components in these relatively young patients and a subsequent high risk of revision surgery in the future (4). Replacement of the osteochondral unit with an auto/allograft (8, 9) or a focal knee resurfacing implant (FKRI) (10) does not rely on cartilage regeneration and could offer joint preservation. However, both auto- and allografting are hampered by drawbacks such as donor-site morbidity and limited availability (11). FKRIs are an emerging group of implants typically intended for the treatment of cartilage defects in middle-aged patients, which may fill the treatment gap (10, 12, 13) .

Most FKRIs approved for clinical use are metal-based, comprising a highly polished cobalt-chromium articulating top to substitute cartilage and a titanium bone-substituting base (10, 12, 14, 15). A primary prerequisite for FKRIs is adequate fixation in the subchondral bone, in order to maintain congruency to the articular surface (12, 16). Multiple studies have proven excellent osseointegration of titanium-based FKRIs (12, 16, 17). However, there are concerns regarding the articulation of native cartilage with metal implants. Damage to the cartilage opposing the metal implant has been shown (17-19), presumably as a result of the large mismatch between the mechanical properties of cartilage and the implant. Biomaterial advancements have led to the development of a hybrid implant comprised of a titanium base and a polymer top layer with an elastic modulus much more similar to the modulus of cartilage (13). However, the metal base still leads to scattering on computed tomography (CT) and magnetic resonance imaging (MRI) (20). Future clinical decision making is thus impaired, which is of great concern in case of new traumatic knee injuries (21) and routine clinical follow-up of the knee joint.

Non-degradable thermoplastic polyurethane (TPU) is increasingly applied as a bearing surface in orthopaedic applications (22-24), and is available in various Shore hardnesses (Sh). Bionate® II 80A (Sh) is the lowest stiffness medical grade Bionate® TPU, and approximates the compressive modulus of cartilage (23). Bionate® 75D (Sh) is the highest stiffness medical grade TPU and could potentially be used as a bone-substitute (25). An osteochondral implant fully composed of TPU has the potential to overcome the typical drawbacks of metal-based implants, as it is MRI-compatible and theoretically would not lead to excessive stress in opposing cartilage (23, 26). Polymers however, pose specific chal-

lenges when implanted in bone. A low mechanical stiffness relative to bone can lead to micromotion between the implant and surrounding bone (27), which can subsequently lead to delayed healing, fibrous encapsulation and an inflammatory response with possible osteolysis (28). Consequently, there is no evidence of successful osseointegration of TPU to date.

The purpose of this study is to develop and evaluate a method to improve the osseointegrative potential of TPU. Next to the elastic modulus, numerous other factors contribute to implant osseointegration, such as the macro- and microscopic surface chemico-physical characteristics, initial implant stability, and the magnitude of applied loads (29). Modification of the implant's bone-contacting surface roughness is a commonly described method to prevent implant fibrous encapsulation and achieve adequate implant osseointegration (28). For titanium implants, a large body of literature supports the application of ≥ 1-2 µm R_a surface roughness to enhance osseointegration (28, 30), yet there is little data that confirms that these recommendations also apply to polymers (31, 32). In addition, various polymer studies have demonstrated the necessity of surface functionalization using bioactive coatings (31, 33, 34). Polyethylene for instance, has been used as an unbacked acetabular cup in hip arthroplasty (33). Without coating, the polyethylene cup was shown to be encapsulated by fibrous tissue in humans upon *post-mortem* analysis (33), whereas the addition of a calcium phosphate coating greatly enhanced its osseointegration in an animal trial (35). Polyetheretherketone (PEEK) has a long-standing history as an interbody cage in spinal fusion, where the so-called 'PEEK-Halo' is often observed (36). This phenomenon is indicative of a fibrous tissue interface between the spinal PEEK cage and vertebral bone (36). Preclinical studies have recently shown the beneficial effects of surface roughness modification and a calcium phosphate coating to promote osseointegration of PEEK (31, 32). Calcium phosphate coatings such as hydroxyapatite (HA) and biphasic calcium phosphate (BCP) have been shown to accelerate implant-bone fixation and to lead to bridging of implant-bone voids of 1-2 mm for metal implants under stable mechanical conditions (37). BCP has been successfully proven as a coating material to promote implant-bone bridging, and is therefore recommended for implant purposes (38).

In the current study, we hypothesize that surface roughness modification and application of a BCP coating to TPU implants will accelerate bone apposition and improve osseointegration. The first objective of this study is to evaluate the effect of surface roughness modification and BCP-coating density variation of TPU discs on *in vitro* human bone marrow derived mesenchymal stem cell (hBMSC) viability and cell-mediated calcification. The second objective is to compare *in vivo* osseointegration of BCP-coated to uncoated TPU implants and titanium-based controls in a 12-week caprine large animal osteochondral model, as a proof of concept. The primary outcome measure of the *in vivo* study is the

bone-to-implant contact area (BIC), determined through bone histomorphometrical analysis. Positron emission tomography PET/CT-scanning with the bone-seeking tracer ^{18}F sodium fluoride (^{18}F-NaF) is a nuclear modality that is able to non-destructively assess peri-implant bone metabolism well before morphological changes are observed (39, 40). A secondary objective of the *in vivo* study is to visualize the bone metabolism surrounding to the implant using *in vivo* ^{18}F-NaF PET/CT-scanning at two timepoints and to determine whether this modality has a predictive discriminating potential for ultimate osseointegration.

2. Materials and methods

2.1. In Vitro Disc and In Vivo Implant manufacturing

Bionate® 75D (DSM Biomedical, Geleen, the Netherlands) TPU plates (80x80x2 mm) were injection moulded. Three different degrees of surface roughness were melt-pressed onto the plates with roughness based on the Verein Deutscher Ingenieure (VDI) 3400 scale (41): low-roughness (R_a 0.14 µm), medium-roughness (R_a 6.3 µm) and high-roughness (R_a 18 µm). Surface roughness applied to the TPU was evaluated using white light interferometry (Wyko NT1100, Veeco, New York, NY, USA). Circular discs with a diameter of 20 mm were subsequently punched out. The surface roughness groups were then subdivided into three groups, each with a different degree of BCP coating: uncoated, double- or eight times dip-coated. Dip-coating was performed using a suspension of 5 wt% BCP particles (80% hydroxyapatite / 20% β-tricalcium phosphate, particle size distribution (d50) of 5.86 µm, CamBioCeramics, Leiden, the Netherlands) in tetrahydrofuran (1.09731, Merck, Kenilworth, NJ, USA) without further additives. The suspension was made by vigorously stirring with a magnetic stirring bar, until shortly before use to prevent settling of particles. Dip-coating was performed by dipping the TPU discs in the BCP/THF suspension within 45 seconds after stopping of stirring. Double or eight times dipping was performed, with a drying time of 5 minutes between cycles. After the last dipping cycle, the discs were rinsed with ethanol and dried overnight at 40-60 °C under vacuum. The two different BCP-coating densities were assessed using scanning electron microscopy. In total, nine types of TPU discs with varying surface modifications were evaluated: uncoated low-, medium-, and high-roughness TPU discs; double dip-coated low-, medium-, and high-roughness TPU discs; and eight times dip-coated low-, medium-, and high-roughness TPU discs. Titanium (Ti_6Al_4V) discs of the same dimensions were manufactured and served as control. Titanium discs were corundum blasted to an approximate R_a of 2-3 µm (OHST Medizintechnik AG, Rathenow, Germany). All discs were sterilized using ethylene oxide, followed by aeration to remove residuals and then aseptically placed in 12-wells plates prior to the addition of culture medium and cells.

For the *in vivo* study, bilayered Bionate® TPU osteochondral implants were manufactured using a Xplore IM12 injection moulding machine (Xplore Instruments BV, Sittard, the Netherlands). The IM12 comprises a heated barrel to melt the polymer pellets, a piston to press the melted polymer from the barrel into a mould and a heated mould with inserts that determine the shape of the implant. Implants were cylindrical, measuring 8 mm in height and 6.1 mm in diameter. The articulating top layer (2 mm height) possessed a double-curvature to match the approximate respective sagittal and coronal curvatures of the goat knee, and was composed of Bionate® II 80A. The bottom layer (6 mm height) was composed of Bionate® 75D, and intended to osseointegrate with the subchondral/trabecular bone. Implants were produced in a two-step injection moulding procedure. In the first step, the top cavity was blocked with an insert that introduces macroscopic roughness to the interface layer to increase the interlocking surface for chemical and mechanical bonding, and only the bottom layers was moulded. Typically, about 5 grams of Bionate® 75D pellets were melted at 235 °C in the barrel. After 30 seconds the semi-molten polymer was compressed to force out the air by applying a 4.5 bar pressure for 3 seconds. The bottom layer was then moulded using an 80 °C mould temperature and 15 bar injection pressure for 2.2 seconds, followed by a packing pressure of 12 bar for 10 seconds. For the second injection-moulding step, the insert blocking the top cavity was replaced with an insert featuring a highly polished cavity for moulding the double-curvature top layer onto the bottom layer of the implant. About 5 grams of Bionate® II 80A pellets were melted at 235 °C for 5 minutes, including prepacking of the semi-molten polymer after 30 seconds. Then, the Bionate® II 80A was injected at 4.5 bar pressure for 5 seconds. The complete mould was taken from the machine and allowed to cool for 1-1.5 minutes to allow the Bionate® II 80A to solidify and prevent sticking and deformation of the material during removal of the full implant from the mould. After injection moulding, the implants were washed consecutively with water, ethanol, heptane, isopropanol, and again ethanol under ultrasonic conditions for 10 minutes. Finally, the implants were dried overnight at 80 °C and reduced pressure (< 200 mBar) while maintaining a small N_2 flow. The surface topography of the Bionate® 75D layer was tailored based on the *in vitro* study results to enhance its osseointegration, see *in vitro* results (section 3.2); VDI3400 medium-roughness was applied to the inside of the injection mould for fabrication of all TPU implants (n=16). The Bionate® 75D bottom layer was dip-coated with BCP for half of the TPU implants (n=8), while the other half (n=8) was left uncoated. Implant dip-coating was performed for eight cycles in accordance with the method applied for the dip-coated discs. SEM analysis confirmed that a homogenous coating was attained, with a similar density as for the discs. After the last dipping cycle, the implants were rinsed with ethanol and dried overnight at 40-60 °C under vacuum.

Metal control implants (n=8) with analogous dimensions were manufactured (OHST Medizintechnik AG, Rathenow, Germany) with a corundum blasted (R_a 2-3 µm) titanium (Ti_6Al_4V) stem and a polished (R_a <0.05 µm) cobalt-chromium top.

All implants were double-packed and subsequently sterilized using ethylene oxide treatment, followed by aeration to remove residual ethylene oxide.

2.2. In Vitro Cell Viability and Calcification Assessment

2.2.1. Cell Culture

Human bone marrow derived mesenchymal stem cells (hBMSCs) from seven female adolescent donors were pooled and expanded to reach sufficient cell numbers (Institutional Review Board approval MUMC+ 08-4-056). Care was taken not to culture beyond passage five. Cells were plated at 5,000 cells/cm^2 in 1000 µL proliferation medium per well in 12-wells plates. Proliferation medium consisted of alpha-MEM (22-571-038, Thermo Fisher Scientific™, Waltham, MA, USA), antibiotic-antimycotic 1% (v/v) (P4333, Sigma-Aldrich, Saint-Louis, MO, USA) and EmbryoMax® ES Fetal Bovine Serum 10% (v/v) (EMD, Merck, Philadelphia, NJ, USA). Cells were allowed to attach for 24 hours, and the proliferation medium was subsequently replaced by 1000 µL osteogenic medium to differentiate the hBMSCs into the osteogenic lineage. The osteogenic medium consisted of proliferation medium supplemented with 10 nM dexamethasone (D8893, Saint-Louis, MO, USA), 10 mM b-glycerol phosphate (50020, Saint-Louis, MO, USA) and 0.2 mM ascorbic acid 2-phosphate (A8960, Saint-Louis, MO, USA). Osteogenic medium was refreshed every 2-3 days. HBMSCs were plated in three wells per biomaterial conditions, and two wells with only culture medium were included to control for background signal per biomaterial conditions. For the cell viability assay, a biomaterial-free well was also included as positive control.

2.2.2. Cell viability

Cell viability was assessed after 24 hours in proliferation medium using PrestoBlue® (Thermo Fisher Scientific™, Waltham, MA, USA). Cells were washed twice using phosphate buffered saline (PBS). Then, 100 µL PrestoBlue® reagent was added to 900 µL of fresh medium for each well and cultured for four hours. Samples of 100 µL PrestoBlue-cultured medium supernatant were measured in triplicate in microtiter plates using absorbance at 560 nm with reference wavelength set at 595 nm using a plate reader (Multiskan™ FC Microplate Photometer, Thermo Fisher Scientific™, Waltham, MA, USA).

2.2.3. Cell-mediated Calcification

Cell-mediated calcification assessment was performed after 24 hours in proliferation medium and after 21 days in osteogenic medium using the Alizarin red-S assay. Alizarin

red-S staining solution (ARS) was made by dissolving alizarin red-S dye (A5533, Sigma-Aldrich, Saint-Louis, MO, USA) in dH_2O to a final concentration of 40 mM. The pH of ARS was set to 4.2 using ammonium hydroxide (338818, Sigma-Aldrich, Saint-Louis, MO, USA). Cells were washed three times using PBS and fixed in 10% (v/v) formaldehyde (47608, Sigma-Aldrich, Saint-Louis, MO, USA) at room temperature for 15 minutes. An excess of dH_2O was used to wash the cells after which the plates were allowed to air dry. ARS (1000 µL) was added to each well. Plates with ARS were incubated at room temperature with gentle agitation for 30 minutes. Wells were washed five times using an excess of dH_2O, after which the discs were carefully inverted, and wells were washed again. Plates were allowed to air dry. In order to ensure complete dye extraction, discs were scraped, and the solution plus cells were transferred to a 1.5 mL microcentrifuge tube, vortexed for 30 seconds, and then covered with 500 µL mineral oil (M5904, Sigma-Aldrich, Saint-Louis, MO, USA). The tubes were heated to 85 °C for 10 minutes, transferred to ice for five minutes and then centrifuged at 20,000*g* for 15 minutes. Then, 500 µL of the supernatant was transferred to a fresh 1.5 mL microcentrifuge tube and 200 µL of 10% (v/v) ammonium hydroxide was added to neutralize the solution. 150 µL of the individual samples were subsequently spectrophotometrically analysed in triplicate at 405 nm absorbance in microtiter plates using a plate reader. A calibration series was taken along in these measurements. Absorbance of the samples was quantified to absolute bound Alizarin Red-S amount per disc, using the calibration series.

2.3. In Vivo Osseointegration Assessment of Osteochondral Implants

2.3.1. Surgery

The animal trial protocol was approved by the central commission for animal testing (in accordance with the EU directive 2010/63/EU for animal experiments) and local animal welfare committee of Maastricht University (Project License: AVD107002016514). Twelve skeletally mature Dutch milk goats, aged 3.28 ± 0.49 years (mean ± SD) and weighing 84.75 ± 12.13 kg, were used for this study. Implants were bilaterally placed in the medial femoral condyles of the stifle joints of the goats. Block randomization was used to randomly assign the implants to the different knees (n=24). Goats were allowed an acclimatization period of two weeks prior to surgery.

After intravenous injection of 10-20mg/kg thiopental and endotracheal intubation, anaesthesia and analgesia was maintained by continuous administration of propofol (0.3-0.6 mg/kg/min) and sufentanyl (6 µg/kg/h). An intravenous bolus of 1-4 mg/kg propofol, and 5 µg/kg sufentanyl was initially administered. Prophylactic antibiotics (single dose of 1 gram amoxicilline/clavulanic acid) were given 15 minutes prior to the incision. Both hind legs were shaved, disinfected using 0.5 % (v/v) chlorohexidine, and covered using standard surgical sheets. A medial parapatellar incision was used to open the knee

joint. Hoffa's fat pad was partially removed to increase visibility. A specifically designed Kirschner-wire guide was used to determine the angle perpendicular to the centre of the medial femoral condyle. A 2.4 mm Kirschner-wire was drilled to serve as guide for the cannulated drill. A specifically designed depth-controller allowed for incremental increases of drilling depth, aiming at a flush to a slightly recessed implant position. After confirmation of the depth using an undersized dummy implant, the implant was unpacked and hammered into the defect. The wound was closed in layers using resorbable sutures. An overview of the most important surgical steps is provided in figure 1 (A-D).

After recovering from anaesthesia, animals were allowed for immediate weight bearing. Postoperatively, pain medication consisted of intramuscular buprenorphine (5-10 µg/kg) and subcutaneous carprofen (2-4 mg/kg) injections for three days or longer if indicated. Subcutaneous amoxicillin/clavulanic acid (100mg/mL, 1 mL / 20kg) administration was continued for 5 days. The animals were housed in outdoor stables when sufficiently ambulant after surgery. Grain pellets, hay and water were provided *ad libitum*. The animal welfare officer continuously monitored the general health and care of the animals. Twelve weeks post-surgery animals were sacrificed by an intravenous pentobarbital overdose (200 mg/kg).

2.3.2. Microscopic Analysis of Osseointegration

After sacrifice, the knees were excised *en bloc* using a oscillating saw and subsequently dissected. The medial condyles were cut out, put in jars with neutral buffered formalin (NBF: formaldehyde 3,7% (v/v) in PBS), and stored on a rocking platform at 4 °C for at least two weeks. Fixed specimens were dehydrated by incubation in increasing concentrations of ethanol in water up to 100% ethanol. Then, specimens were embedded in blocks using Epoxy resin (EpoThin 2, Buehler, Lake Bluff, IL, USA) under vacuum. Resin blocks were cut using a band saw to orient the implants horizontally, and blocks were then mounted in a diamond saw (SP1600, Leica, München, Germany) using ultra low viscosity cyanoacrylate glue. A cut through the middle of the implant was made. Masson Goldner Trichrome (Carl Roth, Karlsruhe, Germany) staining was applied. Tissues were gently wiped dry and allowed to air dry for five-ten minutes. A glass coverslip was glued to the tissue using cyanoacrylate glue. Sections of 50-70 µm were cut and glued to a glass slide with cyanoacrylate glue. The sections were scanned using bright light microscopy at a magnification of 200x (M8 Microscope, Precipoint, Freising, Germany). A custom written MATLAB script (MathWorks, Natick, MA, USA) was used to determine the BIC, the percentage of the implant surface which is in direct contact with bone. This assessment was performed by two observers (RJ & AK), and the average values were used for comparison.

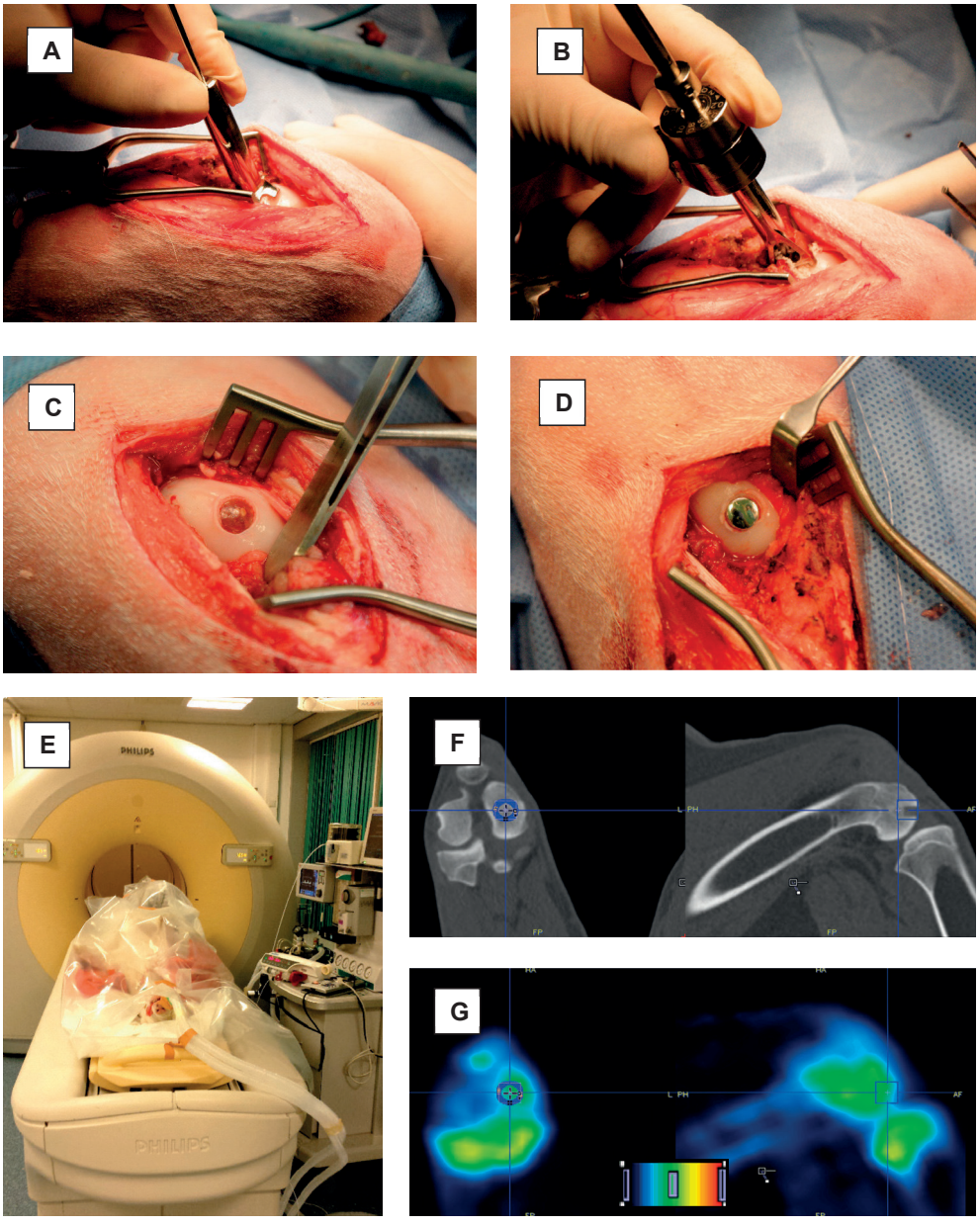

Fig. 1. (A-D) Intraoperative steps of implantation: (A) Kirschner-wire guide with a footprint that matches the curvature of the goat knee for perpendicular implantation; (B) Depth-controller with cannulated drill inside for incremental steps of drilling depth; (C) thermoplastic polyurethane implant after implantation; and (D) metal implant after implantation; (E-G) Positron emission tomography CT-scanning of the goats and analyses: (E) Positioning and fixation of the anaesthetized goat in supine position; (F) The cylindrical volume of interest (VOI) measuring 10mm in diameter and 15mm in length is drawn in the diagnostic CT-scan. The bone is then isocontoured using a threshold value (not shown); (G) The VOI is then transferred to the static positron emission tomography image to obtain standardized uptake values in the bone within VOI.

2.3.3. Peri-implant Bone Metabolism assessed by ^{18}F-NaF PET/CT scans

PET/CT-scanning was performed at three weeks and 12 weeks after surgery under general anesthesia. PET and CT images were acquired with an integrated PET/CT scanner (Gemini TF 64 PET/CT, Philips, Eindhoven, the Netherlands). The goats were supine positioned on the PET/CT bed, while anesthesia was maintained. Sixty minutes after intravenous injection of 146 – 262.16 MBq (median 178.75 MBq) ^{18}F-NaF at three weeks, and 182.73 – 248.6 MBq (median 196.18 MBq) ^{18}F-NaF at 12 weeks, PET and CT images were acquired. After a low-dose CT acquisition scan of both knees (120 kV, 30 mAs, slice thickness 4 mm), a static PET scan of two bed positions of five minutes each was acquired. This was immediately followed by a diagnostic CT scan (64-slice helical, 120 kV, 250 mAs, slice thickness 1 mm, increment 0.8 mm). Scans were viewed on clinical software (EBW, Philips, Eindhoven, the Netherlands), and further analyzed using image quantification software (PMOD 3.0, PMOD Technologies Ltd, Zürich, Switzerland). PET images were reconstructed into CT-based attenuation corrected images. The resulting bimodal ^{18}F-NaF PET/CT scans were evaluated. In each diagnostic CT scan, a cylinder of 10 mm in diameter and 15 mm in length was manually drawn surrounding the implant. A threshold was applied to the cylinder to obtain a volume of interest (VOI) that included the bone surrounding the implant. Figure 1 (E-G) shows the animal position during PET/CT scanning and subsequent VOI computations. A control VOI was drawn in the fifth lumbar vertebrae (L5). The VOI's were subsequently transferred to the static PET image to obtain the standardized uptake value (SUV) within each VOI, by correcting the measured radioactivity concentration (A [kBq/ml]) in the VOI for the injected dose of ^{18}F-NaF (ID [MBq]) and the body weight of the goat (m [kg]) according to formula **(1)**. To correct for individual baseline bone metabolism of the goat, the maximum SUV values within each VOI were divided by the mean SUV values in the control region according to formula **(2)**, creating the corrected SUVmax (cSUVmax).

$(\mathbf{1})\ SUV = (A/(ID\ /\ m))$
$(\mathbf{2})\ corrected\ SUVmax\ (cSUVmax)$ $= ((SUVmax\ implant\ VOI)/(SUVmean\ lumbar\ vertebrae\ 5)$

PET/CT analyses were used to determine the change in cSUVmax between the two timepoints, the differences between the conditions, and correlation with histology.

2.4. Statistical Analyses

Statistical analyses were performed using SPSS 23 (IBM Analytics, New York, NY, USA). Normal distribution was tested using Shapiro-Wilk test for all data. For the *in vitro* experiment, the one-way ANOVA was employed after the Levene's test confirmed equality of variance. The *post hoc* Bonferroni correction was used to determine the significant differences between each condition. For the bone histomorphometry and PET/CT scans, Student's T-test

was used in case of normal distribution, and the Mann-Whitney U-test was used in case of non-normal distribution. Interobserver reliability was assessed using the Crohnbach-alpha Intraclass Correlation Coefficient (ICC). ICC greater than 0.6 indicating good interobserver reliability. For all analyses, *P*-values lower than 0.05 were considered significant.

3. Results

3.1 In Vitro Disc and In Vivo Implant manufacturing

The average R_a of the TPU discs after melt-pressing as assessed by white light interferometry was 0.12 µm, 8.40 µm and 22.72 µm for the low-roughness, medium-roughness and high roughness conditions respectively.

Dip-coating of the *in vivo* implants resulted on average in a weight increase of 1.7 (± 0.3) milligrams for a surface area of 128 mm^2 (13.3 ± 2.3 µg/mm^2). Figure 2 shows the different applied TPU surface modifications. Figure 3 shows the bilayered composition of the TPU implant and embedding of BCP particles.

3.2. In Vitro Cell Viability and Calcification Assessment

All uncoated, the double dip-coated medium- and high-roughness, and the eight times dip-coated high-roughness TPU discs showed significantly lower cell viability compared to the biomaterial-free condition (all $P < 0.05$). Cell viability of the biomaterial-free condition was not significantly different from the titanium and eight times dip-coated low- and medium-roughness TPU discs.

None of the tested conditions showed significant calcification after 24 hours. After 21 days, the medium-roughness uncoated TPU discs showed significantly more calcification compared to titanium ($P < 0.02$). The medium-roughness eight times dip-coated TPU discs showed significantly more calcification compared to the six other TPU conditions and titanium (all $P < 0.04$). All other biomaterial conditions did not lead to significantly increased calcification compared to other conditions. Figure 4 shows the graphs of the *in vitro* quantified cell viability and calcification results.

The TPU condition with the highest amount of calcification were selected as the optimal condition, and chosen to proceed in the *in vivo* study (see methods, section 2.1). Although the medium-rough uncoated TPU's showed decreased cell viability after 24 hours, this material was also selected for implant manufacturing (see methods section 2.1.) to investigate the *in vivo* difference between uncoated and BCP-coated TPU with similar surface roughness.

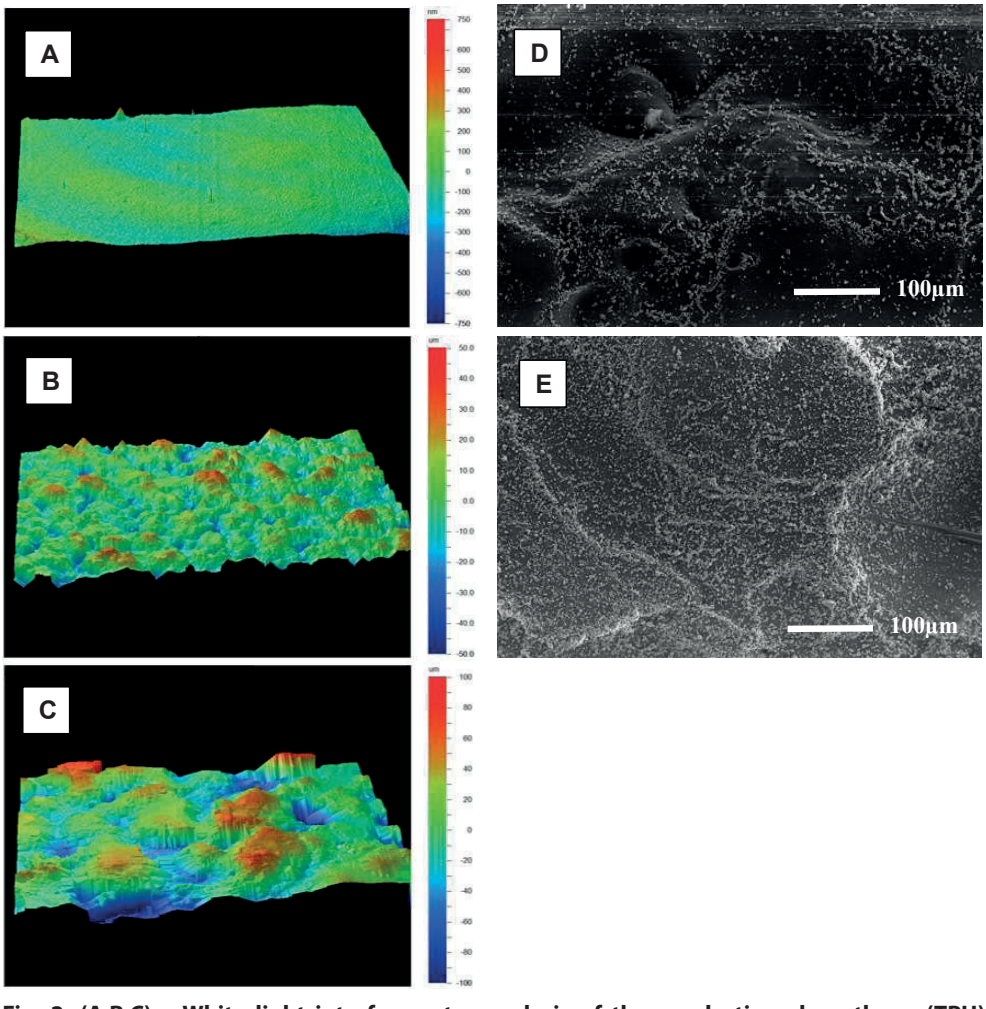

Fig. 2. (A,B,C) – White light interferometry analysis of thermoplastic polyurethane (TPU) plates after melt-pressing different degrees of surface roughness, showing an: (A) R_a of 0.12 µm after melt-pressing with an R_a 0.14 µm; (B) R_a of 8.4 µm after melt-pressing with an R_a 6.3 µm; (C) R_a of 22.72 µm after melt-pressing with an R_a 18.0 µm. (D,E) Scanning electron microscopy of TPU discs with an R_a of 8.4 µm at a magnification of 200x after the TPU was: (D) single dip-coated with BCP particles in tetrahydrofuran; or (E) double dip-coated with BCP particles in tetrahydrofuran.

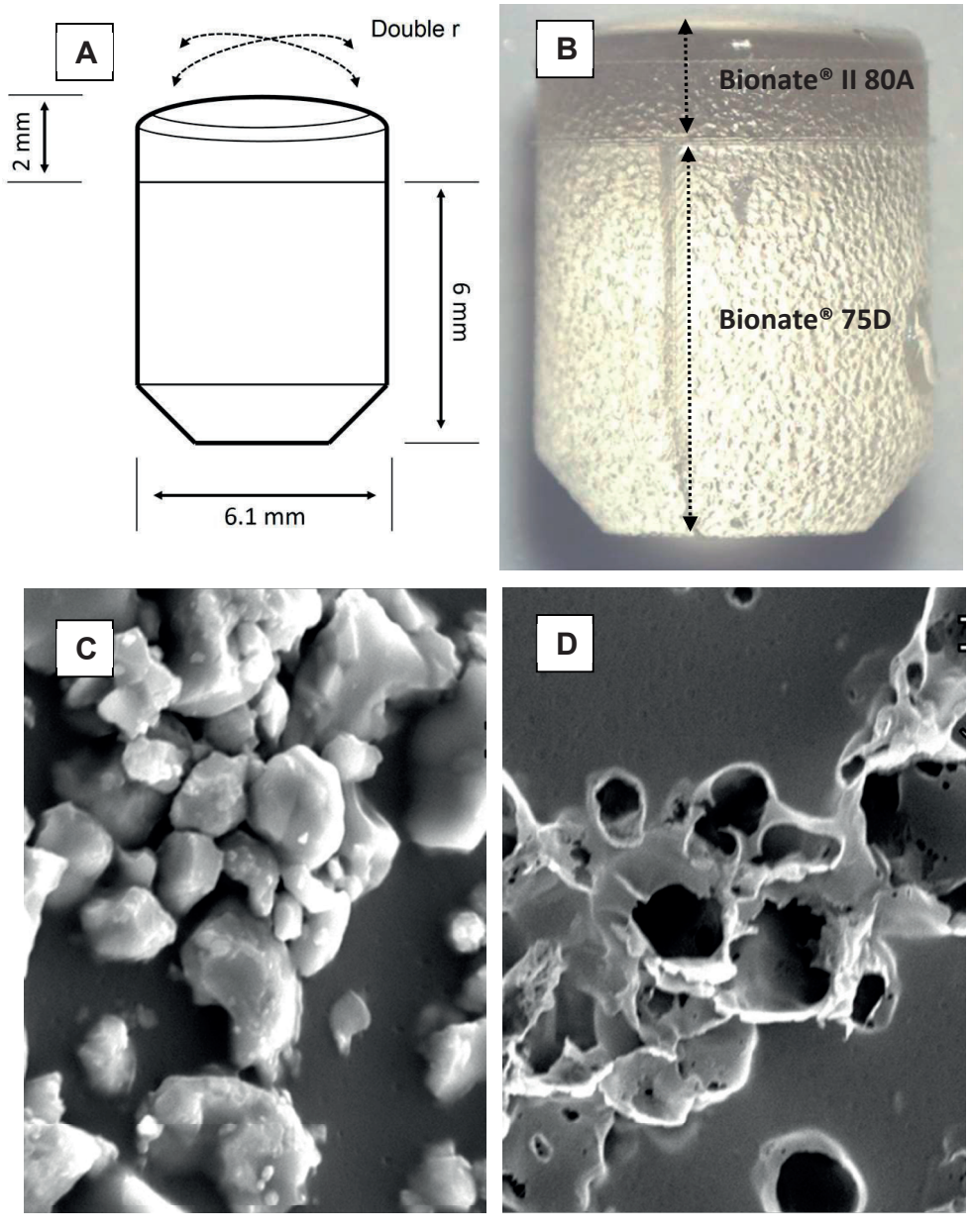

Fig. 3. (**A**) Schematic drawing of the implant; (**B**) Optical microscopy of an uncoated thermoplastic polyurethane (TPU) implant showing the bilayered construction of Bionate® (DSM Biomedical, Geleen, the Netherlands) II 80A and 75D and medium surface roughness with an R_a 8.40 µm; (**C**) Electron microscopy 5000x magnification of a biphasic calcium phosphate (BCP) coated implant showing the BCP particles embedded on the TPU; (**D**) Electron microscopy 5000x magnification of the same implant as in (C) after the BCP particles were dissolved using 1M hydrochloric acid revealing crater-like holes which confirmed deep embedding of the particles in the TPU surface.

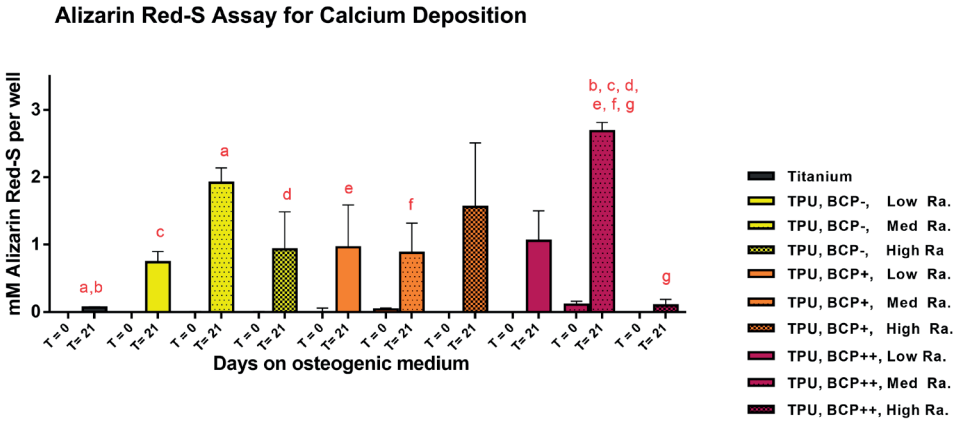

Fig. 4. Thermoplastic polyurethane (TPU) discs with different surface modifications and corundum-blasted titanium control discs were tested *in vitro*. TPU surface modifications included three different degrees of surface roughness combined with three degrees of biphasic calcium phosphate (BCP)-coating; low R_a: low surface roughness R_a 0.12 µm; med R_a: medium surface roughness R_a 8.4 µm, high R_a: high surface roughness R_a 22.72 µm; TPU, BCP-: uncoated TPU; TPU, BCP+: BCP dip-coated TPU; TPU, BCP++: twice BCP dip-coated TPU. HBMSCs were plated and (A) 24 hours after culture on proliferation medium cell viability was determined using a PrestoBlue assay. Results were normalized to the biomaterial-free well. (B) Additional discs with seeded hBMSCs were either sampled directly, or cultured for 21 days in osteogenic medium and calcification on the discs of both time points was colorimetrically quantified using an alizarin red-S assay. (A/B) Significant differences ($P < 0.05$) were determined using ANOVA and Bonferroni *post-hoc* correction. A pair of similar alphabetic letters depicts a significant difference between two conditions.

3.3. In Vivo Osseointegration Assessment of Osteochondral Implants

3.3.1. Animal Health

All surgical procedures were successfully concluded, without occurrence of intraoperative complications. Animals regained normal gait 12 ± 7 (mean ± SD) days after surgery. Fourteen days after surgery, one of the animals started limping with one leg. Due to prolonged discomfort the animal was sacrificed (metal and uncoated TPU implant). Upon *post-mortem* examination, it was discovered that suture resorption had led to medial retinaculum loosening and hence patellar luxation. Two animals were prophylactically revised with non-resorbable sutures (Ethibond Excel, Ethicon, Johnson & Johnson Medical N.V. Belgium) after physical examination showed the ability to dislocate the patella laterally. One knee (metal implant) was not included for analysis due to a recurrent mild unilateral joint infection, which was subdued using antibiotic administration. Two animals suffered from superficial wound infections which resolved after antibiotic therapy.

The weight change during the 12-week period was -7.7 ± 9.6 kg (mean ± SD). There were six metal, seven uncoated TPU implants and eight BCP-coated TPU implants remaining for evaluation at 12 weeks follow-up.

3.3.2. Microscopic Analysis of Osseointegration

None of the implants showed signs of loosening, both macroscopically, as well as upon probing after sacrifice. However, histology showed bone loss with fibrous encapsulation of the implant in two out of the six metal implants, three out of the eight BCP-coated TPU implants and six out of the seven uncoated TPU implants.

The BIC of 10.27 ± 4.50% (mean ± SD) for the BCP-coated TPU implants was significantly higher than the 4.50% ± 2.61 for the uncoated TPU implants (P = 0.03). The BIC of the uncoated TPU implants was significantly (P=0.04) lower than the 12.81 ± 7.55% for the titanium in metal implants. There was no significant difference between BCP-coated TPU implants and the titanium in metal implants (P=0.68). The ICC of the BIC assessment was excellent (0.868, P=0.00). Typical examples of histological findings are shown in figure 5a-c, and BIC-values are depicted in figure 5d.

3.3.3. Peri-implant Bone Metabolism assessed by ^{18}F-NaF PET/CT scans

The cSUVmax values for the metal implants significantly decreased over time from 13.92 ± 2.21 (mean ± SD) to 4.34 ± 2.96 (P = 0.04). BCP-coated TPU implants followed a similar trend, with a decreasing cSUVmax from 9.13 ± 2.93 to 4.83 ± 2.76, although this decline did not reach statistical significance (P=0.07). The cSUVmax for uncoated TPU implants decreased from 8.22 ± 2.89 to 6.47 ± 2.68, without statistical significance (P=0.31).

At three weeks, the cSUVmax for the metal implants was significantly higher compared to the uncoated TPU implants (P=0.04), but not significantly different from the cSUVmax for the BCP-coated TPU implants (P=0.10). At three weeks, the cSUVmax was not significantly different between the BCP-coated and uncoated TPU (P=*0.79*). At 12 weeks, the cSUVmax for all implant groups did not significantly differ from each other (All P>0.32)). Figure 6 shows the cSUVmax values at 3 and 12 weeks.

The cSUVmax values at three weeks did not correlate to the BIC at 12 weeks (Pearson's R = 0.17, P = 0.591). There was a strong correlation between the cSUVmax values at 12 weeks and the BIC values at 12 weeks (Pearson's R 0.74, P=0.001).

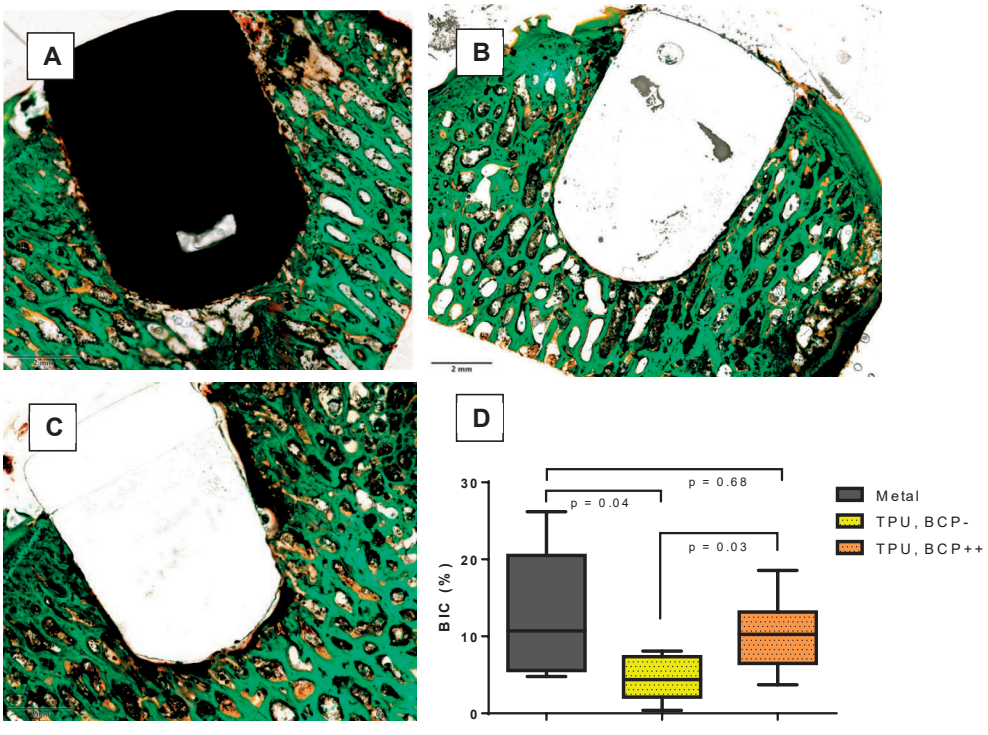

Fig. 5. ***Post-mortem*** **assessment of implant osseointegration by bone histomorphometry: Coloured images show the typical non-decalcified histology examples of (A) metal implant; (B) biphasic calcium phosphate-coated thermoplastic polyurethane (TPU) implant; and (C) uncoated TPU implant. Note that for (A) and (B) there is bone directly in contact with the implant whereas in C there is a persistent gap between the implant and the surrounding subchondral bone with fibrous encapsulation of the implant. Graph (D): Boxplot showing the histomorphometry analysis of osseointegration of the three different implants using the bone-implant contact (BIC) percentage.**

TPU, BCP-: Thermoplastic polyurethane implants without coating. TPU, BCP++: Thermoplastic polyurethane implants with biphasic calcium phosphate coating. Given values are the average of two observers (Intraclass Correlation Coefficient 0.851).

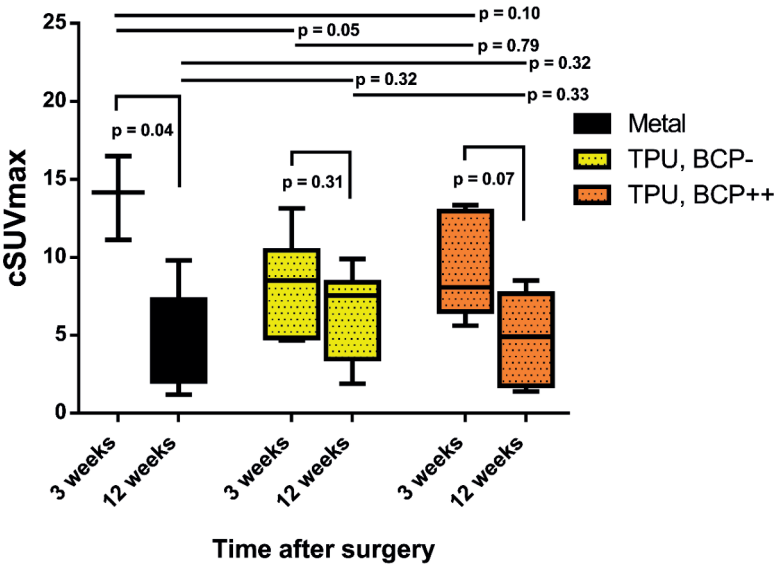

Fig. 6. Peri-implant corrected standard uptake value over time assessed by ^{18}F sodium fluoride positron emission tomography CT-scans: the corrected maximum standard uptake value (cSUVmax) of ^{18}F sodium fluoride in a designated bone volume of interest in the close proximity of the implants at 3 and 12 weeks after surgery are depicted. There was a statistical significant drop in cSUVmax over time for the titanium in metal implants, a similar trend for the biphasic calcium phosphate coated TPU implants (TPU, BCP++) and no statistical significant drop for the uncoated TPU implants (TPU, BCP-).

4. Discussion

The purpose of this study was to develop and evaluate a surface modification and coating method to improve the osseointegration potential of TPU. The first goal was to assess the *in vitro* cell viability and cell-mediated calcification of different TPU surface modifications and coating densities. The addition of the eight times dipped BCP-coating combined with medium surface roughness (R_a 8.4 µm) did not have a negative effect on cell viability and resulted in significantly increased *in vitro* cell-mediated calcification. The second goal was to assess *in vivo* osseointegration at the site of the intended FKRI application. Bone-implant-contact in the BCP-coated TPU implants was not significantly different from the titanium in metal implants, and was significantly higher in comparison to the uncoated TPU implants after 12 weeks. Comparing week three and twelve ^{18}F-NaF PET/CT results, a similar pattern of declining ^{18}F-NaF tracer uptake was observed in the BCP-coated TPU and metal implants, indicating similar osseointegration processes.

This was the first study to investigate osseointegration of non-degradable TPU with surface modifications to improve osseointegration. Previous research on the osseointe-

gration of titanium recommends the application of surface roughness with an R_a of approximately ≥1-2 µm (28, 30). Deng et al. (31) applied an R_a of 1.96 µm on dental PEEK in accordance to these titanium recommendations and compared it to polished PEEK using a canine model. There was a significantly higher BIC after 8 weeks for the roughened PEEK compared to the polished condition. Suska et al. (32) found high BIC values using either a HA or titanium coating on PEEK in an unloaded rabbit model. Surface roughness ranged up to R_a 6.85 µm for the HA-coated PEEK. In the current study, an R_a of 8.4 µm was used which is relatively high in comparison to previous studies. However, comparison to the implants in the study by Deng et al. and Suska et al. is difficult due to use of different polymers, loading conditions and animal models. Further evidence on the effect of the variation of polymer surface roughness on osseointegration should be gathered before conclusive recommendations for polymer surface roughness application can be made. In addition, application of the BCP coating led to significantly better *in vitro* calcification and *in vivo* osseointegration compared to uncoated TPU. Based on the SEM analysis of the coated implants, we hypothesize that the dip-coating method allowed for sufficient dissolvement of the Bionate® 75D surface, allowing for partial enclosure of BCP particles for mechanical stability, while still allowing for biological interaction. Although calcium phosphate coatings are known to be relatively stable in body fluid and initiate apatite formation (42), the mechanical strength and biological stability of the specific coating procedure in present study needs to be further evaluated.

The BIC magnitudes found in this study were in line with Custers et al. (43), who investigated the use of oxidized zirconium FKRIs in goats, and found a BIC of 14.6 ± 5.4% after 26 weeks. Uncoated PEEK has also been tested as bone-substitute in a canine FKRI study, but histological results showed socket expansion in 50% of the implants after three months, indicating severe osteolysis (34). FKRI studies with other implants have shown varying BIC magnitudes, but comparison is impossible due to much longer follow-up periods (16, 44). Overall, higher BIC values are well-correlated with better mechanical stability of an implant (45). Given this correlation, high BIC values should ideally be obtained at long-term to reassure permanent fixation. The interference fit of 0.1mm for press-fit fixation used in this study was selected based on cadaver experiments (not shown), and enabled easy implantation, but may have allowed excessive micromotion. It is known that osseointegration is encouraged when less than 50 microns of micromotion occurs at the bone-implant interface (46). Hence, a higher interference may be recommended in the future to further enhance osseointegration (47).

^{18}F-NaF PET/CT-scans were obtained at 3 and 12 weeks *in vivo*. There was a strong correlation between bone histomorphometry and the cSUVmax at 12 weeks, which is in line with earlier findings (48). In the metal implant group, the cSUVmax was typically high at three weeks and then decreased significantly at 12 weeks. A similar trend was observed for the

BCP-coated TPU implants, whereas this trend was not seen in the uncoated TPU implants. Ogawa et al. (49) implanted titanium implants in the proximal tibial metaphysis in rats, and assessed the BIC and ^{18}F-NaF tracer uptake up to 25 days. All implants showed a BIC > 40% after 25 days, indicating good osseointegration in that study. An initial increase of ^{18}F-NaF tracer uptake after surgery (t=3 days) was also followed by a significant drop in ^{18}F-NaF tracer uptake at 25 days. The results of Ogawa et al. and the results in current study both indicate that successful implant osseointegration is characterized by a high initial ^{18}F-NaF tracer uptake with a relatively fast drop. Ullmark et al. (50) demonstrated in a clinical study that ^{18}F-NaF PET/CT can also be used to distinguish in-growth patterns between HA-coated and uncoated acetabular cups after 1 week and 4 months. In the current study, there was a significant difference in cSUVmax between the metal and uncoated implants at 3 weeks. However, the 3-week cSUVmax values did not correlate to the BIC values at 12 weeks. We hypothesize that the effects of the surgical trauma were still too profound at 3 weeks to distinguish between biomaterial-related differences and variations in surgical trauma. Scanning at a later time point, such as six weeks postoperatively, would have been better suited to assess the predictive value of ^{18}F-NaF PET/CT as a non-destructive determination of osseointegration.

The strength of this proof-of-concept study was that it demonstrated the feasibility of achieving osseointegration of a TPU implant by means of surface modification and BCP-coating. There were also several limitations. The current *in vitro* set up was intended to allow for identification of the surface modification which provided the most cell-mediated calcification in order to select the most suitable candidate for the subsequent *in vivo* study. Although the *in vivo* study confirmed the *in vitro* findings, the osteoinduction and osteoconduction processes of BCP-coated TPU should be explored in future studies. Although BIC values and mechanical implant stability correlate well (51, 52), *ex vivo* pull-out tests could be obtained in future research and may provide a more clinical comparison between the different conditions. Another limitation is the unknown effect of the re-operations for patellar luxation in two goats on findings related to osseointegration.

The ultimate goal of FKRIs is postponing or eliminating the need for a TKA. This requires preservation of the opposing and adjacent tissues in the knee. If and to what extent a TPU implant is capable of preserving the native knee joint still needs to be determined. *In vitro* studies investigating the tribological effects of TPU against articular cartilage revealed low coefficient of friction values (23, 53), which decreased even further upon interaction with the synovial fluid proteins (23). A preclinical goat study investigating a TPU artificial meniscal implant found no difference in histologic degenerative scores between the cartilage that articulated with the implant or against the native meniscus in the unoperated control knee after 6 months (54). Long-term follow-up FKRI studies are warranted to investigate the joint preserving potential of TPU.

In conclusion, osseointegration of BCP-coated TPU implants was not significantly different to titanium implants. TPU closely approximates the elastic modulus of articular cartilage, does not cause scattering on MRI or CT scans and can be tailored to enable osseointegration, rendering it is a promising candidate material for use in FKRIs. In contrast to metal, implants fully composed of non-degradable TPU with BCP-coating and surface modifications offer a potential solution in the quest for a permanent, knee-preserving and imaging-compatible FKRI. The promising results of present study may also provide opportunities for further application of TPU in the field of bone and joint reconstruction in orthopaedic and trauma surgery. This proof-of-concept study justifies future investigations on the use of BCP-coated TPU implants.

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Disclosures

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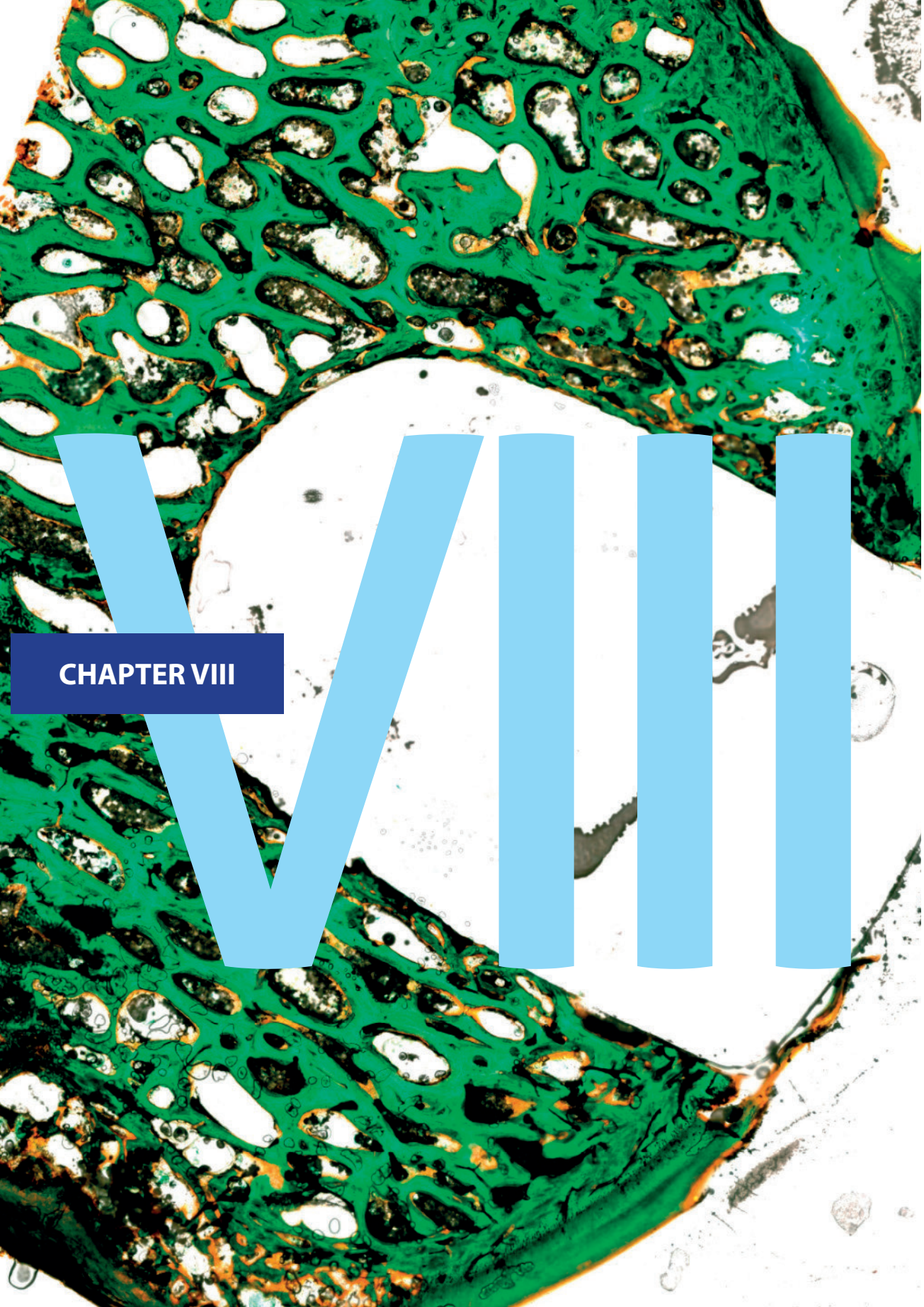

CHAPTER VIII

In vitro and *in vivo* evaluation of the osseointegration capacity of a polycarbonate-urethane zirconium-oxide composite material for application in a focal knee resurfacing implant.

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Abstract

Introduction Currently available focal knee resurfacing implants (FKRIs) are fully or partially composed of metals, which show a large disparity in elastic modulus relative to bone and cartilage tissue. Although titanium is known for its excellent osseointegration, the application in FKRIs can lead to potential stress-shielding and metal implants can cause degeneration of the opposing articulating cartilage due to the high resulting contact stresses. Furthermore, metal implants do not allow for follow-up using magnetic resonance imaging (MRI).To overcome the drawbacks of using metal based FKRIs, a biomimetic and MRI compatible bi-layered non-resorbable thermoplastic polycarbonate-urethane (PCU)-based FKRI was developed. The objective of this preclinical study was to evaluate the mechanical properties, biocompatibility and osteoconduction of a novel Bionate® 75D - zirconium oxide (B75D-ZrO_2) composite material *in vitro* and the osseointegration of a B75D-ZrO_2 composite stem PCU implant in a caprine animal model.

Methods The tensile strength and elastic modulus of the B75D-ZrO_2 composite were characterized through *in vitro* mechanical tests under ambient and physiological conditions. *In vitro* biocompatibility and osteoconductivity were evaluated by exposing human mesenchymal stem cells to the B75D-ZrO_2 composite and culturing the cells under osteogenic conditions. Cell activity and mineralization were assessed and compared to Bionate® 75D (B75D) and titanium disks. The *in vivo* osseointegration of implants containing a B75D-ZrO_2 stem was compared to implants with a B75D stem and titanium stem in a caprine large animal model. After a follow-up of six months, bone histomorphometry was performed to assess the bone-to-implant contact area (BIC).

Results Mechanical testing showed that the B75D-ZrO_2 composite material possesses an elastic modulus in the range of the elastic modulus reported for trabecular bone. The B75D-ZrO_2 composite material facilitated cell mediated mineralization to a comparable extent as titanium. A significantly higher bone-to-implant contact (BIC) score was observed in the B75D-ZrO_2 implants compared to the B75D implants. The BIC of B75D-ZrO_2 implants was not significantly different compared to titanium implants.

Conclusion A biocompatible B75D-ZrO_2 composite approximating the elastic modulus of trabecular bone was developed by compounding B75D with zirconium oxide. *In vivo* evaluation showed an significant increase of osseointegration for B75D-ZrO_2 composite stem implants compared to B75D polymer stem PCU implants. The osseointegration of B75D-ZrO_2 composite stem PCU implants was not significantly different in comparison to analogous titanium stem metal implants.

1. Introduction

Cartilage defects cause substantial pain as well as functional impairment, and often progress to osteoarthritis (OA) if left untreated(1-7). Biological cartilage repair shows excellent results in young patients, but outcomes seem to decrease with increasing patient age(8-11).Opting for total joint replacement surgery in the relatively young, middle-aged patient results in a high risk of revision surgery later in life.[12] Non-resorbable knee resurfacing implants can potentially bridge the treatment gap between biological repair techniques and total knee arthroplasty for middle-aged patients(11,13). Increasingly long-term evidence is becoming available for these focal knee resurfacing implants (FKRIs), which show satisfactory functional outcomes in middle-aged patients in terms of pain reduction, functional improvement and joint preservation(14-17).

Restoration of the native stress distribution at the site of injury and durable integration with the recipient site's bone tissue to ensure implant stability are the primary requirements for effective performance of a FKRI(18,19). Currently available FKRIs are fully or partially composed of different metals, thus showing disparities in terms of mechanical stiffness relative to both cartilage and trabecular bone(20,21). A large disparity between the elastic modulus of cartilage and the FKRI top layer may result in high contact stress, which can cause degradation of opposing or surrounding cartilage and thus progression to OA(22-26). The disparity between the elastic moduli of trabecular bone (150-700 MPa) and titanium (Ti_6Al_4V,110 GPa) can result in stress shielding(27-32). As dictated by Wolff's Law, bone tissue is remodeled after load is propagated mainly through the stiffer implant and bone is unloaded, resulting in bone resorption around the implant. Due to the loss of bone stock, implant loosening may occur in the long term(33,34), and revision surgery becomes more challenging if bone loss management is required(35). Substantiating these concerns, a metallic shoulder resurfacing implant retrieval study reported clear signs of stress shielding with an inhomogeneous as well as reduced bone stock below the implant(36,37). Next to concerns related to stress shielding, metal components cause scattering on CT and do not allow for MR imaging, impairing clinical follow-up(38,39).

A polycarbonate-urethane (PCU) FKRI was previously developed to address these disadvantages of currently available FKRI's(40). An osteochondral FKRI fully composed of PCU-based materials has the potential to overcome the typical drawbacks of metal based implants, as it facilitates restoration of near-normal contact stress distributions in cartilage(23,41) and it is magnetic resonance imaging (MRI) compatible. Although a short-term animal trial has shown promising osseointegration potential of BCP-coated PCU implants, concerns of long term adverse effects remained since the elastic modulus of the implant's stem material fell within the lower margins of the elastic modulus reported for trabecular bone(40). Bionate® 75D (Shore hardness) is the highest hardness grade

commercially available medical-grade polycarbonate-urethane, with an elastic modulus of approximately 200 MPa under physiological conditions. An elastic modulus lower than the apparent modulus of trabecular bone can lead to micromotion between the implant and adjacent bone tissue(42), subsequently leading to fibrous encapsulation and possible osteolysis(43). Closely approaching the elastic modulus of bone tissue has proven to prevent the phenomenon of stress shielding for uncemented orthopedic implants(44).

For these reasons, a novel Bionate® 75D - zirconium oxide (B75D-ZrO_2) composite material was developed. The first objective was to characterize the mechanical properties under ambient and physiological conditions to show that it better approximates the elastic modulus of trabecular bone, and to evaluate the biocompatibility and osteoconductivity of the B75D-ZrO_2 *in vitro,* by using human bone marrow derived mesenchymal stem cells (hBMSCs). Results are compared to standard Bionate® 75D (B75D) and corundum-blasted titanium. Samples with and without an osteoconductive coating for PCU implants, previously developed for uncemented implant applications, were included in the experimental setup(40). The osteoconductive coating was carefully analyzed in terms of applied coating volume and density in order to verify that conditions were uniform.

The second objective of this study was to evaluate the osseointegration of a B75D-ZrO_2 composite stem PCU implant with coating in a caprine animal model and draw a comparison to B75D stem PCU implants and titanium stem metal implants. The primary outcome measure of the *in vivo* study is the bone-to-implant contact area (BIC), determined through bone histomorphometrical analysis.

2. Material and Methods

2.1. Biomaterial Compounding

The Bionate® 75D/zirconium oxide (40/60 wt%) composite was created by compounding Bionate® 75D (DSM Biomedical, Geleen, The Netherlands) and zirconium oxide (ZrO_2) (Merck KGaA, Darmstadt, Germany) using a ZSK Mc^{18} Twin Screw Extruder (Coperion GmbH, Stuttgart, Germany). Metal screws rotating at a speed of 200 rpm and at a temperature of 210 °C compounded B75D granulate and ZrO_2 powder (particle size 0.5 – 10µm) which were fed separately to the screws. A vacuum was applied to de-gas the material during the compounding. After compounding, the material was pressed through a die plate with a single 3 mm hole. The formed strand was immediately water cooled by guiding it through a water bath before being pelletized.

2.2. Injection Molding

2.2.1. Tensile Bars

All samples were injection molded by DSM Biomedical (Geleen, The Netherlands) using an Xplore IM12 injection molding machine (Xplore Instruments BV, Sittard, the Netherlands). The IM12 heats polymer pallets in a barrel to melt them before using a piston to press the melted polymer into a pre-heated mold determining sample shape and dimensions. Both B75D and B75D-ZrO_2 composite tensile bars with a gauge length of 25 mm (2 mm thickness and 5 mm width) were manufactured. 8 grams of B75D pellets were melted at 230 ˚C for 5 minutes before being injected into the tensile bar mold of 80 ˚C. The injection pressure at 15 bars for 2.2 seconds was followed by a packing pressure of 15 bars for 10 seconds. The B75D-ZrO_2 composite was melted at 200 ˚C for 5 minutes and injected into the mold at 100 ˚C with the same injection and packing pressures and durations. After injection molding all samples were annealed at 80 ˚C under N_2 flow overnight.

2.2.2. Disks for in vitro biocompatibility and osteoconduction evaluation

Based on previous experiments, B75D and B75D-ZrO_2 composite disks (20 mm diameter x 2 mm thickness) were injection-molded with a Verein Deutscher Ingenieure (VDI) grade 3400 roughness on one side of the disks(40). Corundum blasted, titanium (Ti_6Al_4V) disks with the same dimensions (R_a 2-3 µm) were produced by Sailer BV (Sittard, the Netherlands).

2.2.3. Implants for in vivo evaluation of osseointegration

Mushroom-shaped implants (9.9 mm top and 6.0 mm stem diameter) with a total length of 10.5 mm were injection molded by DSM Biomedical (Geleen, The Netherlands) (Fig 1). The bi-layered implants contain a double-curved articulating surface (radii 18 and 11 mm) to match the sagittal and coronal plane curvatures of the goat knee and a stem with a VDI3400 roughness. The first group of implants were composed of two different grades of non-resorbable thermoplastic polycarbonate-urethane: a Bionate® II Shore hardness 80A (B80A) top layer and a B75D bottom layer, and are referred to as polymer-stem PCU implants. The second group of implants also contains a B80A top layer, but the bottom layer was composed of the B75D-ZrO_2 composite material. This group of implants is referred to as composite-stem PCU implants. Injection molding took place in a two-step fashion. First the stem was injection molded using B75D or the B75D-ZrO_2 composite with a VDI grade 3400 surface roughness, followed by overmolding a B80A top layer. Before overmolding the top layer, the stems were coated and a radiopaque tantalum marker (4 x 0.5 mm) was inserted into the designated recipient hole in the stem. Metal control implants with analogous dimensions were manufactured (OHST Medizintechnik AG,

Rathenow, Germany) and were composed of a corundum blasted (Ra 2–3 µm) titanium (Ti_6Al_4V) stem and a polished (Ra < 0.05 µm) cobalt-chromium articulating top surface.

2.3. BCP-Coating application

After injection molding, the disks and implant stems were cleaned in an ultrasonic bath using demi water, ethanol (96%), n-heptane, iso-propanol (99%) and ethanol consecutively for 10 minutes. After drying overnight in a 80 °C vacuum oven with slight nitrogen flow, the disks were first spray coated on one side with tetrahydrofuran (THF) for 10 seconds to soften the top surface. A suspension containing 5 wt% biphasic calcium phosphate (BCP) powder (80% hydroxyapatite, 20%, β-tricalcium phosphate, custom batch number 14Cam03.68A, particle volume mean diameter 6.79 µm, CAM Bioceramics BV, Leiden, the Netherlands) in THF was made. Discs were sprayed with this suspension for 20 seconds.

After the cleaning and drying process, the stems were secured on a mechanical stirrer rotating at 360 rpm and sprayed for 10 to 20 seconds using the same BCP / THF suspension. Implant spinning was continued for two minutes after stopping of spray coating to allow for air drying. After coating, the disks and stems were annealed in a 80 °C vacuum oven with slight nitrogen flow over night. After coating, implants were double packed and disks were packed in a 12-well plate before being sterilized using ethylene oxide treatment. Samples were allowed to aerate for 2 weeks to remove residual ethylene oxide.

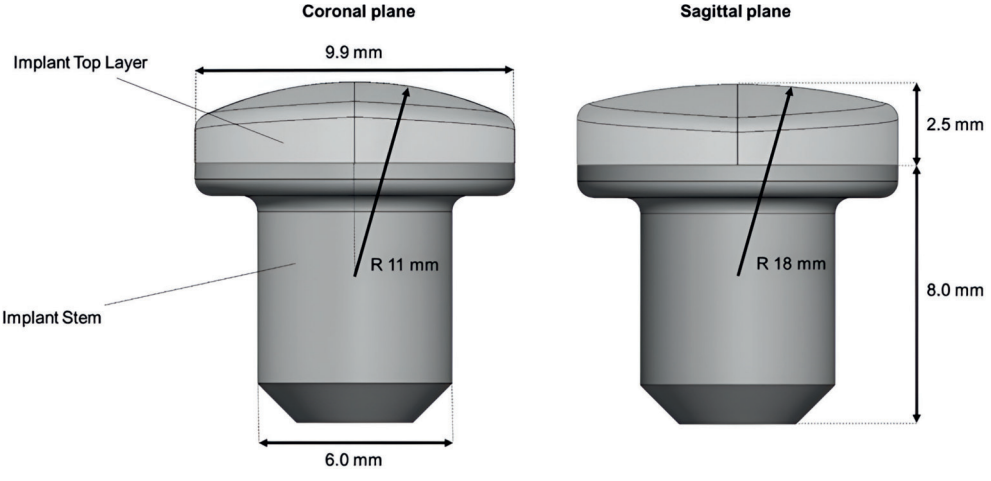

Figure 1: *Schematic overview of the implant Design. The articulating surface was shaped to match the curvature in the mediolateral and anteroposterior plane of the average medial femoral condyle in goats.*

2.4. Mechanical Evaluation

Tensile bars were tested in atmospheric (dry, 20 °C) and physiological (wet, 37 °C) conditions, mimicking surgical implantation and physiologic conditions respectively. Four

samples were tested for each condition. Wet samples were conditioned in a 37 °C water bath until no weight increase was observed before being transferred to a temperature-controlled chamber maintained at 37 °C. Tensile strength tests were performed on a Z010 testing machine (Zwick Roell Group, Ulm, Germany). Samples were fixed in grips, with a grip to grip separation of 54 mm at the start position. A pre-load of 0.5 N was applied before the tensile experiments started. E-Moduli were determined within the first 0.05 and 0.25 % of strain, at a speed of 1 mm/min. Strain was measured with an extensometer until elongation of 60%. Strain was increased until breaking point to measure elongation at break.

2.5. Coating Evaluation

2.5.1. SEM Analysis

The disks and implant surfaces were imaged using scanning electron microscopy to quantify the amount of surface area coated by the BCP-coating. The images were acquired at a magnification of 750x via environmental scanning electron microscope (ESEM, 10kV, Quanta 600, FEI Co. Hillsboro, OR) in low vacuum mode. The investigations were carried on four B75D disks, four B75D-ZrO_2 composite disks, two polymer stem PCU implants and two composite stem PCU implants. Five images were captured of each disk while six images were taken from the stem part of each implant. The acquired images were segmented to quantify the amount of surface coverage by using thresholding via a custom written MATLAB script.

2.5.2. ICP Analysis

To quantify the volume of BCP-coating applied to the samples, the calcium (Ca) and phosphorus (P) were measured using inductively coupled plasma atomic emission spectrometry (ICP-AES) after dissolving the coating using hydrochloric acid (HCl). The investigations were carried on four B75D disks, four B75D-ZrO_2 composite disks, two polymer stem PCU implants and two composite stem PCU implants. Samples were first dried overnight in a vacuum oven at 200 mbar and 80 °C under a slight nitrogen flow and were then weighed. Samples were then placed into a vial containing 10 ml of 1 M HCl. The vials containing the test samples and HCl solution were shaken a few times over a 30-minute period. Next the HCl solution was removed from the vial and stored separately. A second portion of 10 ml of 1 M HCl was added to the vial containing the sample and the procedure was repeated. The two HCl solutions were combined and diluted.

Before analysis, 2.5 mL of the sample solution was combined with 1 mL of HCl (30% HCl) and then further diluted with 21.5 mL MilliQ water. All solutions were measured in one sequence by means of ICP-AES using a spectrometer iCAP 6500 (iCAP 6500, Thermo Fisher Scientific, Waltham, Massachusetts). In between measurements, the instrument

was rinsed for 10 seconds with 0.25% HNO_3 and 0.25% HCl. Both axial (end-on viewing of plasma), as well as radial (side-on viewing of plasma) configurations were used, measuring at various wavelengths as stated below. The concentrations reported are based on the radial results of the wavelengths 393.366 nm (Ca) and 177.495 nm (P). The other wavelengths and the axial results were used to cross-check the results.

2.6. *In vitro* biocompatibility and osteoconductivity evaluation

2.6.1. Cell Culture

Human bone marrow derived mesenchymal stem cells (hBMSCs) from eight donors were pooled and seeded in a 12-well plate containing disks from one of the experimental groups or an empty well (table 1). Cells were plated at 10,000 cells/cm^2 in 1500 µl proliferation medium. Proliferation medium consisted of alpha-MEM (22–571-038, Thermo Fisher ScientificTM, Waltham, Massachusetts), antibiotic- antimycotic 1% (v/v) (P4333, Sigma-Aldrich, Saint-Louis, MO) and EmbryoMax® ES Fetal Bovine Serum 10% (v/v) (EMD, Merck, Philadelphia, PA). Cells were allowed to attach for 24 hours, and the proliferation medium was subsequently replaced by 1.500µl osteogenic medium, inducing differentiation of the hBMSCs. The osteogenic medium consisted of proliferation medium supplemented with 10 nM dexamethasone (D8893, Saint-Louis, MO), 10 mM b-glycerol phosphate (50,020, Saint-Louis, MO) and 0.2 mM ascorbic acid 2-phosphate (A8960, Saint-Louis, MO). The medium was refreshed every 2–3 days. Cells were plated in five wells per biomaterial condition with two wells containing biomaterial and medium without cells serving as control for background signal. Cell viability was referenced with biomaterial-free wells.

Disk composition and surface treatment variation for the *in vitro* biocompatibility and osteoconduction evaluation		**Implant composition and surface treatment variation for *in vivo* evaluation of osseointegration**			
Group	**Surface Treatment**	**Group**	**Top layer**	**Stem**	**Surface Treatment**
B75D	None	B75D	Bionate® II 80A	B75D	BCP coating
	BCP coating	B75D-ZrO_2	Bionate® II 80A	B75D-ZrO_2	BCP coating
B75D-ZrO_2	None	Metal Control	Cobalt Chromium	Titanium	Corundum blasted
	BCP coating	Sham Control		Sham Surgery	
Titanium	Corundum blasted				

Table 1: *Overview of the experimental and control groups in the in vitro and in vivo experimental setup. B75D: Bionate® 75D, B75D-ZrO_2: Bionate® 75D - zirconium oxide composite (40/60 wt.%)*

2.6.2. Cell-viability and Mineralization

Cell viability was assessed after 24 hours using a PrestoBlue® (Thermo Fisher ScientificTM, Waltham, Massachusetts) assay. Cells were washed twice using phosphate buffered saline (PBS) before 100 µl PrestoBlue® reagent was added to 900 µl of fresh medium for each well and cultured for three hours. PBS was manufactured by dissolving 87,52 g sodium chloride (VWR International, Radnor, Pennsylvania, USA), 14,16 g disodium phosphate (VWR International, Radnor, Pennsylvania, USA) and 2,15 g monopotassium phosphate (VWR International, Radnor, Pennsylvania, USA) in 500ml deionized water. Before use the concentrated PBS solution was diluted twenty times using deionized water. Samples of 100 µl PrestoBlue-cultured medium supernatant were measured using absorbance at 560 nm with reference wavelength set at 595 nm using a plate reader (MultiskanTM FC Microplate Photometer, Thermo Fisher ScientificTM, Waltham, Massachusetts).

Cell-mediated mineralization was assessed after four weeks using a calcium assay (Randox, Crumlin, United Kingdom) measuring calcium concentration. Cells were washed twice using phosphate buffered saline (PBS) to remove any free calcium. Next, the cells were lysed in 0.2M hydrochloric acid for three hours on a rocking platform dissolving mineralized calcium crystals. After the addition of 250 µL reagent and an incubation period of 5 minutes the absorbance was measured at 560 nm with reference wavelength set at 595 nm using a plate reader (MultiskanTM FC Microplate Photometer, Thermo Fisher ScientificTM, Waltham, Massachusetts). To correct for background signal due to the applied BCP coating the average calcium concentration in the cell free wells was subtracted from each experimental well.

2.7. *In vivo* implant osseointegration evaluation

2.7.1. Surgical Procedure

The animal trial protocol was approved by the Dutch Central Commission for Animal Testing (in accordance with the EU directive 2010/63/EU for animal experiments) and local animal welfare committee of Maastricht University (project license: AVD107002016514). Sixteen skeletally mature Dutch milk goats, were used for this study. Surgeries were performed bilaterally in the medial femoral condyles of the stifle joints. Block randomization was used to randomly assign one of the implants or a sham surgery to the different knees (n = 32). The animals were allowed an acclimatization period of 2 weeks prior to surgery. The four treatment groups are: (i) Sham surgery (n=8), B75D implant (n=8), metal implant (n=8), and B75D-ZrO2 (n=8).

After intravenous injection of 10-20 mg/kg thiopental and endotracheal intubation, anaesthesia and analgesia was maintained by continuous administration of propofol (0.3–0.6 mg/kg/min) and sufentanyl (6 µg/kg/h). An intravenous bolus of 1–4 mg/kg pro-

pofol, and 5 µg/kg sufentanyl was initially administered. Prophylactic antibiotics (single dose of 1 g amoxicilline/clavulanic acid) were given 15 min prior to the incision. Both hind legs were shaven, disinfected using 0.5% (v/v) chlorohexidine, and covered using standard surgical sheets.

A low morbidity medial parapatellar approach was used to approach the medial femoral condyle.[45] A specifically designed Kirschner-wire guide was used to determine the angle perpendicular to the center of the medial femoral condyle. A 1.6 mm threaded tip Kirschner-wire was introduced into the condyle to serve as guide for the cannulated drill. After incising the cartilage with a K-wire guided cartilage cutter, a specifically designed depth-controller was used to control drill depth, aiming to attain a slightly recessed implant depth. The implants were hammered press-fit into the slightly undersized recipient hole.

After recovering from anesthesia, animals were allowed immediate weight bearing. Postoperatively, pain medication consisted of intramuscular buprenorphine (5–10 µg/kg) and subcutaneous carprofen (2–4 mg/kg) injections for 3 days or longer if indicated. Subcutaneous amoxicillin/clavulanic acid (100 mg/ml, 1 ml / 20 kg) administration was continued for 5 days. The animals were housed in outdoor stables when sufficiently ambulant after surgery. Grain pellets, hay and water were provided ad libitum. The animal welfare officer continuously monitored the general health and care of the animals. Six months post-surgery, the animals were sacrificed by an intravenous pentobarbital overdose (200 mg/kg). After sacrifice, stifle joints were resected and examined for macroscopic signs of implant loosening and failure.

2.7.2 Bone histomorphometric analysis

After sacrifice and dissection of the stifle joint, the medial condyles containing implants were cut out using a band saw and were fixed with neutral buffered formalin (NBF) at 4°C for at least 2 weeks. Fixed specimens were dehydrated by subsequently increasing concentrations of ethanol in water up to 100% ethanol. Specimens were embedded using a HEMA-based plastic-embedding system (Technovit® 8100 KULZER GmbH, Hanau, Germany). Embedded samples were then mounted in a diamond saw (SP1600, Leica, München, Germany) using an acrylic glue (105, Permabond, Pottstown, USA). A coronal cut through the center of the implant was made. Safranine O (Sigma-Aldrich) stain was applied to the sample for ten minutes and the sample was rinsed with acetic acid for one minute. After rinsing with tap water, Fast Green (Sigma-Aldrich) stain was applied for ten minutes and the sample was again rinsed with acetic acid. The sample was gently wiped dry and allowed to air for 10 minutes. A glass coverslip was glued to the sample using ultraviolet light (UV) curing glue with ten minutes of UV exposure. Coronal sections of 50 – 70 µm were cut and glued to a glass slide using UV glue. This process was repeated

until three technically satisfactory slides were obtained. The slides were scanned using bright light microscopy (M8 Microscope, Precipoint, Freising, Germany). A custom written MATLAB script (MathWorks, Natick, MA) was used to determine the bone to implant contact (BIC). This assessment was performed by two observers (PvH & RJ).

3.8. Statistical Analyses

Statistical analyses were performed using SPSS 23 (IBM Analytics, New York, NY). Normal distribution was tested using Shapiro–Wilk test for all data. For the *in vitro* experiment, the one-way ANOVA was employed after the Levene's test confirmed equality of variance. The post hoc Tukey's test was used to determine the significant differences between each condition. For the histomorphometric bone analysis, the one-way ANOVA was employed after the Levene's test confirmed equality of variance. The post hoc Tukey's test was used to determine the significant differences between each condition. For all analyses, p-values equal to or lower than .05 were considered significant. No statistical analysis was performed regarding the mechanical data.

3. Results

3.1. Mechanical Testing

Tensile testing showed no relevant difference in the elastic modulus between the B75D material and the B75D-ZrO_2 composite material at atmospheric conditions. However, at physiological conditions (wet 37 $^{\circ}$C) the elastic modulus of the B75D-ZrO_2 composite material (480 ± 24 MPa) was substantially higher than the elastic modulus of the B75D material (206 ± 41 MPa). As a result of the compounding, a decrease of elongation-at-break was observed in both atmospheric and *in vivo* conditions to an elongation at break of 10.1 +/- 2.3% and 14.8 +/- 10.6% respectively. Material properties are summarized in table 2.

Material	Condition	E-mod (MPa)	St. Dev	σ-break (MPa)	St. Dev	ε-break (%)	St. Dev
B75D	Dry 20 °C	1492	49	68	13	157	57
B75D	Wet 37 °C	206	41	26	2	192	8
B75D-ZrO_2	Dry 20 °C	1435	35	36	1	10	2
B75D-ZrO_2	Wet 37 °C	480	24	16	1	15	10

Table 2: Overview of the material properties based on tensile strength tests. n = 4 replicates of each condition. B75D: Bionate® 75D, B75D-ZrO_2: Bionate® 75D - zirconium oxide composite (40/60 wt.%)

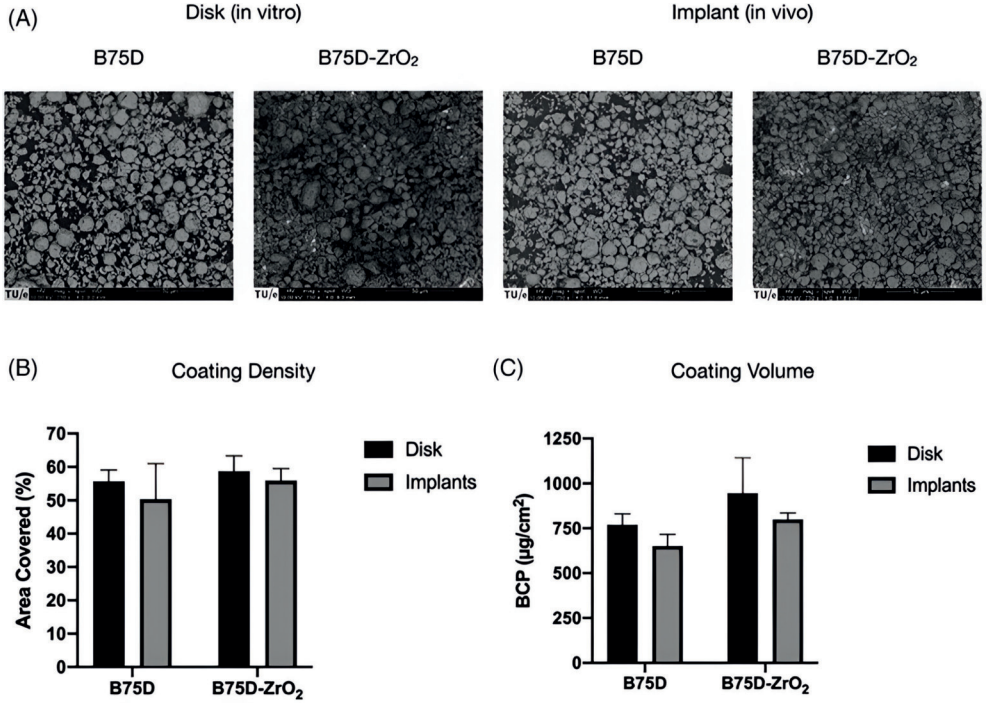

Figure 2: Coating quantification. A: Representative scanning electron microscope (SEM) images illustrating the distribution of the applied BCP surface coating of the disks and implants of both Bionate® 75D (B75D) and Bionate® 75D - zirconium oxide composite (40/60 wt.%) (B75D-ZrO2). B: Coating density analyzed by SEM imaging. Error bars represent standard deviation. n = 4 samples for disks, n = 2 samples for implants. C: Coating volume analyzed using inductively coupled plasma mass spectrometry (ICP) Error bars represent standard deviation. n = 4 samples for disks, n = 2 samples for implants.

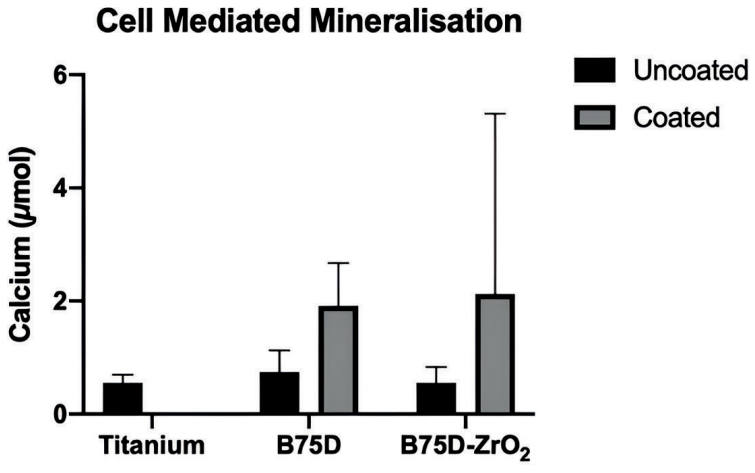

Figure 3: ***In vitro mineralization after four weeks of incubation of human mesenchymal stem cells on the biomaterials. Error bars represent standard deviation. n = 3 replicates per group.***

3.2. Coating Quantification

3.2.1. SEM Analysis

Representative electron microscopy images are shown in figure 2. It can be observed that the BPC particles exhibit similar characteristics in all four groups. The BCP particles are distributed homogenously across the surface leaving small patches of uncoated regions. The image analysis reveals no significant differences in coating density between the B75D-ZrO_2 composite disks and B75D-ZrO_2 implant stems compared to the B75D disks and implant stems as shown in figure 2. For both materials, the disks were coated with a lower standard deviation than the implant stems.

3.2.2. ICP Analysis

No significant difference was observed between the BCP coating volume present on the B75D-ZrO_2 implants (799 +/- 36 µg/mm^2) and the B75D implants (650 +/- 66 µg/mm^2). This also applied to the B75D and B75D-ZrO_2 disks, with an average BCP coating volume of 769 +/- 62 µg/mm^2 and 956 +/- 196 µg/mm^2 respectively. Results are summarized in figure 2.

3.3. Cell-viability and Mineralization

None of the biomaterials induced a decrease in cell viability after a 24 hour exposure when compared to the empty well condition, indicating that biocompatibility is acceptable for all materials. Exposure of cells to both coated B75D and B75D-ZrO_2 composite disks showed no significant difference in cell viability compared to titanium ($p = 0.32$

and p = 0.15 respectively). Cell mediated mineralization was observed in all biomaterial groups indicating facilitation of mineralization after an incubation period of 4 weeks. The mineralization results are shown in figure 3.

3.4. Surgery

Normal joint anatomy was confirmed preoperatively in all goats using macroscopic examination and plain radiograph. No complications during and after surgery were observed. No difference in post-surgery recovery and macroscopic joint healing was observed between sham and intervention groups. All animals were able to load both hind limbs directly postoperatively and no weight loss was observed in any of the animals after a six month follow-up. No signs of implant migration, loosening or failure were observed by macroscopic examination and plain radiography. An example of a post mortem radiograph is shown in figure 4. The B75D-ZrO2 material can be clearly identified on the radiograph. On visual inspection, no visible signs of wear were observed on the articulating surface of the implants.

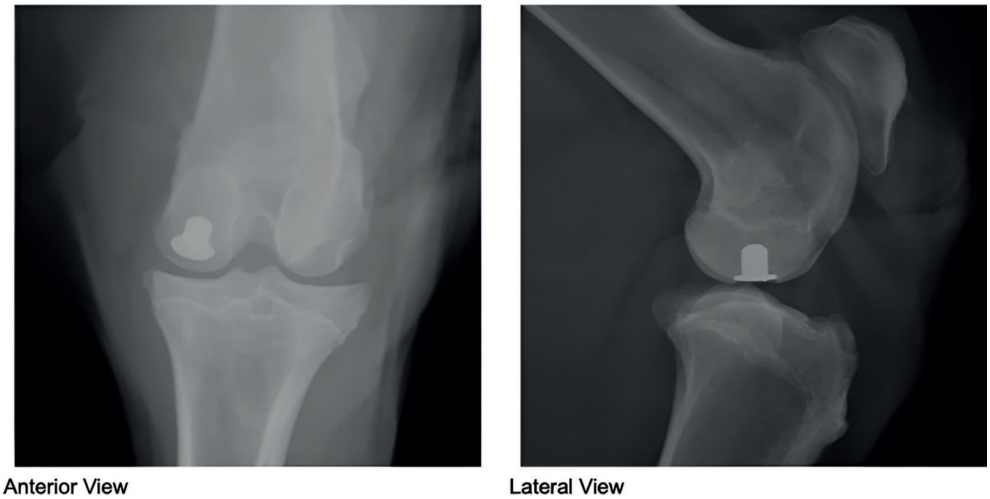

Figure 4: Post mortem plain radiograph of a *B75D-ZrO$_2$* implant in the medial femoral condyle after six month follow-up.

3.5. *In vivo* osseointegration evaluation

No macroscopic signs of implant migration were observed and all implants were assessed to be mechanically stable after manual compression during necropsy. No significant difference between the BIC of titanium stem metal implants (23.6 +/- 14.2%) and composite stem PCU implants (32.5 +/- 16.6%) was found (p = 0.44). The BIC of polymer stem PCU implants (14 +/- 11.3%) was significantly lower when compared to the composite stem PCU implants (p = 0.04). The BIC values are shown in figure 5 combined with typical examples

of each experimental group. Failure of osseointegration, defined by a predominantly fibrous tissue interface between the implant and bone, was observed in 1/8 titanium stem metal implants, 1/8 composite stem PCU implants and 4/8 polymer stem PCU implants. No signs of severe osteolysis, possibly resulting from infection, were observed.

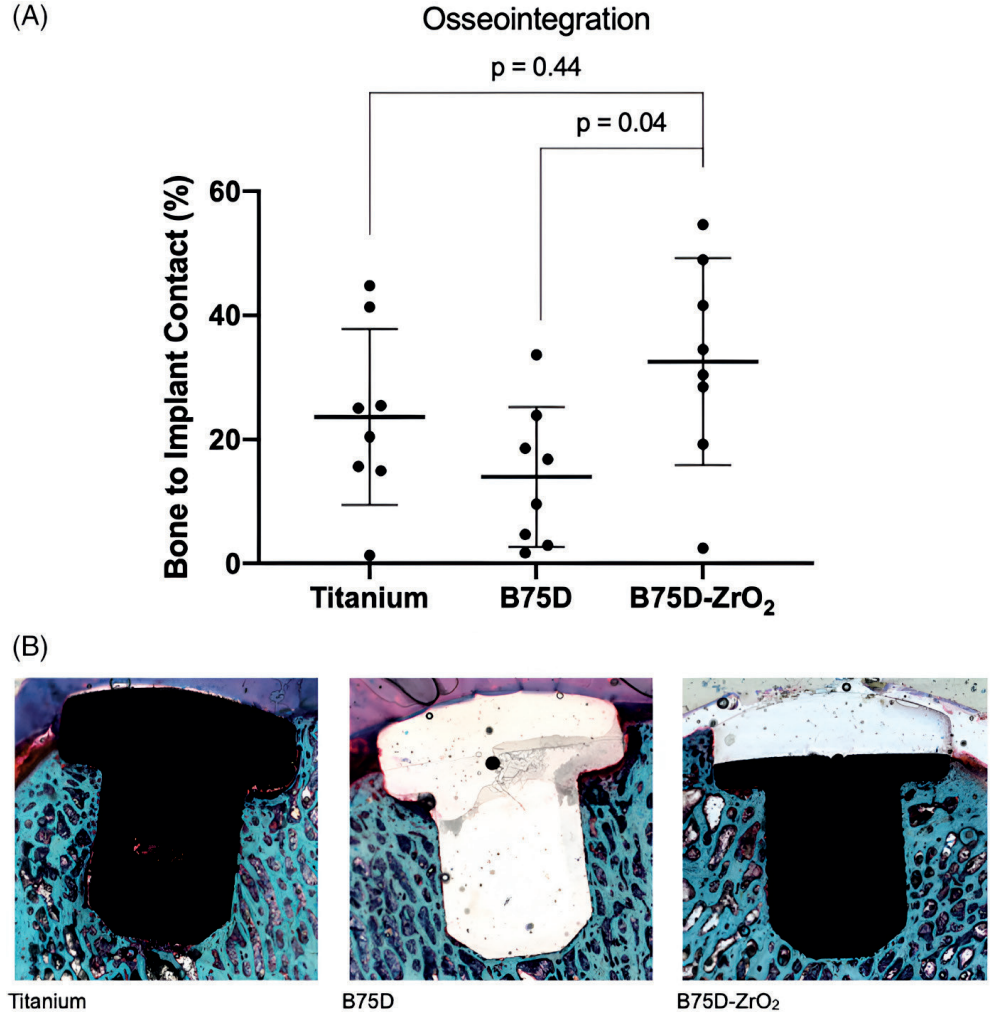

Figure 5: Implant osseointegration assessed through bone histomorphometry. A: Column scatter plot showing the histomorphometric analysis of osseointegration of the three experimental implant groups using the bone-to-implant-contact (BIC) percentage . Error bars represent standard deviation. n = 8 replicates per group. B: Typical example of each implant type.

4. Discussion

The goal of this study was to evaluate the *in vitro* osteoconductive and *in vivo* osseointegrative capacity of a novel Bionate® 75D (40 wt%) and zirconium oxide ceramic (60 wt%) composite material coated with a previously developed BCP coating in the context of a focal knee resurfacing implant(40). *In vitro* mechanical testing has confirmed that the B75D-ZrO_2 composite material possesses an elastic modulus substantially higher than B75D (480 MPa vs 206 MPa) under physiological conditions and well in the range of the elastic modulus reported for trabecular bone (150 - 700 MPa)(32). As a side-effect, adding ZrO_2 decreased the yield stress from 26 MPa for B75D to 16 MPa for B75D-ZrO_2. However, yield stress values are still beyond those that are expected to occur *in vivo*. The absence of a cytotoxic effect on hBMSCs was observed for all materials in an *in vitro* biocompatibility and osteoconduction evaluation. Both B75D and the B75D-ZrO_2 composite material facilitated cell mediated mineralization to a comparable extent as titanium. In an *in vivo* implant osseointegration study, the BIC of composite stem PCU implants was not significantly different compared to titanium stem implants. A significantly higher bone-to-implant contact (BIC) score was observed in the composite stem PCU implants compared to the polymer stem PCU implants.

Zirconium oxide was selected as a filler material to increase the mechanical stiffness of B75D and facilitate future clinical diagnostic imaging. The use of zirconium oxide as a filler material is attractive since the high density renders the material radiopaque for X-ray imaging, while magnetic resonance imaging (MRI) is not impeded due to its non-magnetic properties. Most importantly, the attractive processing properties of zirconium oxide facilitate compounding with B75D, as a low moisture content is achieved and this limits hydrolytic degradation of the PCU material during melt mixing. The use of zirconium oxide powder as a filler material is gaining relevance in orthopaedic applications, for example as a radiopacifier in bone cements(46,47), thanks to its radiopacity and excellent biocompatibility. Biocompatibility of zirconium oxide was previously confirmed *in vitro* and *in vivo*(48-51).

The current study focuses on the biocompatibility and osteoconductive/osseointegrative capacity of the B75D-ZrO_2 composite material, and the positive initial findings in the current study warrant further evaluation of the physical- and mechanical properties of the B75D-ZrO_2 composite material. In this study the mechanical properties of the B75D-ZrO2 composite material were evaluated in tension only. In future studies, compressive mechanical properties, fatigue-, fracture- and impact resistance, and the effects of aging on the mechanical properties should all be evaluated. The effect of varying the zirconium oxide content on the mechanical properties of the B75D-ZrO_2 composite material should also be investigated. A material with a relatively high zirconium oxide content was pur-

posely manufactured in order to obtain a highest possible elastic modulus nearing bone tissue, while embrittlement related manufacturing issues were avoided. The B75D-ZrO_2 compounding process can perhaps be further optimized, which may necessitate lower amounts of ZrO_2 contents to obtain similar mechanical properties in future batches of composite material. Previous efforts have been made to develop B75D-based composite materials for similar orthopedic implant applications(52), but a detailed comparison to the results reported in that study is outside the current scope since we focus on the biological response to the composite material here.

In order to improve the osteoconductive coating homogeneity on PCU materials, a previously developed dip coating technique was adapted to a spray coating technique(40). Osteoconductive coatings are routinely applied to orthopedic implants intended for uncemented applications to ensure implant osseointegration. The biocompatibility and osteoconductive capacity of B75D and the B75D-ZrO_2 composite material was evaluated with and without BCP-coating to capture the effects of all variations. Standardization of the coating process was first confirmed based on homogeneity of coating volume, density and distribution. None of the biomaterials showed cytotoxic effects *in vitro*, and all proved to facilitate cell mediated mineralization.

The *in vivo* osseointegration capacity was evaluated using BIC scoring. The composite stem PCU implant group exhibited satisfactory osseointegration, with a BIC score of 32.54 +/- 16.68%, which significant higher BIC compared to the polymer stem PCU implant group (13.97 +/- 11.30%) and non-significant difference compared to the titanium stem control (23.62 +/- 14.22%). The difference in BIC score between the composite stem PCU implant group and the polymer stem PCU implant group highlights the important role of mechanics in the osseointegration process, as no significant differences in cell mediated mineralization were observed in an *in vitro* setup. The osseointegration of the titanium stem metal implants was in line with currently available literature evaluating metal based implants in a similar animal model and follow-up, with an average BIC of 23 ± 14% against 15 ± 5% reported previously(53). The relatively high standard deviation in our study can be attributed to the fibrous encapsulation and complete failure to osseointegrate observed for one of the titanium stem implants. Previous examples of fibrocartilaginous tissue encapsulation of metal based implants have been described(25,53). Possible reasons for poor osseintegration are bone tissue heat osteonecrosis induced by drilling or insufficient initial fixation due to drilling errors rendering a decreased interference fit. Similar reasons may have caused poor osseintegration of one implant in the composite stem implant group.

Corundum-blasted titanium disks and implant stems were included as a comparison in the *in vitro* and *in vivo* studies respectively. It was beyond the scope of the current study

to make a comparison to other kinds of osteoconductive titanium surface treatments, although numerous variations of surface treatments to enhance the osseointegrative capacity of titanium implants have been described. The comparison between BCP-coated PCU implants and uncoated titanium stem implants therefore possibly favors the PCU groups.

The novel B75D-ZrO_2 composite material offers an attractive alternative to metal based components in orthopedic implant development, with focal knee resurfacing explored in this study as a first possible application. The compatibility with magnetic resonance imaging (MRI), computed tomography imaging (CT), and radiopacity on standard radiography (X-ray) is an additional advantage of B75D-ZrO_2 composite based PCU implants. Without the incorporation of metal parts, these imaging modalities are not hampered by scattering, allowing for valuable future follow-up of patients treated with an FKRI for an (osteo)chondral defect.

With the ultimate goal of postponing or eliminating the need for joint replacement, an effective FKRI should be durable and be able to preserve the native opposing and adjacent cartilage in the knee and should not negatively impact joint homeostasis. Assessing the quality of the opposing and surrounding cartilage and the presence of wear particles in a long-term study is thus warranted.

5. Conclusion

A biocompatible B75D-ZrO_2 composite approximating the elastic modulus of trabecular bone was developed by compounding Bionate 75D with zirconium oxide. *In vitro* biocompatibility and osteoconduction capability was confirmed using osteogenitically-stimulated hBMSCs. Significantly enhanced osseointegration was observed for the composite stem PCU implants in comparison to analogous polymer stem PCU implants *in vivo*, highlighting the importance of approximating the mechanical properties of trabecular bone for uncemented orthopedic implant applications. The osseointegration of BCP-coated composite stem PCU implants was not significantly different in comparison to analogous titanium stem metal implants. Further characterization of the B75D-ZrO_2 composite mechanical properties and evaluation of the effect of the FKRIs on opposing cartilage quality and joint homeostasis to assess the capacity for joint preservation are warranted.

6. Acknowledgements

The authors would like to thank all personnel of the Large Animal Department of the Maastricht University animal facility (CPV) for their assistance throughout the animal trial.

7. Disclosures

This work was performed under the framework of Chemelot InSciTe. Two of the authors (AR, PE) are shareholders of Avalanche Medical BV, the private entity which owns a license to commercially exploit the herein described implant technology. EA is an employee of Avalanche Medical BV. HO and JT are employees of DSM Biomedical BV.

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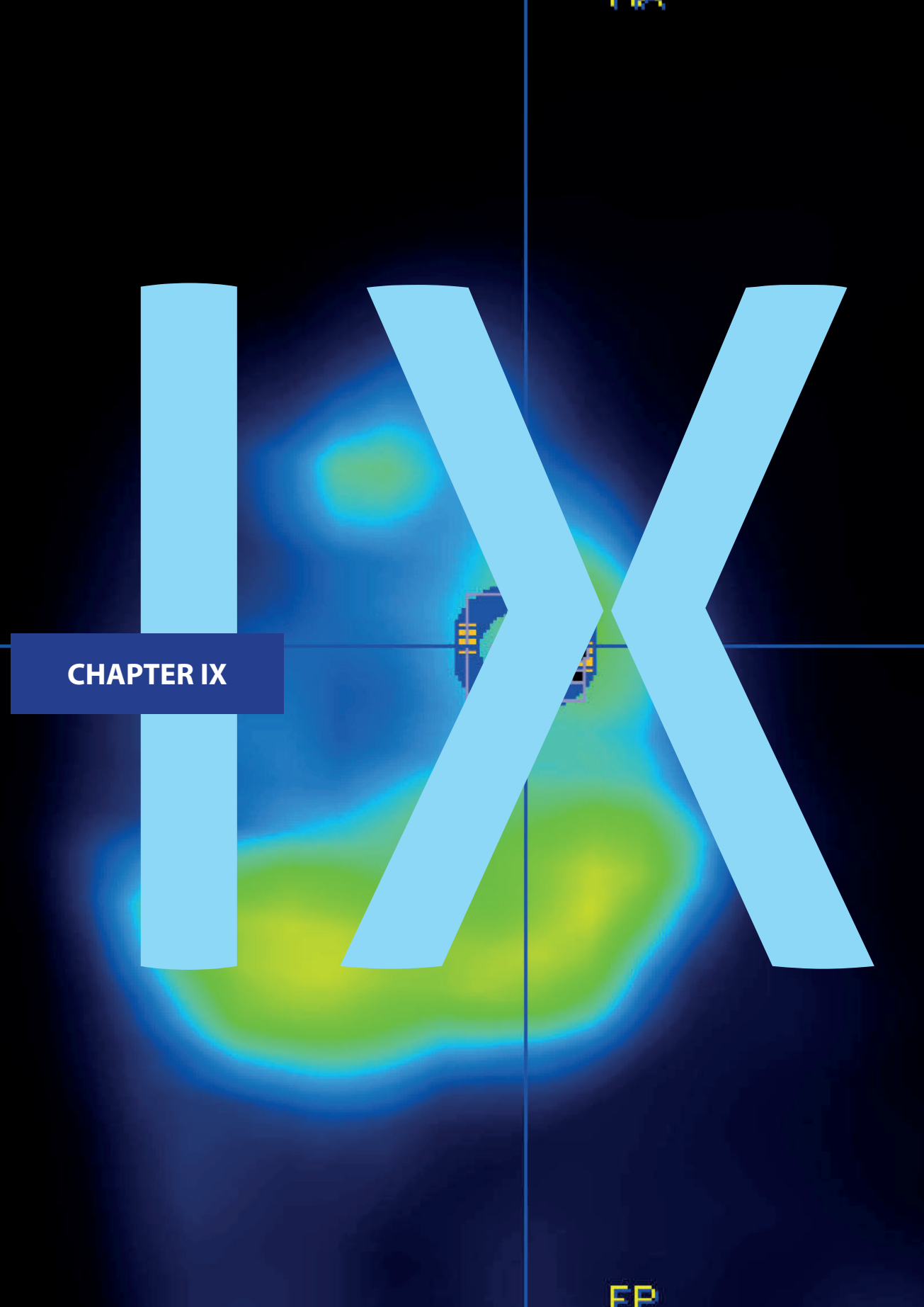

CHAPTER IX

GENERAL DISCUSSION

General Discussion

This thesis contributes to the increasing recognition and acceptance of the importance of joint preservation. The main focus is on addressing the treatment gap that orthopedic surgeons face when treating middle-aged patients with a focal cartilage defect in the knee. These middle-aged patients can benefit substantially from judicious treatment selection and innovative treatments tailored to their needs. This thesis proposes a significant role for focal knee resurfacing implants (FKRIs) within this treatment gap, and it explores their place in the treatment arsenal. The second part of this thesis investigates the primary prerequisites and development of a novel non-degradable polymer FKRI.

Cartilage Repair in Young Patients

Chapter III challenges the belief that loose cartilage fragments are unviable and cannot be re-fixated. At least in children and adolescents. The method of fixation of the shear-off fragments in chapter III was the modified hedgehog technique (1). Chondral fragments without subchondral bone (shear-off fragments) or loose fragments with only a thin bone layer (osteochondritis dissecans (OCD) grade 3-4 type 3) are challenging to re-fixate. It is believed that these fragments lack sufficient bone for osseointegration (2, 3), and bone-to-bone screw fixation is often unstable due to the inadequate bone stock of the fragment. The processing of cartilage, leading to a 'hedgehog appearance' to increase the bottom surface and enhance 'chondro-integration' to the subchondral bone, was first described for cartilage-only allografts by Bardos et al (2, 3). The modifications introduced by our group in chapter III encompass the use of autologous cartilage-only fragments (instead of cartilage-only allografts) and the trimming of the fragment and defect edges in such way that the fragment will interlock with the joint surface. This is possible due to the inherent intra-matrix osmolarity of the cartilage matrix which aids swelling of the cartilage fragment and the edges of the defect. Shaping the swollen fragment increases its initial stability and also removes the less viable chondrocytes, since these are typically located at the edges according to the study by Hembree et al (4). This 'modified hedgehog technique' allows for fixation without metal or resorbable screws, which often necessitates a second removal surgery or results in metabolic waste-induced osteolysis (1). This in turn, demands "chondro-integration", i.e. chondral tissue producing extra-cellular matrix (ECM) adhering to the subchondral bone, and as such, healing of the osteochondral unit capable of transducing mechanical loading in a painless manner. However, misconceptions about the viability of loose cartilage may contribute to their frequent disposal; a study found that 43.5% of shear-off fragments were removed after acute patellar dislocation (5). Our survey in Chapter V found that 84% of Dutch orthopedic surgeons would only refixate chondral shear-off lesions with sufficient bone attachment (i.e.: osteochondral fragments) (6). Concerns about the viability of loose cartilage fragments in the joint may seem irrational since healthy adult cartilage also relies on

metabolic exchange through synovial fluid diffusion (7). It thus seems more rational to assume that these 'floating' cartilage fragments will remain viable in the intra-articular micro-environment. However, these concerns about viability are not entirely unfounded, as studies indicate age-dependent differences in chondrocyte viability favoring younger patients. Hembree et al. observed significant chondrocyte death in chondral fragments from middle-aged patients post-trauma, particularly at the fragment borders, hypothetically due to more direct exposure to pro-inflammatory joint conditions (4). In contrast, Pascual-Garrido et al. found no difference in chondrocyte viability in loose OCD fragments in young patients compared to the viability of healthy cartilage(8). The classification of OCD as a post-traumatic or micro-traumatic disease remains debated,(9) raising questions about the comparability of the cartilage fragments studied by Hembree and Pascual-Garrido. Nonetheless, their findings suggest a more anabolic joint homeostasis in younger individuals. The anabolic potential in youth is supported by *in vitro* analyses from Pestka et al. (2011), who identified age as the sole determinant of elevated chondrogenic markers, such as CD44, collagen type II, and aggrecan, in cells harvested for ACI (10). Additionally, children and adolescents possess more mesenchymal stem cells (MSCs) and better cartilage nutrition, facilitating chondrogenesis through immunomodulatory and trophic factors (11-13). The presence of an open tidemark in adolescents also allows a better penetrable diffusion from the subchondral bone side of nutrition to the articular cartilage, enhancing nutrition compared to the less effective diffusion from synovial fluid after tidemark closure (11). Based on the findings in Chapter III, it can be cautiously concluded that the refixation of loose cartilage fragments is generally successful in children and adolescents. However, due to the limited number of cases and short follow-up period, this study should be viewed as proof-of-concept, demonstrating the high regenerative potential in young patients. Larger studies with extended follow-up and comparisons to established cartilage repair methods are needed to validate these findings and determine if the modified Hedgehog technique is comparable. Its simplicity and low cost suggest it could be a valuable alternative for select patients. High-field MRI or second-look arthroscopy studies with small histological samples could provide detailed information on the 'chondro-integration' of refixed cartilage.

The success and insights from Chapter III hold broader implications. Although the loose fragments often gain size as a result of increased water uptake due to the lack of confinement by the disrupted collagen matrix (1), some fragments might appear much smaller than the defect site due to wear of both the fragment and defect edges. An alternative to placing back the small fragment in such case, is the use of the minced/particulated cartilage technique(14). Using this technique, the loose fragment is minced to a paste-like structure, which is then fixated in the defect site using fibrin glue. The utilization of small autologous cartilage fragments for the treatment of osteochondral lesions was initially demonstrated by Albrecht et al. in 1983, employing an experimental rabbit model(15).

The minced cartilage technique shares some mechanisms with the modified hedgehog technique. Both aim to provide hyaline articular cartilage or its fragments with viable chondrocytes to the injury site for reattachment, i.e. chondrocytes embedded in their ECM. This involves intentionally damaging the extracellular matrix by incising or mincing the fragment to facilitate chondrocyte migration from the ECM. Although not widely accepted and still under research, evidence suggests that chondrocytes can migrate in injured cartilage, similar to their behavior *in vitro(16)*. Chondrocyte migration has been confirmed in human *ex vivo* studies(16-18). Lyman et al (2012) for example showed that chondrocytes migrated to the margin of an *ex vivo* created cartilage defect using human osteochondral explants obtained during knee arthroplasties(17). Also, the use of fibrin glue as adhesive has been shown to be favorable for cell adhesion and proliferation(19). Fueled by the results of the modified hedgehog technique in young patients and growing literature on minced cartilage, our group has gained significant experience with applying the minced cartilage technique. In 2022, our group published the outcomes after cartilage repair using the minced cartilage technique in 18 patients, showing good functional- and patient reported outcomes measures (PROMs) and MRI scores at 1 year postoperatively(14). The average age at surgery was 23 years, i.e. young but skeletally mature. Whether or not these techniques are equally successful in older individuals should be the subject of future research. Furthermore, it is also conceivable that injectables or additives will be introduced to manipulate the joint homeostasis and chondrogenic differentiation in minced cartilage, potentially allowing the use of minced cartilage in repairs up to middle age. The viability of cartilage fragments and the technique used to prepare the graft (whether open or arthroscopic) for the Modified Hedgehog technique and minced cartilage repair remain subjects of future research(20).

Chapter III highlights the high regenerative potential in children and adolescents and also challenges the long-held belief that loose chondral shear-off lesions are unviable and incapable of reattaching to the subchondral bone.

Cartilage Repair in Middle-aged: The Treatment Gap

The review conducted in Chapter IV delved into the influence of advancing age on the efficacy of cartilage repair treatments for focal cartilage defects, comparing clinical outcomes between young and middle-aged patients.

In bone marrow stimulation treatments, transitioning from an abraded defect site with microfracture holes to durable reparative scar tissue that prevents the “pothole effect” is particularly challenging in middle-aged individuals as described before. Additionally, therapies like microfracture can disrupt subchondral bone architecture, leading to subchondral sclerosis and compromised load distribution (21). The potential for bone marrow stimulation techniques to negatively impact subsequent cartilage repair treatments

should make orthopedic surgeons cautious in using them opportunistically in middle-aged patients (22-25). Cell-based techniques such as autologous chondrocyte implantation (ACI) and bone marrow aspirate concentrate (BMAC), may offer better outcomes by providing more potent cells to the defect site (21). However, the evidence for efficacy of these approaches in middle-aged patients remains limited, necessitating further research. Bone-based techniques, such as FKRIs and osteochondral grafts, provide an alternative by implanting loadable osteochondral grafts or implants that rely on osseointegration, a process that resembles the fracture healing responses and remains viable even with advanced age due to the bone's inherent regenerative capacity (21, 26-28). Therefore, such bone-based techniques may be more suitable for middle-aged patients. However, the absence of a well-designed randomized trial (RCT) comparing outcomes of various treatments specifically in middle-aged cohorts persists to date. Conducting a level-I study in this field presents challenges due to the extensive variability in knee cartilage lesion size and location, concomitant injuries in the affected knee, the many available treatments and commercial product variations, varying reimbursement regulations and the individualized nature of patients. One potential solution to bypass the labor- and cost-intensive demands of RCTs is leveraging real-world data. In other words, establishing registries where all joint preservation therapies and patient characteristics are meticulously recorded. Building such a comprehensive registry could provide invaluable insights into individualized care and offer a pathway towards elucidating optimal treatment strategies.

The perspectives of orthopedic surgeons in survey studies underscore the knowledge- and treatment-gap for middle-aged patients. Li et al. (2013) conducted a survey among 173 Canadian orthopedic surgeons regarding the treatment of middle-aged patients with early osteoarthritis (OA). This survey revealed that the majority (84%) of respondents acknowledged the necessity for non-arthroplasty interventions for patients under 60 years-old, to prevent or delay initial arthroplasty. Furthermore, a significant proportion (68%) of respondents identified a 'treatment gap' for middle-aged patients. In Chapter V, we conducted a similar survey (2023) among 115 Dutch orthopedic surgeons specialized in knee surgery, with the objective of exploring utilization of knee cartilage repair techniques with a special emphasis on age-related considerations. The majority of orthopedic surgeons reported to perform cartilage repair procedures in the age group of 18 to 40 years. Only 21% of respondents reported performing cartilage repair in patients aged 40-60 years. Apparently, indications for cartilage repair significantly diminish after the age of 40 according to the survey response. This is in line with a similar European survey amongst German speaking orthopedic surgeons, in which one third upheld an upper patient age limit of 50 years (29). The imposition of this age limit may reflect existing guidelines or the lack of evidence specifically for middle-aged patients, influenced by the prevalent practice of implementing strict upper age-based inclusion criteria in cartilage repair studies (30). Nevertheless, the discrepancy between the preference for

non-arthroplasty treatments for individuals below the age of 60 and the diminishing utilization of regenerative therapies in patients over 40 defines what is known as the "middle-aged treatment gap."

There also appears to be a lack of knowledge underlying this treatment gap. In our survey (Chapter V), orthopedic surgeons indicated to not believe that advancing age adversely affects any cartilage repair treatments. Remarkably, one-third of the German-speaking respondents also did not see age as a limiting factor for cartilage repair surgery (29). More than 60% of the Dutch respondents also indicated that if they treat patients over 40 years of age, they would use microfracture. This is line with recent retrospective database number from the United States(31). Moreover, 41% indicated commonly using microfracture in ICRS grade 3-4 defects of 2-3 cm^2, despite long-standing evidence contradicting these practices (32, 33). Similarly, findings from German-speaking orthopedic surgeons indicated that microfracture was utilized across a broad spectrum of patient ages, including individuals up to 70 years old (29). Consequently, it is plausible that when middle-aged patients undergo joint preservation surgery, they may receive inappropriate treatment due to a lack of knowledge amongst orthopedic surgeons. A plausible rationale for the results of our survey (Chapter V) could be attributed to the recent centralization of cartilage care in the Netherlands: complex cases are now recommended for referral to specialized tertiary centers where advanced techniques like ACI and combined surgeries are undertaken. Nonetheless, a prerequisite for effective care centralization should be clarity among general orthopedic surgeons regarding the guidelines for cartilage repair options available outside of the expert centers. This is particularly important since the most readily available, technically easy treatment is microfracture. Especially in this regard, there appears to be a particular benefit to be attained from improved education.

The primary limitation of the survey study in Chapter V is its reliance on subjective responses, as orthopedic surgeons were asked what they would do rather than providing objective data on their actual practices. In the Netherlands, cartilage repair procedures are not separately registered, highlighting – once again – a key area for future improvement: encouraging knee surgeons to participate in registries as highlighted previously.

The Development of a Polymer Focal Knee Resurfacing Implant to Fill the Treatment Gap

The systematic review and survey presented in this thesis (Chapters IV and V) highlight the need for improved cartilage repair treatments for the middle-aged patient group(6, 21). In Chapter VI, we reviewed the literature on biomaterial options to potentially enhance cartilage repair in this age group(26). Chapter VI summarizes that technical advancements have led to several methods for improving cartilage repair, including the use of natural or synthetic, degradable or non-degradable polymers. Degradable polymers

are often employed to tissue engineered constructs that mimic the structure and biomechanical properties of the native tissue(26). These constructs are frequently 3D printed, either with embedded cells and bioactive molecules or morphologically designed to attract these molecules in vivo(26). Theoretically, when using the right cells and providing the appropriate environment, the cells should proliferate and differentiate into the intended phenotype (26). Ultimately, those cells should produce the extracellular matrix that replaces the function of the degradable scaffold and functions as the desired tissue.

Unfortunately, tissue engineering cartilage constructs faces several major challenges. Many promising constructs investigated in preclinical research fail to reach clinical application due to the use of novel polymers, which require extensive characterization and render the regulatory approval process for human use even more complex and costly (34). Other "bench-to-bedside" challenges include the significant financial investment, time, and resources needed, which necessitate a well-developed business plan and clear future outlook (35). This could be the reason why Chapter VI found many different polymers in the preclinical setting but a monotonous selection in clinical research (36). Additionally, tissue engineering research is often fragmented (34), typically focusing for instance on specific variables. For instance, some preclinical studies use Wakitani histopathological grading system(37) and some do cell counting as outcome measure,(38) making comparison difficult and hard to extrapolate to the clinic. Moreover, the success of tissue engineering in cartilage repair depends on the timely development of tissue with appropriate mechanical properties. While construct properties are important, the host's response, heavily influenced by factors such as age, plays a crucial role in determining tissue quality(26). As discussed in Chapter IV, cell-based or tissue-engineering treatments for cartilage repair may therefore be less effective and reliable as age increases, whereas a reliable cartilage repair treatment should ideally yield predictable outcomes regardless of age. Despite decades of research, tissue engineering has yet to achieve consistent and durable cartilage repair with true hyaline properties, for which current evidence is even limited in ideal patients or laboratory animals (26).

Permanent implants composed of non-degradable biomaterials could offer a durable solution for cartilage repair in middle-aged patients. Permanent implants do not rely on aligning the timing of biomaterial breakdown and repair tissue maturation, making them potentially more predictable and less dependent on the host's regenerative capacity. Permanent implants rely on osseointegration rather than regeneration(21). In Chapter VI, we found that established orthopedic metals such as titanium and cobalt-chromium are primarily used for FKRIs(26). There are also hybrid constructs using polymers and metals: an implant with a titanium base and an ultra-high-molecular-weight polyethylene and hyaluronic acid articulating surface has been developed(39).

Although there is considerable evidence supporting the success of metal FKRIs (40, 41), concerns remain regarding the disparity in elastic modulus relative to native joint tissue such as articular cartilage and the meniscus (42-45). This holds true for both the metal and hybrid examples. Stiff biomaterials will lead to high contact pressures in opposing tissues(43-45), and may lead to stress-shielding bone remodeling and ultimately implant loosening(46). The varying success (up to 49.5%% conversion rate to arthroplasty within 5 years in the registries of Australia and Denmark(47, 48)) of first generation metal FKRIs (HemiCAP®, Arthrosurface, Anika Therapeutics Inc., Bedford, MA, USA) might be explained by mechanical mismatches that do not allow for standardized geometries. Poor implant surface congruency with adjacent cartilage, as a result of either mismatching curvature or poor implant placement, has been shown to lead to extensive opposing cartilage damage in large animal models(43). Other reasons for failure could be the wrong indication, surgeon's learning curve, etc. Additionally, since FKRIs are used as a joint preservation treatment, patients may require future imaging, such as MRI. Metal FKRIs however, impede detailed views of knee anatomy, compromising future clinical decision-making.

A second-generation metal FKRI (Episurf®, Stockholm, Sweden) utilizes personalized, custom-made curvatures based on MRI scans of the defect site, thereby eliminating the potential harmful effects of a protruding implant(41). Although these procedures are costly and logistically complex, the short- and mid-term results of personalized FKRIs are very promising(40, 41). However, next to the time and costs required for these personalized implants, another significant concern remains: the mismatch in mechanical properties between the implant and the adjacent and opposing cartilage. For example, the Young's modulus of cartilage is approximately 1 MPa (49), whereas that of cobalt chromium (CoCr; the top layer material of a metal FKRI) is about 230 GPa(50), making it 230,000 times stiffer than articular cartilage.

Hence, the development of an all-polymer non-degradable focal knee resurfacing implant could offer three important advantages over (partially) metal FKRIs. First, polymers can be selected to match the mechanical properties of joint tissues more closely. By reducing the disparity in elastic modulus relative to native joint tissues substantially, the impact of implant protrusion may be reduced substantially. Polymers exhibit creep deformation and may therefore conform to the native joint morphology under loading. These factors potentially make off-the-shelf curvatures sufficient and eliminating the need for extensive preoperative planning. Besides that, being more 'forgiving' also reducing the impact of a learning curve. Second, the disparity also accounts for the bony side of the implant: by creating bone mimicking mechanical properties the potential stress-shielding related problem is also addressed. Third, they allow for follow-up using MRI scans, enhancing postoperative monitoring and assessment.

In terms of non-degradable, compliant, and biocompatible polymers, polycarbonate-urethane (PCU) is an outstanding candidate (51). Considerable research has focused on the intra-articular use of PCU, particularly in the context of total meniscal replacements(52). PCU is available in various Shore hardnesses, with Bionate® II 80A and 75D possessing mechanical properties closely resembling articular cartilage and trabecular bone respectively (51). For example, Bionate® II 80A has a Young's (elastic) modulus of 11 MPa, making it only 11 times stiffer than native cartilage(53) (compared to 230,000 times for cobalt-chromium(50)). Bionate® II 80A also has a very low coefficient of friction (COF) (another FKRI prerequisite from chapter VI), particularly when applied in synovial fluid (51, 54, 55). This COF is lower than CoCr against cartilage(56). Therefore, the use of Bionate® PCU appears to be a logical step forward in terms of cartilage substitute. However, as described in chapter VI, the first prerequisite for a successful FKRI is durable fixation. This means that PCU should osseointegrate in the subchondral bone.

Proof of concept study; Animal Trial I (chapter VII)

The last part of this thesis explored the feasibility of an all-polymer FKRI composed of PCU. As stated in chapter I, this was done under the framework of the public-private collaboration Chemelot Institute for Science and Technology (InSciTe) Biomedical. InSciTe was specifically initiated to enhance the translational aspect of product development in medicine.

While there is extensive literature on the osseointegration of conventional orthopedic metals such as stainless steel and titanium(57), research on the osseointegration of polymers is relatively sparse. Insights from both orthopedic and dental literature suggest that the osseointegration of implants is influenced by factors such as initial stability, macroscopic and microscopic surface roughness, porosity, coatings, and wettability (57). From work on other polymers such as polyethylene(58) and polyetheretherketone(59, 60) it has become clear that polymers often require surface modifications to sufficiently osseointegrate. Chapter VII explores the osseointegration of Bionate® PCU. Due to the lack of prior efforts on achieving osseointegration of PCU, a 'proof of concept study' was conducted to evaluate if PCU is able to osseointegrate or determine if and what type of surface modification is needed to achieve osseointegration. Bionate® 75D (B75D) is the highest hardness grade of commercially available medical-grade PCU and was used as it most closely approximates the elastic modulus of trabecular bone. It has a Young's modulus of approximately 200 MPa under physiological conditions, which falls within the range of the elastic modulus of trabecular bone (150-700 MPa)(46). Nine different surface modifications were tested *in vitro*, involving three degrees of surface texturization and three types of coating, applied to discs specially designed for evaluation of osseoconduction capacity using *in vitro* cell culture experiments.

The surface modification that showed the best performance in the *in vitro* study was the biphasic calcium phosphate (BCP) coating, applied with eight dips and combined with medium surface roughness (Ra 8.4 µm). This modification was subsequently applied to the stem of a bilayered cylindrical FKRI (the top was composed of a Bionate® II 80A double curvature articulating layer) in a caprine model, and tested against an uncoated control and a titanium control. After 12 weeks, there was a significantly larger histologically assessed bone-to-implant contact (BIC) score for the coated B75D (10.27 ± 4.50%, mean ± SD) compared to the uncoated B75D (4.50% ± 2.61, mean ± SD), with no statistically significant difference between the coated B75D and the titanium control (12.81 ± 7.55%, mean ± SD). As a proof-of-concept study, Chapter VII provides the first evidence of successful osseointegration of a PCU FKRI. The most important finding was that PCU required surface modification to support a level of osseointegration comparable to uncoated titanium.

Next, several important secondary findings emerged. First, a large number of implants exhibited osteolysis and fibrous encapsulation, particularly in the uncoated PCU implant group. Second, the fibrous encapsulation resulted in large standard deviations in histologically assessed osseointegration, i.e. BIC. Third, the obtained BIC values after 12 weeks were relatively low compared to other studies, particularly when considering metal implants. FRKI preclinical studies showed a range of 14.6 – 92.3 % of BIC for different time-points, biomaterials and animal models(43, 44, 61, 62).

We hypothesized that the following occurred in our caprine study of Chapter VII, explaining our relatively low BIC values: the initial prerequisite for osseointegration, mechanical fixation, was insufficiently met. Mechanical instability is known to result in fibrous tissue interface formation, not osseointegration(63). This lack of osseointegration could also have left voids, allowing synovial fluid to penetrate alongside the implant, resulting in increased osteolysis(64, 65). Also, drilling errors could have led to oval prepared implant recipient holes, potentially leaving voids adjacent to the implant. Substantial research has been conducted on the critical level of micromotion, predominantly focusing on titanium(63). However, only two studies have assessed a polymer (PMMA), and none have utilized FKRIs as a model(63). Consequently, the applicability of titanium micromotion levels to PCU FKRI remains uncertain.

The oversizing of the implant in chapter VII relative to the drilled hole was based on a manual pre-test (not published), without quantification of implant push-out force and this interference fit was potentially too low. Second, although the Young's modulus of Bionate® 75D is within the normal range of trabecular bone, it is at the lower border. It is well known that bone becomes denser towards the cortex and joint line, and literature reports that Young's modulus of the subchondral bone plate is approximately 1150

MPa(66). For an FKRI with a stem reaching from the dense subchondral bone plate towards the more porous trabecular bone, the elastic modulus should ideally approximate the properties of the tissue it replaces at a macroscopic level (apparent modulus). It has been shown that there is a strong correlation between an implant's Young's modulus and its osseointegration(67, 68). The higher BIC values and reduced fibrous tissue interface in the coated PCU group compared to the uncoated group suggest that the coating facilitated faster osteoconduction, stabilizing the implant before animals increased ambulation and accompanying micromotion at the bone-implant interface also increased.

Currently, there is no established minimum BIC value for ensuring durable fixation, but it is evident that a higher BIC value (such as 90%) is more desirable. Ex vivo human retrieval studies have confirmed such correlation between mechanical stability and BIC values exists (69). Previous studies using CoCr, oxidized zirconium, or hydroxyapatite-coated titanium FKRIs reported BIC values ranging from 36% to 92%(62, 70). Thus, the data presented in chapter VII support the use of a non-degradable polymeric FKRI by showing no difference between the non-degradable polymeric FKRI and the titanium FKRI in terms of osseointegration. However, chapter VII also identified several important areas for improvement, to increase the osseointegration capacity of a PCU FKRI. Since Chapter VII was a proof-of-concept study, future research should focus on optimizing and industrializing the manufacturing process of the composite material and implant injection molding process.

The role of PET-CT scan in in vivo assessment of Osseointegration

Histology remains the gold standard for assessing osseointegration in the absence of advanced non-invasive measurement tools(71). When the research involves understanding the chronology of osseointegration, multiple histological evaluations at various time points are required, necessitating the sacrifice of several animals for each time point. A key secondary aim of the proof-of-concept study in Chapter VII was to determine whether positron emission tomography computed tomography (PET-CT) imaging, using ^{18}F-sodium fluoride, could accurately predict histological outcomes of osseointegration. Since this approach supports longitudinal assessment in the same laboratory animal, it could significantly reduce the number of animals required, along with associated costs and environmental impact.

Chapter VII demonstrated a PET-CT trend in implants with successful osseointegration. Both the titanium and coated PCU implant groups exhibited a significant decrease in ^{18}F-sodium fluoride tracer uptake from 3 to 12 weeks post-implantation. This reduction suggests a rapid increase followed by a 'cooling off' of bone metabolism once the implant was mechanically stabilized and histologically integrated through adjacent bone deposition. In contrast, uncoated PCU implants did not show this decline in tracer uptake,

indicating ongoing bone remodeling. However, this pattern of early increase and rapid decrease did not achieve sufficient statistical significance when correlated with the ultimate histological osseointegration at 12 weeks.

Recent studies have observed similar patterns of tracer uptake varying with different biomaterials. For example, research in mice with magnesium and titanium implants identified earlier peaks and declines in standard uptake value (SUV) uptake across multiple time points (1, 3, 7, 14, 28, and 45 days)(72). Clinical studies in humans, investigating the osseointegration of spinal cages, reported much slower decreases over nearly two years. These findings suggest that ^{18}F-sodium fluoride PET-CT patterns are likely species-specific and dependent on both the biomaterial and the implantation site(73). Consequently, the routine use of PET-CT scans for osseointegration assessment is limited by low specificity, labor intensity, high costs, and radiation exposure

Improving PCU FKRI Osseointegration; Animal Trial II (Chapter VIII)

The purpose of Chapter VIII was to explore options to enhance the osseointegration of the PCU FKRI, measured as BIC, by refining the biomaterial properties and the surgical technique.

To increase the stiffness of the implant stem layer, a composite material was developed. The composite was manufactured from Bionate® 75D and zirconium oxide, Bionate® 75D/zirconium oxide (40/60 wt%) composite: B75D-ZrO2. The ceramic zirconium oxide was chosen for several reasons: it has a high hardness, it is radiopaque yet non-magnetic allowing good x-ray and MRI visibility, it is biocompatible and potentially shows favorable compounding properties, causing limited hydrolytic degradation to the PCU substrate (46, 74, 75).

The new biomaterial, B75D-ZrO2, underwent in vitro testing to assess its mechanical properties, biocompatibility and osteoconduction. These tests confirmed the biocompatibility and osteoconductive properties of the B75D-ZrO2 composite and showed that it had a substantially higher elastic modulus than B75D used in chapter VII (480 MPa vs. 206 MPa) under physiological conditions. Subsequently, a six-month caprine study was conducted to evaluate in vivo osseointegration characteristics.

The surgical technique was also refined by increasing the interference fit and incorporating the use of a dilator to prepare the recipient site. In Chapter VII, the interference fit was 3.5% under sizing of the implant's cross-sectional area. Based on unpublished laboratory testing, this was increased to 9.8%. Additionally, the drill used for the recipient hole was made smaller, with the final widening performed using a metal dilator (impactor rod) creating clear and impacted bone at the borders of the hole. This adjustment resulted

in a more consistently round recipient hole and, theoretically, a higher bone density surrounding the implant. However, the optimal under sizing of the implant is difficult to determine. The literature on this topic is sparse and the optimal under sizing also depends on the bone density and location of the implant(76). For FKRI application, particularly in polymer FKRIs, this should be topic of future research.

The composite stem B75D-ZrO2 implant group demonstrated satisfactory osseointegration, with a histologically assessed BIC value of 32.54 ± 16.68%. This was significantly higher than the B75D implant group with the same surface modification (13.97 ± 11.30%) and not significantly different from the titanium stem control (23.62 ± 14.22%).

There are several FKRI studies in literature to compare with. Custers et al. found BIC values of 47.5% and 59.6% for oxidized zirconium (OxZr) and cobalt-chromium (CoCr) implants, respectively, after 4 weeks in rabbits(61). In a similar 4-week rabbit study, Custer et al. found BIC values of 63.2% and 62.5% for uncoated OxZr and CoCr implants, respectively(45). The same authors found BIC values of 39.5% and 42.3% for OxZr and CoCr respectively (44), this time in a 1-year caprine study, more comparable to our animal model in chapter VIII. Hence, our results seem comparable to those by Custers et al. In another caprine study by the same authors a BIC value of 14.6% was reported for an OxZr implant after 6 months(43). Martinez-Carranza from the Swedish Episurf group obtained BIC values as high as 90.6% and 92.3% after 6 and 12 months respectively for a hydroxyapatite coated titanium FKRI in sheep(62). BIC values are influenced by numerous factors, including implant location, loading pattern, animal model, and follow-up duration. Long-term follow-up studies, ideally incorporating radiostereometric and pull-out / push-out analyses, are necessary to establish the minimal BIC value required for effective FKRI application. Such long-term follow-up studies should be conducted only after industrialization of production process on a device substantially equivalent to the devices envisioned for first-in-human clinical trials.

Based on the results from Chapter VII and the subsequent enhancements in Chapter VIII, it can be concluded that the first prerequisite for a successful novel FKRI biomaterial, namely osseointegration, has been achieved for PCU. However, there is room for improvement to ensure more predictable results with smaller standard deviations, through advancements in implant design, implant manufacturing, and the surgical technique. The use of automatically water-cooled drills could avoid potential heat necrosis. Additionally, optimizing the tools to standardize the implantation depth can improve and standardize the loading pattern. While simple and pragmatic implant geometries were employed in Chapters VII and VIII, the B75D-ZrO2 composite material should be extensively tested to help determine the optimal and safest geometries under physiological loading. Fortunately, many

of these improvements can be explored in preclinical settings using techniques such as finite element analysis.

The effects of Bionate® II 80A articulation on the opposing cartilage, the second prerequisite of a successful FKRI, are beyond the scope of this thesis. However, preliminary results appear promising and the degree to which PCU preserves opposing cartilage surpasses CoCr. Future research will determine if a PCU-based FKRI can fulfill all the criteria for a successful FKRI.

Advancing the Field of Knee Joint Preservation

Based on the studies presented in this thesis, there are several important opportunities on national and international level to improve joint preservation care in the future:

Improving diagnostics

Many middle-aged patients present with no history of trauma, apparently normal macroscopic leg alignment, and relatively normal plain knee radiographs. Only upon MRI examination do focal cartilage defects, degenerative meniscal tears, and other abnormalities become evident. Additionally, subtle malalignments, such as abnormalities in the medial proximal tibial angle (MPTA) or lateral distal femoral angle (LDFA), may only be discernible on dedicated long-axis radiographs. Innovations in diagnostic modalities should focus on cost-efficiently detecting focal cartilage defects in an early stage and alignment abnormalities to enhance individual patient care, decrease health care costs and facilitate large-scale data collection. For instance, the utilization of low-field MRIs(77) and low-dose slit-beam digital radiography systems for lower extremities could be explored(78). Such modalities should easily reveal whether a knee joint is in an early osteoarthritic phase and if extra-articular therapies such as a high tibial osteotomy can be of value. However, their application should be considered only after foundational principles of knee joint preservation, such as maintaining a healthy weight and an optimal movement regimen, have been addressed.

MRI and radiographs are commonly regarded as "dry biomarkers." In contrast, "wet biomarkers" are any biological substances that can function as a potential index of a disease process, measurable in body fluids, such as urine, blood, serum, plasma, or synovial fluid (79). A biomarker set or profile indicative of joint homeostasis would be invaluable. Such biomarkers could differentiate between biological and chronological age and potentially establish a threshold for the utilization of certain therapies. The integration of wet and dry biomarkers would furnish extensive data, capable of predicting specific patterns in joint degradation and determining the appropriate therapeutic interventions at various disease stages of degenerative joint disease.

Advancements in orthobiologics.

Orthobiologics refer to a class of medical substances or treatments derived from biological sources, such as cells, tissues, proteins, or growth factors, which are utilized in orthopedic procedures to enhance healing, repair, or regeneration of musculoskeletal tissues(80). Corticosteroids, hyaluronic acid, and platelet-rich plasma are among the most commonly utilized injectables for early osteoarthritis management (80). Numerous preclinical and fundamental studies are underway investigating novel injectables and injectable systems aimed at modulating the cellular degenerative cascade (81). Orthobiologics may be used in conjunction with surgical interventions or as standalone therapies.

Improving regenerative treatments

The potential impact of aging on the efficacy of bone marrow stimulation techniques is considerable, as shown from the findings of Chapter IV. The therapeutic window for employing microfracture appears to be narrow. Some orthopedic surgeons already suggested the abandonment of microfracture almost a decade ago (82). Despite its inconsistent results in middle-aged, the technical simplicity and single-stage nature of microfracture without the need for commercial products other than simple surgical tools continues to hold appeal. This simplicity likely contributes to its widespread adoption and broad therapeutic window indicated by many orthopedic surgeons, as shown in Chapter V. Attempts to enhance the efficacy of bone marrow stimulation procedures are underway, with researchers focusing on augmenting the healing response following bone marrow stimulation, as elaborated upon in Chapter VI. Various tissue engineering scaffolds have been developed to promote an improved cellular response, with some designed to mimic the native osteochondral architecture through layered configurations and others incorporating bioactive molecules in multilayered spatiotemporal orientations (26).

It is conceivable that in the future, a synergistic approach involving the combination of orthobiologics targeting joint homeostasis and the utilization of multilayered biomimetic scaffolds could enhance a cartilage healing response sufficiently to render bone marrow stimulation suitable as single stage surgery for middle-aged patients.

Shifting the therapeutic paradigm: from cartilage to bone

Approximately 40 % of cartilage defects have a non-traumatic origin and are thus the result of chronic subcritical overload(83). It has been well established that knee malalignment poses a significant risk for the onset and progression of osteoarthritis, particularly in cases of varus knee alignment among obese individuals(84). Malalignment can be the result of degeneration itself (uni-compartmental thinning of cartilage/meniscus), be acquired from abnormal loading patterns during growth, have a genetic or (micro-) traumatic) origin, or can be multi-factorial. However, it seems that malalignment of the proximal tibia (MPTA) leads to the fastest progression of joint degeneration (84, 85). Con-

ceptually, such degeneration 'must' start with a focal cartilage defect or cartilage thinning, the pre-osteoarthritic stage according to Ryd et al. (86). Indeed, in the German Cartilage Registry an association was found between varus angle and the prevalence of cartilage defects on the medial femoral condyle(87). As mentioned in chapter II, a cartilage defect in this pre-osteoarthritic stage can be symptomatic or asymptomatic. A comprehensive patient work-up may reveal both the presence of a cartilage defect in the medial femoral condyle and concurrent extra-articular deformity. Not only the cartilage defect should be repaired in such case, but the bony deformity should be addressed as well. There is no scientific evidence for when the deformity is large enough to justify a high tibial osteotomy, but the generally accepted threshold is 5 degrees of varus in the mechanical hip knee angle(88). This was also indicated by the respondents of our survey (chapter V). The effect of malalignment correction is two-fold: it aims to address the underlying causative factor (mechanical overload in one compartment) in certain cases of atraumatic cartilage defects on the medial femoral condyle, and secondly, it aims to create a more favorable mechanical environment for the healing and maturation of repair tissue. Faber et al. (2021) conducted the only matched-pair analysis where patients with a medial femoral condyle cartilage defect and a ~5 degrees varus angle undergoing either cartilage repair alone were compared to patients who underwent cartilage repair combined with a concomitant high tibial osteotomy. The average age was 42 years in both groups and the follow-up was 3 years. Patient reported outcome measures and satisfaction rates were significantly better during the 3-year follow-up period in the combination treatment group (88). The question remains if the combined therapy group will have longer arthroplasty-free survival in the longer-term follow-up. The study highlights the importance of a holistic diagnostic work-up (including at least lower leg alignment assessment next to detailed knee evaluation) and the collaboration between orthopedic surgeons that are able to perform intra-articular cartilage repair and extra-articular re-alignment procedures. What the optimum threshold is to perform realignment procedures should be part of future research.

Our survey (Chapter V) showed that 57% of the respondents would perform a leg axis correction when indicated. The study conducted by Faber et al. advocate for an even more liberal approach to leg axis corrections concomitant to cartilage repair procedures (88). Enhancing educational initiatives regarding joint preservation is imperative to ensure orthopedic surgeons are aware of the latest research findings and are adept at integrating such insights into their clinical practices

As mentioned in the preceding section (and expounded upon in Chapter IV), the efficacy of bone-based cartilage repair procedures appears to be unaffected by advancing age. Approximately 45% of participants in the survey analysis (detailed in Chapter V) recognized an indication for osteochondral auto- and allografts in patients aged 40-50 years,

while none advocated for such therapies in patients aged 50-60 years. However, the practical application of these preferences appears limited, with only 27% of respondents reporting actual utilization of autografts and a mere 3% employing allografts. Remarkably, only one respondent out of the 117 surveyed indicated access to FKRIs. Notably, both allografts and FKRI face availability constraints within the Netherlands. Allograft availability is impeded by regulatory hurdles, while FKRI systems remain predominantly utilized in small-scale studies. This is also driven by the lack of reimbursement at the moment.

Enhancing data collection.

The recruitment of a homogeneous patient population composed of young, healthy subjects without concurrent injuries in cartilage repair studies often presents challenges in extrapolating findings to real-world clinical scenarios (30). To optimize treatment decision-making for individual patients, it is imperative to establish comprehensive databases containing detailed patient characteristics, including age, sex, medical history (including prior surgeries), level of physical fitness, etc. Such database could form the basis for the development of a predictive model for cartilage repair outcome.

While German-speaking countries have initiated efforts such as the 'Knorpel Register' to collect data on cartilage repairs(87-90), the Netherlands currently lacks a dedicated registry for various cartilage defect treatments. However, relying solely on national cartilage repair registries may not yield sufficiently high enrollment numbers or provide a comprehensive overview of all joint preservation strategies. Ideally, a standardized international joint preservation registry encompassing all non-arthroplasty surgical interventions for the knee, supplemented by detailed patient characteristics, should be established. Such registries could incorporate both wet and dry biomarkers, enabling the development of a comprehensive "digital twin"(91) for each patient. Such a database with predictive value is, of course, a vision for the future, where key parameters must be selected, statistical models tested, the model trained and validated, and ultimately clinically evaluated(92). The International Cartilage Regeneration & Joint Preservation Society (ICRS) has taken commendable steps towards implementing a joint preservation registry at the international level, reflecting a concerted effort to advance orthopedic research and clinical practice

Education

Many patients with pre- or early-stage osteoarthritis are managed conservatively by their general practitioners for years and only seek orthopedic consultation when significant osteoarthritis symptoms and radiographic osteoarthritis are present. This approach is effective for most patients and therefore also most cost-effective for these patients. However, it is crucial that general practitioners provide accurate conservative treatments for

patients, while ensuring that the small percentage of patients with surgically modifiable risk factors are referred to an orthopedic surgeon in a timely manner. Implementing a structured care pathway could be valuable, such as regular visits by orthopedic surgeons to general practitioners to see a pre-selected group of patients. Radiologists could also contribute by identifying signs of dysplasia of the proximal tibia or distal femur or early joint space narrowing. Regular educational updates for general practitioners could further ensure appropriate referrals and awareness of specialized care centers. Among orthopedic surgeons, education on the latest guidelines is essential, particularly emphasizing that the opportunistic use of microfracture in middle-aged patients—still practiced in 2024(31)—is obsolete and associated with negative consequences(21).

Collaboration among pediatric, sports medicine, joint-preserving, and arthroplasty orthopedic surgeons, including those specializing in revisions, is essential to maintaining mobility in an aging population. Safeguarding an optimal patient journey from the perspective of orthopedic care providers remains paramount.

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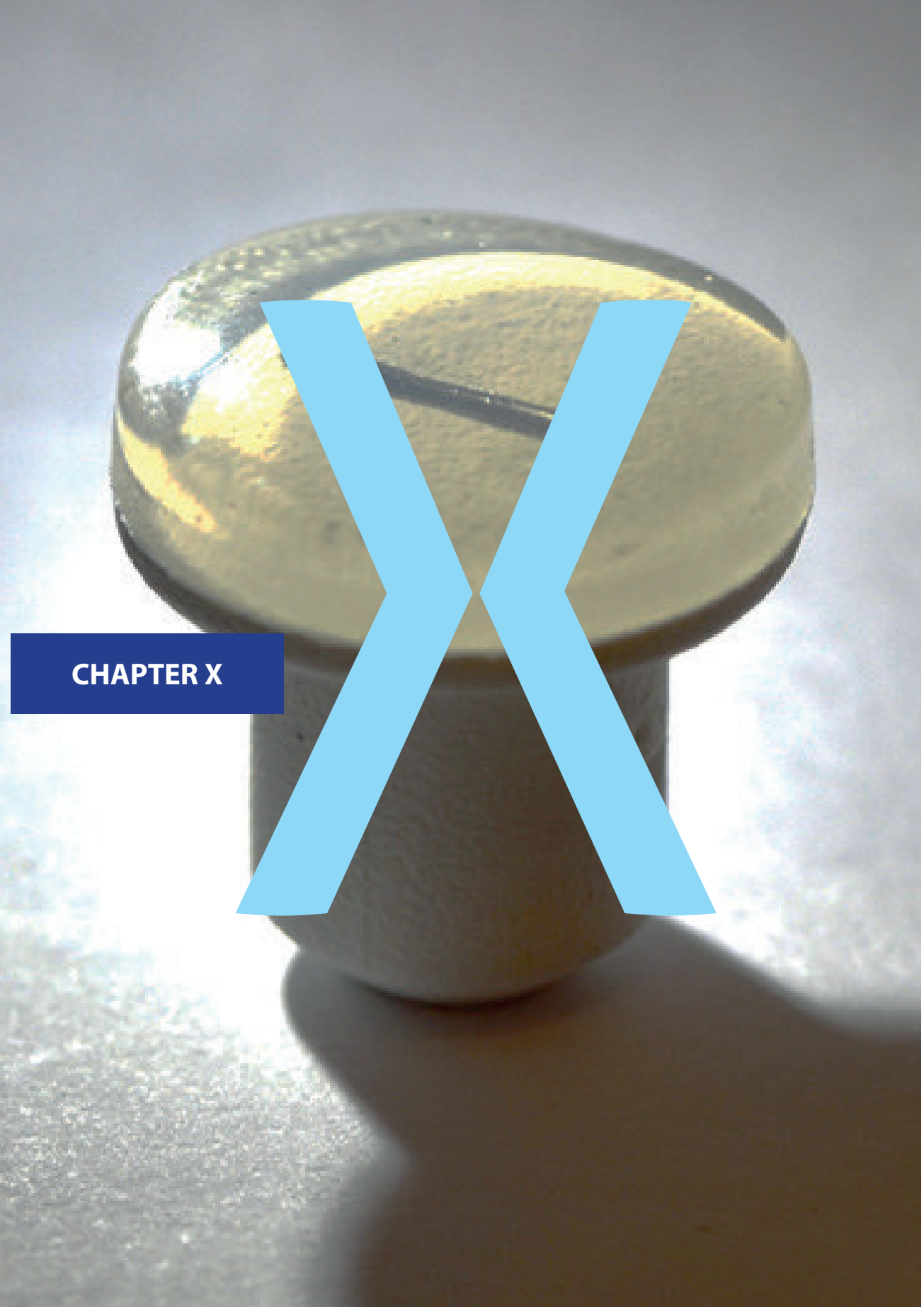
CHAPTER X

IMPACT

Impact

Cartilage Defect Repair and its Downstream Effects on the Burden of Knee Osteoarthritis

Symptomatic focal cartilage defects significantly impact patients' quality of life(1). Research has demonstrated that individuals with cartilage defects experience reductions in quality of life comparable to those of patients with osteoarthritis (OA) awaiting total knee arthroplasty (TKA) (1). The natural history of focal cartilage defects remains incompletely understood and is influenced by various factors, including patient age and defect size (2). Young patients with a cartilage defect may exhibit none to minimal progression toward OA (3). Conversely, a growing body of evidence indicates that middle-aged patients with large focal cartilage defects are more likely to progress to OA, with these defects serving as a significant risk factor for requiring TKA(1, 4). The importance of preserving mobility throughout the patient journey and appropriately treating (osteo)chondral defects with proper concomitant surgery is increasingly recognized, as supported by registries(5-7).

OA is an increasingly prevalent condition, currently afflicting 1.5 million people in the Netherlands(8). Without adequate intervention, this number is anticipated to rise to 3 million by 2050(9), affecting 4 out of 10 people and becoming the most common chronic disease (10). Many patients are in the midst of their working years, forced to endure chronic pain that leads to substantial work absences, with an average of 186 days annually in severe cases(10). Knee OA, therefore imposes a significant economic burden, costing Dutch society €1.2 billion annually (11). Knee OA also has a significant effect on other chronic diseases. Significantly more depression, cardiovascular disease, diabetes, back pain and osteoporosis were found in patients with knee OA(12). Knee OA increasingly affects the relatively young population, particularly the middle-aged population. The surge in knee OA among the middle-aged can be attributed to several factors. Demographic changes, including the growth of the middle-aged population, are a significant contributor(13). The obesity epidemic, which is particularly pronounced among younger individuals, exacerbates the problem by increasing chronic mechanical stress and low-grade adipogenic inflammation across the body(14-16). Furthermore, the modern middle-aged patient is not merely approaching old age, but represents more active and demanding individuals than in previous decades(17). Additionally, increased participation in sports (particularly amongst women (18)) has contributed to the rise in cases. In Western countries, the lifetime risk for a TKA is consequently as high as roughly 1 out of 8 and has increased over the past decades(19).

In 2023, a total of 39,643 knee replacements were performed in the Netherlands of which 5876 under the age of 60 (20). In the United States, knee replacements for individuals

aged 45 to 64 surged by 240% between 2000 and 2017(13). It is predicted that >50% of TKAs in the United States will soon be undertaken in patients aged <65 years (21). Part of the increase in TKA use in young patients is driven by the fact that orthopedic surgeons face a treatment gap at this age, as shown in the first half of this thesis(22-24). Orthopedic surgeons resort to TKAs because literature does not show very effective alternatives, such as cartilage repair, in middle-aged(22, 24). However, younger patients tend to report high dissatisfaction rates (averaging 25%) following arthroplasty (25). Implanting a prosthesis at a younger age also increases the lifetime risk of revision: up to 35% for men under the age of 60 years, with a median time to revision of only 4.4 years in this group (26). Unfortunately, revision surgeries are often associated with diminished functional outcomes, more pain, an increased risk of complications such as periprosthetic joint infections and ultimately higher costs (~ € 35,000) (27-30).

Traumatic knee injuries and chronic mechanical overloading frequently result in focal cartilage defects, which can progress to early-onset OA as described above(1, 31, 32). Knee joint preservation treatments that can postpone the need for TKA by five years have the potential to decrease the number of revision surgeries later in life by 17% (33). Our group also showed via a state-transition model that postponing the need for a TKA by five years amounted to € 7,634 headroom (34). More importantly, joint preservation therapies likely offer patients an enhanced quality of life during their most productive years and improve general health. Ultimately, knee joint preservation could have a significant downstream effect on the (financial) burden of knee OA(29). A large observational study has identified significant and potentially modifiable risk factors for TKA in middle-aged patients, including cartilage defects ≥ 2 cm^2 (16.1% of TKA patients) alongside other factors such as increased body weight(1). Indeed, cost-effective TKA prevention has been shown for osteochondral allografts in cartilage repair(35). If focal knee resurfacing implants (FKRIs) can also successfully delay the need for TKA by at least five years, the socioeconomic impact could be substantial. In the Netherlands alone, such an intervention could potentially save €7 million in annual healthcare costs, given that 16.1% of the 5876 TKAs performed each year are attributable to cartilage defects in middle-aged patients, with an estimated cost-saving headroom of €7,634 per case.

Contribution and Relevance of This Thesis

Orthopedic surgeons have long observed that musculoskeletal injuries tend to heal more effectively in younger patients than in those who are middle-aged or older. This thesis illustrates the high regenerative potential of young patients by first demonstrating that preserving the native joint through the refixation of a loose cartilage fragment can yield favorable outcomes (36). More importantly, it challenges the longstanding belief that cartilage is too metabolically inert to support sufficient biological response for tissue ingrowth. Using loose cartilage fragments for cartilage repair marks a significant shift

from the traditional approach of discarding them. Second, a systematic review has been conducted for the first time to compare existing surgical cartilage repair techniques, differentiating outcomes between younger and older patients. The findings reveal that patients over 40-year benefit less from bone marrow stimulation, may experience reduced efficacy with cell-based techniques, and are likely to achieve more durable outcomes with bone-based restorative methods(24).

The findings in this thesis not only broaden our understanding of cartilage repair across age groups, but also underscore the need for more age-specific approaches in orthopedic surgery. The use of biomarkers with such age-specific approach to assess the biological age of a joint, rather than relying solely on chronological age, remains a prospect for future developments. Low hanging fruit could be the inclusion of a subgroup analyses in cartilage repair studies with a cut-off at 40 years of age. Additionally, new large-scale trials in cartilage repair should include patient cohorts representative of the typical age observed in large cartilage repair databases.

Our survey study in the Netherlands revealed that many orthopedic surgeons predominantly rely on straightforward cartilage repair techniques, typically treat younger patients, but extend these methods, such as microfracture, to middle-aged individuals. This practice should be questioned based on the results of our review and latest research (24, 37). Better awareness about the latest insight could prevent opportunistic use of microfracture, for which we have written an article in the Dutch Journal of Medicine (Nederlands Tijdschrift voor Geneeskunde; NTvG), see chapter XI(5).

However, the most critical finding from this survey study is the lack of a cartilage repair registry in the Netherlands(23). In the Netherlands, cartilage treatments are not separately registered, hindering the assessment of whether surgeons are adhering to the latest insights, if care is adequately centralized, and what the outcomes are for each treatment type or combinations. This underscores the need for an international registry and widespread use of such registry (38), which could ultimately even provide more comprehensive data than traditional randomized controlled trials (39). Such a registry could also serve as the foundation for a predictive model and a digital twin for each patient. The International Cartilage Repair Society has initiated an international registry, but its widespread implementation remains hindered by patient privacy legislation. To address this, policymakers should facilitate the development of international registries for surgical interventions. Cartilage expertise centers, supported by public-private partnerships, are the logical initiators of such an initiative. And perhaps, the use of such registry should be mandatory when surgeons want to perform cartilage repair.

For orthopedic surgeons, treating middle-aged patients with cartilage defects and early-onset OA presents a unique challenge(22-24). Bone-based therapies emerged as the most promising treatment option for the middle-aged population in our systematic review(24). Among these, the metallic Focal Knee Resurfacing Implant (FKRI) presents a viable alternative to autografting and allografting. However, these implants are not without drawbacks, including biomechanical mismatches with native knee tissues and imaging artifacts on CT and MRI scans. As a result, the final chapters of this thesis focus on the development of a non-degradable polycarbonate urethane (PCU)-based FKRI. A key prerequisite for the successful implementation of this implant was the demonstration of adequate bone ingrowth, which was achieved during the second animal trial. However, an equally critical consideration is ensuring that the polymer FKRI does not induce damage to the opposing cartilage surface to render effective joint preservation. Furthermore, the performance of larger implants must be evaluated, particularly with regard to their osseointegration and cartilage-preserving properties. These latter prerequisites warrant future investigation, with many aspects amenable to ex vivo or in silico methodologies, such as mechanical testing and finite element modeling.

Beyond the potential benefits of knee joint preservation through FKRI-based cartilage repair, our animal studies also carry broader implications for the orthopedic field. They reaffirm that surface-modified PCU can successfully integrate with bone. Beyond FKRI applications, a PCU implant could potentially be used for radial head prostheses, metatarsophalangeal joint prostheses, finger prostheses, and even more complex reconstructions, such as 'en bloc' replacement of the distal radius (hemiarthroplasties). Future investigators are encouraged to explore the applicability of non-degradable PCU for these common symptomatic joints.

Target Audience

The findings of this thesis hold significant insights for a wide-ranging audience. The first group includes general orthopedic surgeons and possibly even general practitioners. This thesis highlights the challenges faced by middle-aged patients with cartilage defects. By raising awareness of the problem and treatment options for middle-aged patients with cartilage defects, it is likely that better care pathways will emerge, with age considerations and improved indications for conservative and surgical treatment and better referrals. This will also create societal and financial support for further research into this issue. For this first target audience, a separate article has been published in the NTvG summarizing the key philosophy of this thesis (Appendix)(5).

The second group consists of (fundamental) researchers within the orthopedic field. The goal is to bring attention to the issue of cartilage defects in middle-aged patients/early-

onset OA and to encourage further research into the applicability of non-degradable polyurethane for knee joint preservation, as well as other uses previously described.

A final word of this thesis is reserved for the third group: policy makers. While careful planning, execution, and documentation of studies are essential, the journey from concept to clinical application and subsequent widespread patient access in translational medicine is notoriously lengthy and costly. Progress therefore remains slow despite the explosively growing OA population. To accelerate this process, early guidance on reimbursement decisions is essential. Conditional reimbursement by health care insurance should be considered at an early stage, subject to positive post-marketing surveillance data and recommendations from professional medical institutes such as the NOV (Nederlandse Orthopaedische Vereniging). Establishing programs akin to the FDA's Breakthrough Device Designation (BDD) and Total Product Life Cycle Advisory Program (TAP) at European level can further streamline regulatory approval and reimbursement pathways. Lastly, fostering innovation requires expanding funding opportunities to help startups overcome the critical 'valley of death' and bring promising technologies to clinical practice.

We need more adequate research and product development in degenerative musculoskeletal disease to keep people active and healthy in the nearby future and to prevent unsustainable health care expenditure.

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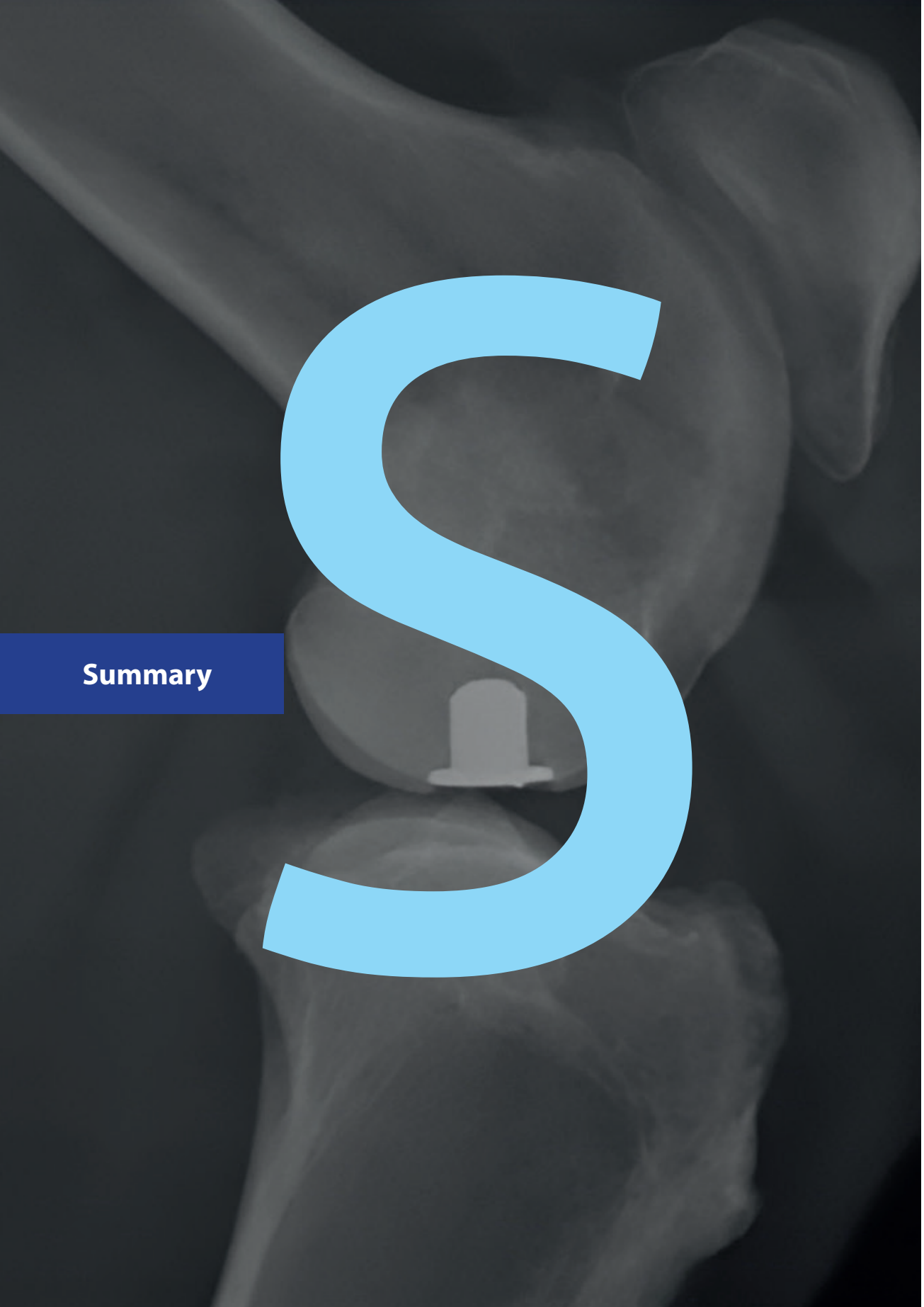

Summary

English Summary

Knee joint health is essential for maintaining mobility, quality of life, and overall well-being. However, cartilage defects, whether caused by trauma, chronic overload, degenerative conditions or a combination of these causes, present a significant challenge, particularly for middle-aged individuals. While younger patients have strong regenerative potential and older patients are good candidates for total knee arthroplasty, treatment options for middle-aged individuals remain complex. The work in this thesis explores the intricacies of knee joint preservation, highlighting treatment strategies across different life stages that could delay or even eliminate the need for knee replacement surgery.

Chapter III introduces the modified hedgehog technique for trimming and refixation of traumatic shear-off chondral fragments in young patients. This method improves fragment integration without using screws or resorbable materials. All three patients achieved stable healing and a full return to sports within a year. This case series demonstrates the high regenerative potential of young patients and underscores the value of reattaching cartilage fragments whenever possible.

Chapter IV systematically reviews cartilage repair techniques in younger adults (<40 years) and middle-aged (40–60 years) patients. Bone marrow stimulation techniques, such as microfracture, show poor outcomes in middle-aged patients, with lower defect filling and lower clinical outcomes after short-term follow-up. Cell-based therapies, such as autologous chondrocyte implantation (ACI), decline in long-term efficacy, likely due to aging chondrocytes and altered joint homeostasis. In contrast, bone-based techniques (including osteochondral autografts and focal knee resurfacing implants (FKRIs)), demonstrate durable outcomes by relying on osseointegration rather than chondrogenesis.

Chapter V surveys Dutch orthopedic surgeons' treatment choices for cartilage defects, revealing frequent deviations from national and international treatment guidelines. Microfracture is still used for larger defects or in older patients, despite evidence of its limitations. Advanced techniques such as ACI and osteochondral allografting are restricted to expert centers or hindered by availability issues. The inconsistent treatment strategies for middle-aged patients highlight the need for improved education, stricter guideline adherence, generation of better clinical evidence by more inclusion of representative study populations in clinical studies, and a national cartilage repair registry.

Chapter VI explores advancements in biomaterials for cartilage repair. While degradable polymers hold promise for tissue engineering, many have failed to advance to clinical trials due to non-successful preclinical studies, regulatory hurdles and technical limitations. Moreover, no current tissue engineering approach successfully replicates the hyaline cartilage phenotype in humans. In contrast, non-degradable polymers offer potential as

permanent replacements for diseased tissue. Demonstrating that implants and materials are capable of withstanding the biomechanical demands of the joint remains crucial.

Chapter VII evaluates non-degradable polycarbonate-urethane (PCU) as a novel FKRI material. PCU has the potential to overcome the typical drawbacks of metal-based FKRIs, including opposing cartilage degeneration, stress shielding, and imaging artifacts. In this proof-of-principle study, osseointegration of a PCU implant was tested with different surface modifications. Both *in vitro* and *in vivo* (goat model) experiments demonstrated that biphasic calcium phosphate and surface texturization enhance cell-mediated calcification and bone to implant contact as assessed by histology.

Chapter VIII describes a second-generation PCU-based prototype FKRI, composed of a PCU-zirconium oxide composite stem to enhance mechanical properties and osseointegration. The composite's mechanical properties were optimized to match the elastic modulus of trabecular bone, improving osseointegration. *In vitro*, it demonstrated good biocompatibility, supporting cell viability and mineralization. *In vivo*, composite implants showed osseointegration comparable to titanium implants in a goat model, outperforming the native PCU implants analogous to the prototype implants tested in Chapter 7. Additionally, the PCU-zirconium oxide composite material is MRI-compatible and radiopaque, improving clinical imaging.

Overall, the work in this thesis underscores a key challenge recognized by orthopedic surgeons: cartilage defect management is complex and particularly in middle-aged patients. By thoroughly analyzing this issue, treatment strategies can be improved. More studies stratified by age and registry data are needed to refine clinical decision-making. This decision-making involves proper patient selection and personalized treatment plans (e.g. by combining cartilage repair with concomitant surgeries and lifestyle interventions). Additionally, non-degradable materials may play an increasingly vital role in middle-aged patients, as bone's biological response remains more resilient to aging than cartilage regeneration. With the development of a PCU FKRI, this dissertation introduces a novel approach to knee joint preservation.

Nederlandse Samenvatting

Gezonde kniegewrichten zijn essentieel voor het behoud van mobiliteit, kwaliteit van leven en het algeheel welzijn. Kraakbeendefecten, ontstaan door trauma, chronische overbelasting, degeneratieve aandoeningen of een combinatie hiervan, vormen echter een serieuze uitdaging voor orthopeden, met name bij patiënten van middelbare leeftijd. Waar jongere patiënten beschikken over een groot regeneratief vermogen en oudere patiënten vaak in aanmerking komen voor een totale knieprothese, is de behandeling van patiënten van middelbare leeftijd complex. Het werk in dit proefschrift onderzoekt de mogelijkheden van gewricht sparende behandelingen van de knie, met als doel het uitstellen of zelfs voorkomen van een knieprothese.

In **hoofdstuk III** wordt de gemodificeerde 'hedgehog-techniek' beschreven voor het bijwerken en opnieuw vastzetten van traumatische afgeschoven kraakbeenfragmenten bij jonge patiënten. Deze techniek verbetert de integratie van het fragment zonder gebruik van schroeven of resorbeerbare materialen. Bij alle drie patiënten trad stabiele genezing op en was er binnen een jaar een volledige terugkeer naar sportactiviteiten. Deze casusreeks toont het hoge regeneratieve potentieel van jonge patiënten aan en onderstreept het belang van het vastzetten van kraakbeenfragmenten wanneer dit technisch mogelijk is.

Hoofdstuk IV bevat een systematisch overzicht van kraakbeenhersteltechnieken bij (jong)volwassenen (<40 jaar) en patiënten van middelbare leeftijd (40–60 jaar). Beenmergstimulatietechnieken, zoals microfracturering, laten bij de middelbare leeftijdsgroep ongunstige resultaten zien: het defect vult zich minder goed en de klinische uitkomsten zijn slechter op de korte termijn. Op cellen gebaseerde therapieën, zoals autologe chondrocyte implantatie (ACI), verliezen hun effectiviteit op langere termijn, waarschijnlijk door veroudering van chondrocyten en veranderingen in de gewrichtshomeostase. Daartegenover laten op bot gebaseerde technieken, zoals osteochondrale autografts en *focal knee resurfacing implants* (FKRI's), duurzamere resultaten zien doordat ze gebaseerd zijn op osseointegratie in plaats van chondrogenese.

In **hoofdstuk V** wordt verslag gedaan van een enquête onder Nederlandse orthopedisch chirurgen over hun behandelkeuzes bij kraakbeendefecten. Hieruit blijkt dat er frequent wordt afgeweken van nationale en internationale richtlijnen. Microfracturing wordt nog steeds toegepast bij grotere defecten of bij oudere patiënten, ondanks de bekende beperkingen. Geavanceerde technieken, zoals ACI en osteochondrale allografts, zijn beperkt beschikbaar en meestal voorbehouden aan expertisecentra. De inconsistente behandelstrategieën voor middelbare leeftijdspatiënten onderstrepen de noodzaak van betere scholing, striktere richtlijnnaleving, meer representatieve patiëntengroepen in klinische studies en de opzet van een nationaal kraakbeenregister.

Hoofdstuk VI bespreekt de ontwikkeling van biomaterialen voor kraakbeenherstel. Hoewel afbreekbare polymeren veelbelovend zijn binnen de wereld van weefselregeneratie, stagneert de doorontwikkeling vaak door teleurstellende preklinische resultaten, strenge regelgeving en technische beperkingen. Bovendien is er nog geen enkele weefselregeneratietechniek die het hyaline kraakbeenfenotype bij de mens succesvol weet te repliceren. Niet-afbreekbare polymeren daarentegen bieden perspectief als blijvende vervanging van ziek weefsel. Een belangrijke voorwaarde blijft dat implantaten en materialen de biomechanische belasting van het gewricht kunnen doorstaan.

In **hoofdstuk VII** wordt polycarbonaat-urethaan (PCU) onderzocht als nieuw materiaal voor FKRI's. PCU kan de typische nadelen van de op metaal gebaseerde FKRI's, zoals degeneratie van het tegenoverliggende kraakbeen, *stress shielding* en beeldvormingsartefacten, mogelijk verbeteren. In dit *proof-of-principle*-onderzoek werd de osseointegratie van een PCU-implantaat getest met verschillende oppervlaktebehandelingen. Zowel *in vitro* als *in vivo* (geitenmodel) bleek dat bifasisch calciumfosfaat en oppervlakte-textuering de bot-implantaatinteractie en celgemedieerde calcificatie verbeterden.

Hoofdstuk VIII beschrijft een tweede generatie van de op PCU gebaseerde prototype FKRI, bestaande uit een PCU-zirkoniumoxidecomposiet. Dit composiet verbetert de mechanische eigenschappen en osseointegratie doordat het een elasticiteitsmodulus heeft die beter aansluit bij die van trabeculair bot. *In vitro* liet het materiaal goede biocompatibiliteit zien, met behoud van cel-viabiliteit en mineralisatie. *In vivo* waren de osseointegratie resultaten vergelijkbaar met die van titanium implantaten en beter dan die van de oorspronkelijke PCU-implantaten uit hoofdstuk VII. Daarnaast is het PCU-zirkoniumoxidecomposiet MRI-compatibel en radio-opaak, wat de klinische beeldvorming ten goede komt.

Samenvattend benadrukt dit proefschrift een belangrijke uitdaging binnen de orthopedie: de behandeling van kraakbeendefecten is complex, met name bij patiënten van middelbare leeftijd. Een grondige analyse van dit probleem maakt het mogelijk behandelstrategieën te verbeteren. Toekomstig onderzoek, met leeftijdsstratificatie en registratiedata, is nodig om de klinische besluitvorming te verfijnen. Dit vraagt om zorgvuldige patiëntselectie en gepersonaliseerde behandelplannen, bijvoorbeeld door kraakbeenherstel te combineren met bijkomende operaties en leefstijlinterventies. Bovendien kunnen niet-afbreekbare materialen een steeds belangrijkere rol spelen bij middelbare leeftijdspatiënten, omdat de biologische respons van bot beter behouden blijft bij veroudering dan het regeneratief vermogen van kraakbeen. Met de ontwikkeling van een PCU-FKRI levert dit proefschrift een nieuwe behandeling voor gewricht sparende behandeling van de knie.

Appendix

A

Gewrichtssparende behandelingen van de knie

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Abstract

Middle-aged patients with early onset arthritis or cartilage defects are difficult to treat. These patients are relatively young for joint replacement and relatively old for regenerative therapies, i.e.: a treatment gap. Therefore, the concept of joint preservation has emerged with the main goal to delay of even prevent joint replacement. Several novel surgical techniques and treatment algorithms for cartilage repair are developed. New insights show that knee joint preservation is best achieved by combining treatments, for instance cartilage repair and mechanical (alignment) correction. Since combining different surgeries introduces more complexity, a guideline has been drafted, which states that (part of) this care is perhaps best centralised. It is therefore important, to treat patients eligible for joint preservation in collaboration with expert centres. This brief clinical lesson provides the latest insights in the Dutch knee joint preservation care supported by the latest guideline.

Samenvatting

Bij patiënten van middelbare leeftijd met beginnende artrose of kraakbeenafwijkingen zijn de behandelmogelijkheden beperkt. Deze patiënten zijn te jong voor een gewrichtsvervanging en te oud voor regeneratieve behandelingen. Daardoor is er sprake van een 'gat' in de behandeling ('treatment gap'). Om dit gat te vullen is het concept van gewrichtsbehoud ('joint preservation') ontstaan. Dat heeft als belangrijkste doel om een gewrichtsvervanging uit te stellen of zelfs te voorkomen. Er zijn verschillende nieuwe operatietechnieken en behandelalgoritmen ontwikkeld voor kraakbeenherstel. De gewrichtssparende behandeling is het effectiefst wanneer verschillende behandelingen worden gecombineerd, bijvoorbeeld kraakbeenherstel in combinatie met beenascorrectie. Vanwege de complexiteit van deze gecombineerde ingrepen stelt de richtlijn dat deze zorg wellicht het beste gecentraliseerd kan worden. Het is daarom belangrijk om patiënten die hiervoor in aanmerking komen te behandelen in samenwerking met een expertisecentrum. In dit artikel geven wij een overzicht van de nieuwste inzichten op het gebied van de gewrichtssparende behandeling van de knie.

Beste collega's, in dit artikel gaan we het hebben over de gewrichtssparende behandeling van de knie. Deze omvat alle conservatieve en operatieve interventies die een gewrichtsvervanging kunnen uitstellen. Een overzicht van de huidige inzichten.

Gewrichtsvervanging middels een totale knieprothese (TKP) is een succesvolle behandeling voor patiënten van gevorderde leeftijd met knieartrose in het eindstadium. Patiënten van middelbare leeftijd met beginnende degeneratie van de knie of acute letsels die kunnen leiden tot 'een jonge patiënt met een oud gewricht' vormen een toenemende uitdaging, zowel voor de huisarts als voor de orthopedisch chirurg. Met het herziene standpunt van de Nederlandse Orthopaedische Vereniging (NOV) uit 2019 ten aanzien van kraakbeenbehandeling en de toenemende centralisatie van kraakbeendefectbehandelingen is de behandeling van knieartrose in Nederland veranderd. In dit artikel lichten wij het nieuwe begrip 'gewrichtssparende behandeling' toe aan de hand van een illustratieve casus.

Het concept 'gewrichtssparende behandeling' omvat alle conservatieve en operatieve behandelingen die een gewrichtsvervanging kunnen uitstellen of voorkomen. Dit berust op meerdere principes: (a) verbeteren van de kraakbeenbelasting – denk aan gewichtsverlies en leefstijlaanpassingen, beenascorrectie, correctie van de patellastand of -sporing of herstel van meniscusletsel; (b) het verbeteren van de stabiliteit van het gewricht door fysiotherapie en herstel van ligamentair letsel of meniscusletsel; en (c) het herstellen van kraakbeendefecten – en zo nodig het onderliggende bot – met bestaande en nieuwe kraakbeenbehandelingen. Dit artikel borduurt voort op een eerder artikel in het *NTVG*

uit 2013 (A5719). Inmiddels is de behandeling van kraakbeendefecten geëvolueerd naar een bredere aanpak. Daarin worden de gehele knie als orgaan en patiëntkenmerken meegenomen, en niet alleen kenmerken van het kraakbeendefect.

De behandeling van een kraakbeendefect is onder andere afhankelijk van de leeftijd. Op basis de nieuwste inzichten en consensus worden verschillende groepen onderscheiden. Kinderen en jongvolwassenen hebben nog een lange levensverwachting. Bij hen pogen we het kraakbeen te herstellen; we kiezen dus voor een regeneratieve behandeling. Bij ouderen (leeftijd: 60-65 jaar) is dit niet zinvol, aangezien hun regeneratief potentieel laag is. Deze 'ouderen' kunnen we goed behandelen met een gewrichtsvervanging, dus door een knieprothese te plaatsen.[1]

De meest uitdagende groep zijn de patiënten van middelbare leeftijd (leeftijd: 40 tot 60 jaar). Aan de ene kant zijn er aanwijzingen dat zij 'te oud' zijn voor succesvolle regeneratieve therapieën.[2] Aan de andere kant zien we dat patiënten die wél een totale knieprothese (TKP) krijgen vóór de leeftijd van 60 jaar een verhoogd risico hebben op een revisieoperatie later in hun leven; dit leidt tot inferieure uitkomsten en gaat gepaard met hogere kosten.[1] Bovendien is tot 20% van de patiënten ontevreden na plaatsing van een TKP, en een jonge leeftijd verhoogt het risico hierop.[1,3]

Dit leidt tot een 'gat' in de behandeling ('treatment gap') bij patiënten van middelbare leeftijd.[2] Juist bij deze patiëntengroep is gewrichtssparende behandeling dus essentieel, zeker gezien de grootte van deze groep. Uitstel van de eerste gewrichtsvervanging met vijf jaar is al voldoende om het aantal TKP-revisieoperaties te reduceren met 17%.[4] Tegelijkertijd vormt deze grote groep patiënten van middelbare leeftijd een uitdaging voor de huisarts en orthopedisch chirurg, doordat de gewrichtssparende behandelmogelijkheden zich snel ontwikkelen, niet overal voorhanden zijn en huisartsen en orthopedisch chirurgen niet altijd op de hoogte zijn van de nieuwste inzichten.

Patiënt A, een 47-jarige vrouw met een abdominale, gynaecologische en orthopedische voorgeschiedenis, kwam op de polikliniek Orthopedie vanwege klachten van beide knieën.

Op basis van de anamnese, het lichamelijk en het aanvullend onderzoek concludeerden wij dat er links sprake was van mediale meniscusdegeneratie met een degeneratieve meniscusscheur. Verder had zij een centraal kraakbeendefect van de mediale femurcondyl links en stond het linker been in 4,4 graden varus (figuur 1a).

Wij behandelden de patiënte conservatief gedurende vijf maanden, maar de klachten hielden aan. Daarom besloten wij om de patiënte gewrichtssparend te behandelen aan

de linker knie met een partiële meniscectomie, een mozaïekplastiek van het kraakbeendefect en een correctie van de beenas door middel van een tibiakoposteotomie (figuur 1b).

Vóórdat wij de patiënte op onze polikliniek zagen had zij elders een partiële meniscectomie van de rechter knie ondergaan. Tijdens een artroscopie en op de postoperatieve MRI-scan van de knie was toen al een kraakbeendefect van de mediale femurcondyl gezien; dit defect werd in het betreffende ziekenhuis ongemoeid gelaten. Toen de patiënte daarna bij ons kwam wilde zij eerst de linker knie laten behandelen, aangezien zij daarvan de meeste klachten ondervond. De rechter knie reageerde goed op conservatieve behandeling met een valgiserende brace.

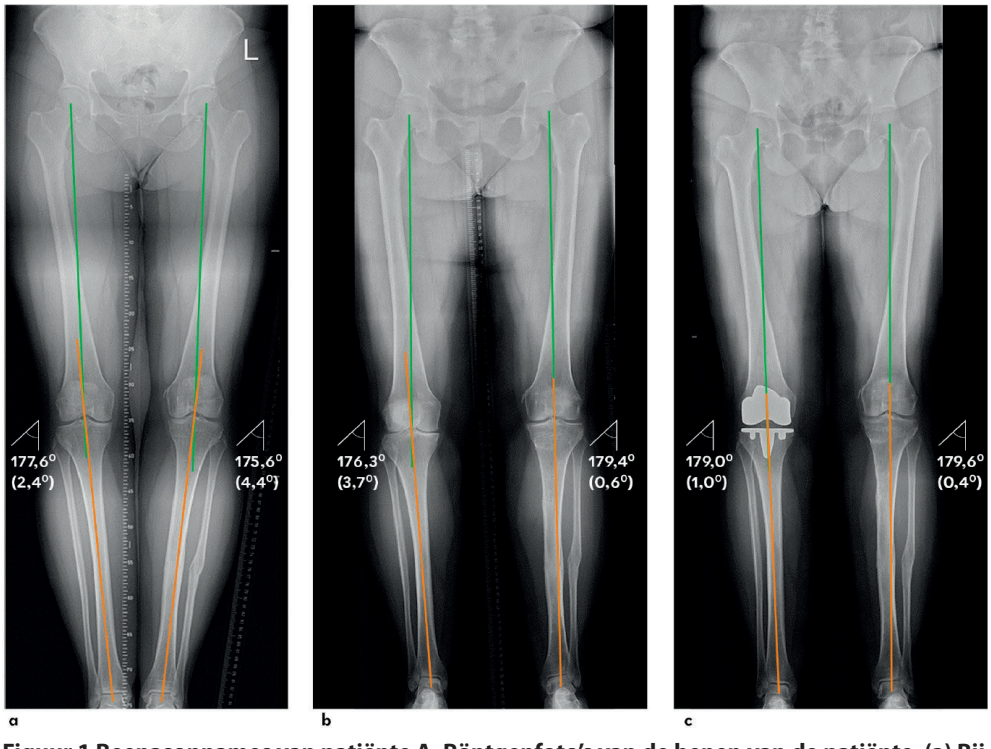
Na ruim tien jaar van niet-chirurgische behandeling ontwikkelde de patiënte progressieve klachten van de rechter knie op basis van mediale gonartrose. Vanwege de invaliderende klachten behandelden wij de patiënte met een totale knieprothese rechts (figuur 1c). Elf jaar na de gewrichtssparende operatie liet de linker knie klinisch en radiologisch nog geen kenmerken van artrose zien, waardoor een gewrichtsvervanging niet nodig was.

Beschouwing

Steeds vaker komen patiënten bij de huisarts met knieartrose. In de eerste lijn kunnen deze patiënten aanvankelijk goed worden behandeld met gewichtsreductie, fysiotherapie en een intra-articulaire injectie. Wanneer deze aanpak onvoldoende effect heeft, is het belangrijk de patiënt te verwijzen naar een orthopedisch chirurg, zeker bij een relatief jonge patiënt.

Onze patiënte had kraakbeenschade in beide knieën. Gezien het goede klinische beloop van de linker knie, is het de vraag of chirurgisch ingrijpen bij lichte klachten, zoals aanvankelijk aan de rechterzijde was gebeurd, ook destijds al was gerechtvaardigd. Vanzelfsprekend is deze casus slechts één voorbeeld van het belang om patiënten met een verhoogd risico op progressieve artrose vroegtijdig te herkennen. Denk hierbij aan patiënten met meniscus- of kruisbandletsel, een beenasafwijking, een afwijkend stand of slechte sporing ('maltracking') van de patella, of een kraakbeendefect.

Dit maakt dat er, naast de klassieke criteria voor fulminante artrose, nu ook classificatiecriteria zijn opgesteld voor artrose in een vroeg stadium (vroegartrose): (a) pijn, symptomen of stijfheid, functiebeperking in het dagelijks leven en beperking van de kniegerelateerde kwaliteit van leven; (b) pijn in de gewrichtsspleet bij palpatie of crepitaties; en (c) graad 0- of graad 1-artrose op basis van radiologische criteria.[5]



Figuur 1 Beenasopnames van patiënte A. Röntgenfoto's van de benen van de patiënte. (a) Bij het eerste polikliniekbezoek op 47-jarige leeftijd is er sprake van lichte gonartrose beiderzijds. Er is een varusstand van het rechter been van 2,4 graden. Het linker been staat in 4,4 graden varus. (b) Na correctie van de linker beenas met een valgiserende tibiakoposteotomie op 47-jarige leeftijd staat het linker been in 0,6 graden varus. Er is sprake van gevorderde gonartrose rechts met een varusstand van 3,7 graden. (c) Na plaatsing van een totale knieprothese rechts op 58-jarige leeftijd staat het rechter been in 1,0 graden varus. De linker knie laat geen kenmerken zien van fulminante artrose; de linker beenas is nog steeds vrijwel neutraal (0,4 graden varus).

De prevalentie van 'artrose' stijgt met de leeftijd. In Nederland bedraagt die 1,25% bij mensen van 40-44 jaar; 2,85% bij 45-49-jarigen; 5,9% bij 50-54-jarigen; en 10,8% bij 55-59 jarigen. Daarbij wordt voor de komende twee decennia een stijging van 36% verwacht.[6] Voor de diagnose 'vroegartrose' of risicofactoren daarvoor, zoals kraakbeendefecten of beenasafwijkingen, zijn in Nederland helaas nog geen objectieve getallen beschikbaar. Door de veranderde zorgvraag van patiënten van middelbare leeftijd, de obesitasepidemie bij jongeren en hogere sportparticipatie met bijkomende letsels is het aannemelijk dat de voorspelde prevalentiestijging ook vooral de groep van middelbare leeftijd treft.

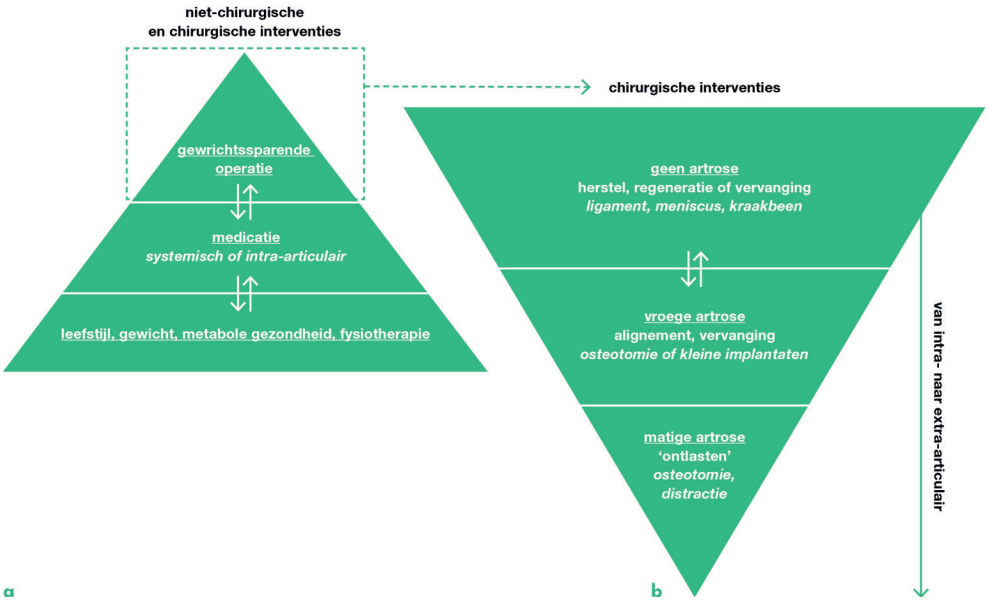
Gewrichtssparend behandelen

Het doel van een chirurgische behandeling is om het gewrichtsoppervlak te herstellen en de biomechanische gewrichtsfunctie te normaliseren of op z'n minst te optimaliseren. Het belangrijkste primaire doel is om een gewrichtsvervangende operatie te voorkomen, of om een leeftijd te bereiken waarbij het risico op een revisie van een gewrichtsprothese minimaal is (figuur 2).

Steeds vaker wordt chirurgische behandeling van een osteochondraal defect gecombineerd met een peri-articulaire osteotomie. Het doel hiervan is om de belasting op het behandelde kraakbeendefect te verminderen. Daarnaast is het belangrijk dat, indien nodig, de stand en de sporing van de patella wordt geoptimaliseerd. Een recent onderzoek toonde aan dat patiënten die naast kraakbeenherstel ook een tibiakoposteotomie ondergingen, een hogere functionele kniescore, een hoger tevredenheidspercentage en een lager pijnniveau hadden na een periode van drie jaar vergeleken met patiënten bij wie alleen een kraakbeenbehandeling was uitgevoerd.[7] Verder kunnen kruisbandreconstructie of meniscusaugmentatie of -transplantatie worden gecombineerd met behandeling van een kraakbeendefect, aangezien de uitkomst van het kraakbeenherstel inferieur is bij blijvende instabiliteit.[8]

Het leeuwendeel van de gewrichtssparende behandeling betreft voorlichting en leefstijlaanpassingen, eventueel aangevuld met een beenascorrectie; deze therapieën kunnen uitstekend worden toegepast in respectievelijk de eerste en tweede lijn.[9] Complexere behandelingen, zoals de behandeling van grote kraakbeendefecten of gecombineerde chirurgische ingrepen, worden idealiter uitgevoerd in óf in samenspraak met een kraakbeenexpertisecentrum.

Er zijn verschillende operatietechnieken voor de behandeling van een kraakbeendefect; de meesten zijn al eerder beschreven in het *NTVG* (A5719). Het vernieuwde standpunt benoemd echter ook aanvullingen op al bestaande technieken; met name de autologe-chondrocytenimplantatie (ACI) van de vierde generatie, allogene osteochondrale transplantaten ('allografts') en kraakbeenimplantaten ('focal knee resurfacing implants'; FKRI) worden toegelicht. Bij de vierdegeneratie-ACI wordt er gebruik gemaakt van sferoïden; deze behandeling is kosteneffectief gebleken bij kraakbeenlaesies met oppervlakte groter dan 2 cm^2. Kraakbeenimplantaten zijn geschikt voor defecten op verschillende plaatsen en van verschillende groottes. Met name patiënten van 40-60 jaar komen voor deze behandeling in aanmerking, aangezien de werking niet berust op regeneratie maar op vervanging van het kraakbeen en het onderliggende bot.[8]



Figuur 2. Gewrichtssparende behandeling kniedegeneratie. Weergave van (a) het aandeel van niet-chirurgische en chirurgische interventies ter voorkoming of vertraging van degeneratie in het kniegewricht, en (b) het aandeel van chirurgische interventies per stadium van artrose. De leefstijl – de vraag naar belastbaarheid –, het gewicht – mechanische belasting –, de metabole gezondheid – systemische inflammatie – en fysiotherapie – balans, proprioceptie en musculaire demping piekbelasting – zijn de belangrijkste componenten van de niet-chirurgische behandeling. De medicamenteuze behandeling bestaat veelal uit pijnstilling of intra-articulaire injectie van corticosteroïden of hyaluronzuur. Er loopt preklinisch onderzoek naar nieuwe intra-articulaire middelen. Bij patiënten zonder artrose met letsel van meniscus,kruisband of kraakbeen worden hersteloperaties toegepast, zoals een meniscopexie, kruisbandreconstructie of kraakbeenherstel met autologe chondrocytenimplantatie. Bij patiënten met artrose in een vroeg stadium kunnen focale kraakbeendefecten worden vervangen door een allograft of 'focal knee resurfacing implant'. Bij patiënten met vroege of matige artrose kan een eventuele beenasafwijking worden gecorrigeerd.

Centralisatie van zorg

Het vernieuwde standpunt stelt dat patiënten met een osteochondraal defect van 2 cm^2 of groter verwezen dienen te worden naar centra die de beschikking hebben over celtherapieën, zoals ACI. Op dit moment zijn er vier expertisecentra voor kraakbeenherstel aangewezen; Elisabeth-TweeSteden Ziekenhuis (Tilburg), UMCG (Groningen), UMCU (Utrecht) en MUMC+ (Maastricht).

In deze expertisecentra worden patiënten met een groot kraakbeendefect behandeld, bijvoorbeeld met de eerder genoemde ACI- of FKRI-methode, maar ook andere experi-

mentele therapieën, zoals kniedistractie en meniscusimplantaten. Naast de behandeling van kraakbeendefecten worden in de expertisecentra ook gecombineerde chirurgische behandelingen uitgevoerd. Denk hierbij aan een beenascorrectie in combinatie met herstel van ligamentair letsel en behandeling van een kraakbeendefect.[8] Door geïndividualiseerde behandelplannen worden in deze centra voornamelijk gewrichtssparende behandelingen verricht in plaats van uitsluitend kraakbeenbehandelingen.[10] Dit komt overeen met de doelstelling in het Integraal Zorgakkoord. Op basis van een vergelijkbare richtlijn in het Verenigd Koninkrijk zijn er in overleg met de National Health Service in het VK nu veertien gewrichtssparende klinieken opgericht en inmiddels 10.000 patiënten opgeroepen voor een osteotomie.[11]

Beste collega's, artrose van de knie kunnen we niet volledig voorkomen, maar we kunnen wel proberen een gewrichtsvervangende operatie uit te stellen of te voorkomen. Bij patiënten van jonge of middelbare leeftijd is het essentieel om zoveel mogelijk gewrichtssparend te behandelen, door samen te werken en behandeldoelen af te stemmen binnen het zorgtraject. Het is belangrijk om, als dat geïndiceerd is, kraakbeenherstel te combineren met correctie van de beenas of de patellasporing of herstel van ligamentair letsel; veel van deze behandelingen kunnen in de tweede lijn plaatsvinden. De behandeling van complexere kraakbeenletsels of gecombineerde chirurgische ingrepen vinden bij voorkeur plaats in, of in samenwerking met, gespecialiseerde centra. Dit besef en een goede samenwerking tussen de eerste, tweede en derde lijn is cruciaal om een goede registratie op te zetten van het effect van gewrichtssparende behandelingen in Nederland. Daarbij moet een goed lichaamsgewicht en een gezonde levensstijl het uitgangspunt zijn.

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