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ARAGONITE-BASED SCAFFOLD FOR THE TREATMENT OF KNEE OSTEOCHONDRAL DEFECTS: RESULTS OF A PROSPECTIVE MULTI-CENTER TRIAL AT 2 YEARS' FOLLOW-UP

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ABSTRACT

PURPOSE: To evaluate the clinical and MRI outcomes after the implantation of a nanostructured cell free aragonite-based scaffold in patients affected by knee chondral and osteochondral lesions.

METHODS: 126 patients (94 men, 32 women; age 32.7±8.8 years) were included according to the following criteria: grade III or IV chondra/osteochondral lesions in the femoral condyles or throclea; 2) no limb axial deviation (i.e. varus or valgus knee > 5°); 3) no signs of knee instability; 4) no concurrent tibial or patellar chondral/osteochondral defects. All patients were treated by arthrotomic implantation of an aragonite based-scaffold by a press-fit technique. Patients were prospectively evaluated by IKDC, Tegner, Lysholm and KOOS scores preoperatively and then at 6, 12, 18 and 24-months follow-up. MRI was also performed to evaluate the amount of defect filling by regenerated cartilage. Failures were defined as the need for re-intervention in the index knee within the follow-up period.

RESULTS: Average defect size was 2±1.3 cm^2 and in most cases a single scaffold was used. A significant improvement in each clinical score was recorded from basal level to 24 months' follow-up. In particular, the IKDC subjective score increased from 42.14±16 to 70.94±24.69 and the Tegner score improved from 2.95±1.90 to 4.82±1.85 ($p<0.0005$). Lysholm score and all the subscales of KOOS showed a similar trend over time. Age of the patient at implantation, size of the defect and BMI were correlated with lower clinical outcome. The presence of OA didn't influence the clinical results. MRI evaluation showed a significant increase in defect filling over time, with the highest value reached at 24 months. Failures occurred in eleven patients (8.7%).

CONCLUSION: The aragonite-based biomimetic osteochondral scaffold proved to be safe, and encouraging clinical and radiographic outcomes were documented up to 2 years' follow-up.

General Index

GENERAL INTRODUCTION

Osteochondral defects are areas of damage involving the articular cartilage and the subchondral bone. They affect a significant number of people and can cause relevant functional limitation. Every joint can be affected by this pathological condition but the most frequently involved is the knee, followed by the ankle.

Even if this kind of lesion can occur at every age, young and active population is the most affected because the origin of these lesions is mainly traumatic or micro-traumatic. [1] In particular, articular cartilage is the weakest component of the joint from a metabolic point of view: it does not have any vascular, nervous and lymphatic supply. Furthermore, there is a big disproportion between the matrix and the cellular population. This explains the poor regeneration capacity of this tissue [2].

Only small osteochondral lesions are spontaneously filled with fibrous tissue while this does not happen for larger lesions. The loss of the functional properties of cartilage leads to changes also in the subchondral bone and progressive articular degeneration that can result in osteoarthritis [3]. Young patients with osteoarthritis are a challenging population because of a combination of high functional demands due to the age, and limited indications for joint replacement. The same can be said for middle age active patients who want to maintain a high activity level and to postpone or avoid metal resurfacing.

In the last years many therapeutic options to repair the damaged cartilage have been proposed. First, microfractures have been employed, documenting positive outcomes in the short-mid term, but results have been shown to deteriorate over time due to the fibrocartilaginous nature of the repair tissue. Autologous osteochondral transplant is considered a valuable therapeutic option with a high rate of success, but it is possible to collect just a limited amount of tissue, in order to avoid donor site morbidity. A good

alternative to autografts is represented by allogenic osteochondral transplants; however the high demand of tissues cannot be met and infections transmission is a remote but possible risk. The percentage of success of allogenic transplantation is anyway inferior to autograft [4].

An innovative treatment option has been introduced with the development of autologous chondrocyte transplantation (ACT) first, and matrix-assisted ACT then. This approach represented a truly milestone in the field of regenerative medicine: it has shown very good results up to long term evaluation, and the possibility of using arthroscopic implantation contributed to minimizing the trauma for the patients. However this technique presents some limitations concerning the surgical procedure, i.e. the need of two surgical steps, and the extremely high costs of cell expansion in lab [5].

More recently, progress in the field of biotechnology has led to the introduction of one-step approaches by using various biomaterials endowed with the ability of stimulating tissue regeneration. A new biomimetic scaffold has been recently developed, i.e. Agili-C^{TM} (Cartiheal Ltd, Israel) [77]. This is a cell-free, resorbable, bi-phasic scaffold made of inorganic calcium carbonate (aragonite) associated with hyaluronic acid. Aragonite is a biological material similar to human bone in its three-dimensional structure, pore interconnections and crystalline form of calcium carbonate ($CaCO_3$). Aragonite is obtained from exoskeletons of corals, marine invertebrates of the wide Anthozoa class including more than 7.000 species. Different species differ in dimension and interconnectivity of pores, crucial factors in conferring good bone regeneration capacity and coralline biomaterial resorbtion. 100 μm is the pore diameter found to provide a better colonization by osteoid and connective tissues. Pore connections of 100 to 200 μm are instead ideal for the creation of Haversian systems and the entry of blood vessels. Coral has thus been used as a bone substitute in orthopedics due to its osteoconductive and osteogenesis capacities.

The structure of the aragonite has been modified to create the present scaffold, where the most superficial layer is peculiarly able to stimulate also cartilage regeneration through the addition of hyaluronic acid.

The main purpose of this study is to evaluate the outcome obtained on a sample of 126 patients treated by the implantation of the aforementioned scaffold, and evaluated both clinically and radiographically up to 24 months' follow-up

CHAPTER 1

KNEE ANATOMY

1.1 Articular anatomy

The knee is a modified hinge joint (gyglimus), a type of synovial joint, which is composed of three functional compartments: the two mentioned tibiofemoral articulations and the patellofemoral articulation, consisting of the patella, or "kneecap", and the patellar groove on the front of the femur which is concave and through which the patella slides.

The articular portion of the anterior surface of the distal femur, which articulates with the patella, is the trochlea. The trochlear surface of the femur is divided into two facets, the medial and lateral condyles, which are separated by a groove: the intercondylar notch.

The femoral condyles have an antero-posterior diameter bigger than the transversal diameter. The medial condyle is larger, more curved and extend more distally than the lateral condyle. This asymmetry between the medial and lateral condyle of the knee determines the relative mobility of each compartment.

The proximal tibial end has two articular facets flattened in the horizontal plane. The superior articular surfaces of the two condyles are concave, particularly centrally.

Between the medial and lateral condyles there is the intercondylar region of the tibial plateau, centrally it is narrow and raised to form the intercondylar eminence.

Just the central part of the condyles directly supports the pressure of the femoral condyles, the outer margins of the surfaces are flatter and are the regions in contact with the interarticular discs of fibrocartilage (menisci).

Menisci are two cartilaginous laminae that improve congruency between the flat tibial articular surfaces and convex femoral surfaces during joint movements.

Furthermore the meniscus is more elastic than articular cartilage, and therefore absorbs shock.

They are ring shaped with a triangular cross section with three surfaces, the superior one is concave and is in contact with the femoral condyles, one inferior which is almost flat and lies on the edge of tibial condyles, one peripheral which is convex and is attached to the internal wall of the capsule.

The two menisci are incomplete rings forming two crescent shaped structures, their extremities are called anterior and posterior horns.

The lateral meniscus is more circular (the horns are closer together and approximate the ACL); the horns of the medial meniscus are more distant and it has the characteristic crescent or "C" shape.

The lateral meniscus is thicker and larger than the medial one. Both menisci have their external surface adherent to the internal part of the articular capsule except for a small tract of the posterior circumference of the lateral meniscus where it communicates with the popliteus muscle.

Every horn is attached to the tibial condyle respectively in the intercondylar fossa anteriorly and posteriorly. The anterior horns of the two menisci are connected by a fibrous ligament, the anterior (intermeniscal) transverse ligament of the knee.

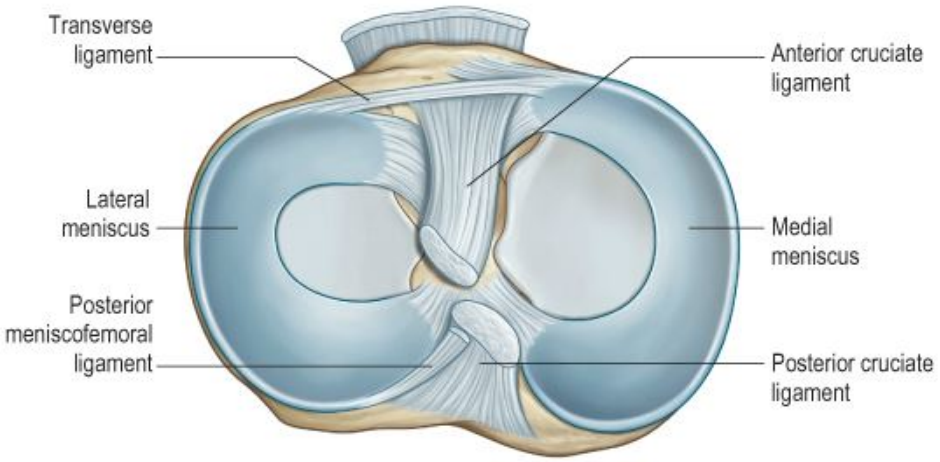


Fig. 1 Superior aspect of the left tibia showing the medial and lateral meniscus

The patella is the largest sesamoid bone in the body and is embedded in the tendon of the quadriceps femoris, lying anterior to the distal femur.

It is flat, distally tapered and proximally curved, it has anterior and articular surfaces, three borders and an apex which is the distal end of the bone.

The posterior surface is covered in cartilage, it has an oval articular area crossed by a smooth vertical ridge, which fits the intercondylar groove on the femoral-patellar surface and divides the patellar articular area into medial and lateral facets.

The base of the patella has a small triangular facet for the insertion of the quadriceps femoris muscle tendon.

The apex represents the attachment of the patellar ligament.

The bony parts of the knee joint are bound together by a thick articular capsule, a fibrous membrane which envelops the distal end of the femur and the proximal end of the tibia keeping them in reciprocal contact. This capsule is the non-bony part of the articular cavity.

This capsule is reinforced by five ligaments (anterior or patellar, external and internal collateral, anterior and posterior cruciate ligament) and is covered by the synovial membrane in its deep surface.

Anteriorly the capsule is replaced by the patellar ligament and does not pass proximal to the patella or over the patellar area. Elsewhere, it lies deep to expansion from vasti medialis and lateralis, separated from them by a plane of vascularized loose connective tissue. The expansions are attached to the patellar margins and patellar ligament extending back to the corresponding collateral (tibial and fibular) ligaments and distally to the tibial condyle. They form medial and lateral patellar retinacula, the lateral being reinforced by the ilio-tibial tract.

Posteriorly, the capsule contains vertical fibers that arise from the articular margins of the femoral condyles and intercondylar fossa and from the proximal tibia. The fibers mainly pass downwards and somewhat medially.

The oblique popliteal ligament is a well-defined thickening across the posteromedial aspect of the capsule and is one of the major extensions from the tendon of the semimembranosus.

The internal surface is covered with synovial membrane that does not cover cartilaginous articular surfaces and menisci.

Distal to the patella, the synovial membrane is separated from the patellar ligament by the infrapatellar fat pad. Where it lies beneath the fat pad, the membrane projects into the joint as two fringes, alar folds, which bear villi. The folds converge posteriorly to form a single infra-patellar fold or plica (ligamentum mucosum), which curves posteriorly to its attachment in the femoral intercondylar fossa.

Patellar ligament is the continuation of the quadriceps muscle tendon directs to the tibial tuberosity, it is a very thick and flat bundle.

Collateral ligaments prevent knee hyperextension and reinforce the articular capsule.

MCL resists valgus angulation and works in concert with ACL to provide restraint to axial rotation.

LCL is a cord-like structure that passes distally, superficial to the popliteus tendon and deep to the lateral retinaculum, to attach to the fibular head, where it blends with the biceps femoris tendon just anterior to the apex of the head of the fibula. It resists varus displacement at 30 degrees of flexion and resists posterolateral rotatory displacement with flexion that is less than approximately 50 degrees.

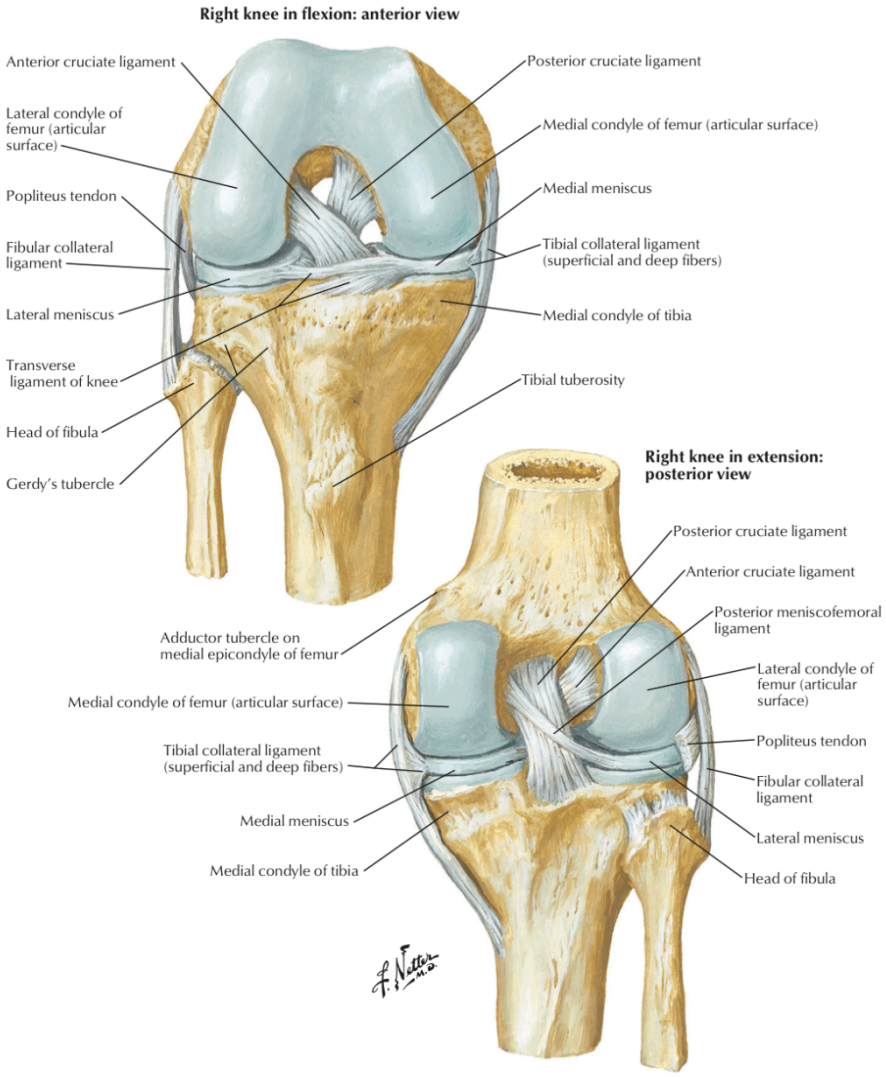


Fig. 2 Articular surfaces and ligaments of the knee.

Cruciate ligaments are very strong, richly innervated intra-capsular structures. The point of crossing is located a little posterior to the articular center. They are named anterior and posterior with

reference to their tibial attachments. A synovial membrane almost surrounds the ligaments but it is reflected posteriorly from the posterior cruciate ligament to adjoining parts of the capsule; the intercondylar part of the posterior region of the fibrous capsule has no synovial covering.

The anterior cruciate ligament (ACL) is attached to the anterior intercondylar area of the tibia, just anterior and slightly lateral to the medial intercondylar tubercle, partly blending with the anterior horn of the lateral meniscus. It ascends postero-laterally, twisting on itself and fanning out to attach high on the posteromedial aspect of the lateral femoral condyle. It resists anterolateral displacement of the tibia on the femur, stabilizes the knee during extension and avoids hyperextension.

The posterior cruciate ligament (PCL) is thicker and stronger that the ACL. It is attached to the lateral surface of the medial femoral condyle and extends up on to the anterior part of the roof of the intercondylar fossa, where its attachment is extensive in the antero-posterior direction. The ligament has a fan like structure in which fibre orientation is variable.

Anterolateral and posteromedial bundles have been defined, the anterolateral bundle tightens in flexion while the posteromedial bundle is tight in extension of the knee.

Each bundle slackens as the other tightens. It is not isometric during knee motion and resists posterior tibial displacement, especially at 90 degrees of flexion.

Dynamic equilibrium of the knee is determined by the combined work of ligaments, muscles and articular surfaces.

Quadriceps femoris muscle, the great extensor of the leg, covers almost all of the front and sides of the femur. It can be divided into four parts, one of them is the rectus femoris which arises from the ilium and travels straight down the middle of the thigh. The other three arise from the shaft of the femur and surround it (apart from the linea aspera) from the trochanter to the condyles: vastus lateralis is lateral to the femur, vastus medialis is medial to it and vastus intermedius lies anterior to the femur. Rectus femoris crosses both hip and knee joints, while the three vasti only cross the knee joint.

The tendons of the four components of the quadriceps femoris unite in the lower part of the thigh to form a single strong tendon attached to the base of the patella, and some fibres continue over it to blend with the patellar ligament.

The flexor muscles of the knee are six. Biceps femoris, semitendinosus and semimembranosus are collectively known as the hamstrings and make up the posterior compartment of the thigh. Sartorius, politeus and gastrocnemius are the other flexors of the knee. All these muscles are bi-articular except for the short head of the biceps and the popliteus that are mono-articular.

Bi-articular muscles are at the same time flexors of the knee, hip extensors and knee rotators.

External rotators are biceps femoris and tensor fasciae latae; internal rotators are instead sartotius, semitendinosus, semimembranosus, internal rectus and popliteus [8] [9] [10] [11].

1.2 Articular biomechanics

The knee works essentially by axial compression under the action of gravity.

However the knee is not a simple hinge joint and small motions in other planes are essential to enable the joint to flex and extend.

The knee is the most complex joint of the human body. From the mechanical point of view it is a compromise, which sets out to reconcile two mutually exclusive requirements:

- To have a great stability in complete extension, when the knee is subject to severe stresses resulting from the body weight and the length of the lever arms involved;
- To have a great mobility after a certain measure of flexion has been achieved. This mobility is essential for running and for the optimal orientation of the foot relative to the irregularities of the ground.

The knee resolves this problem by highly ingenious mechanical devices but the poor degree of interlocking of the surfaces, essential for great mobility, renders it liable to sprains and dislocations.

Flexion is the movement of the posterior aspect of the leg towards the posterior aspect of the thigh. Femoral “roll-back” is fundamental during flexion because rolling alone would cause the knee to

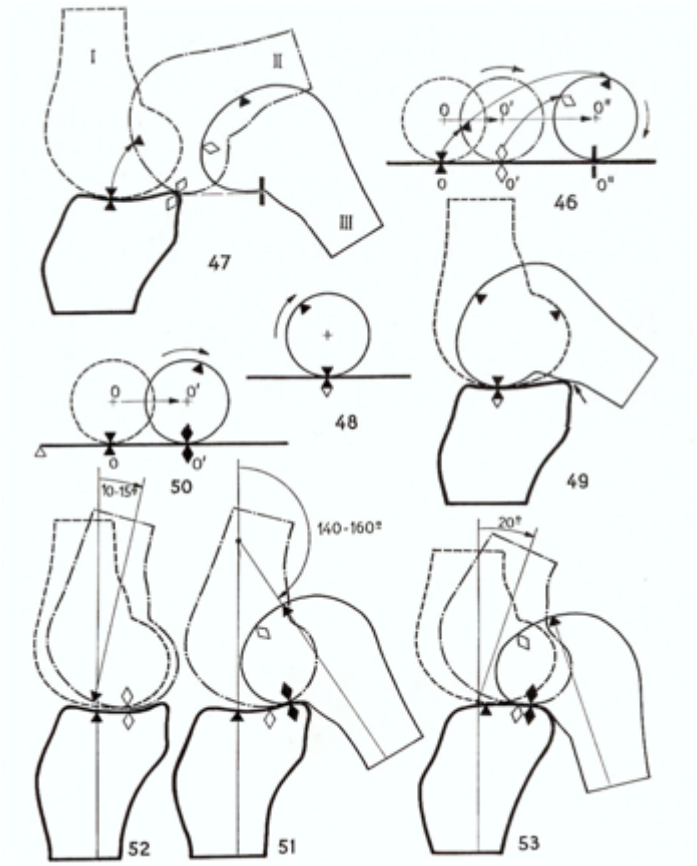


Fig. 3 Knee Biomechanics

dislocate as the distance around the femoral condyles is approximately twice the A/P width of the tibial plateau. Sliding only would cause impingement of the posterior femoral shaft on the posterior tibial plateau and block flexion. Rolling and sliding together allow the knee to remain stable and flex fully (fig. 3).

The patella articulates with the lateral femoral ridge when the knee is in full extension, and slides into the intercondylar notch at full knee flexion. The contact area becomes more proximal on the patella and more distal on the femur with increasing knee flexion. The size of the patella femoral contact area also increases with knee flexion i.e. with increased force of the quadriceps.

The range of knee flexion varies according to the position of the hip according to whether it is active or passive. Active flexion attains a range of 140° if the hip is already flexed and only 120° if

the hip is extended. This difference is due to the fact that the hamstrings loose some of their efficacy with the extension of the hip. Hamstrings muscles are bi-articular and are both extensors and flexors of the knee. When the hip is extended their flexors role is decreased because of a minor functional reserve, this causes a decrease in the functional flexion arch of the knee.

Passive flexion of the knee attains a range of ca 140° and allows the hip to touch the buttock. It is considered a passive range as it can only be achieved with manual assistance.

Extension is defined as the movement of the posterior aspect of the leg away from the posterior surface of the thigh. The patella articulates with the lateral femoral ridge when the knee is in full extension. In full knee extension the knee is internally rotated with reference to the tibia; the cruciate ligament four bar linkage mechanism is tightened and the joint is blocked. The center of weight of the upper body lies anterior to the center of rotation of the joint, and the resultant moment is balanced by passive resistance of the posterior capsule and ligaments. This allows the quadriceps to stop contracting and the position is maintained passively with little energy expenditure.

Perfect extension of the lower limb provides stability and solidity of the whole lower limb both in static and dynamic conditions.

Active extension rarely goes beyond the position of reference and then only slightly (5°) and this depends upon the position of the hip joint. In fact the efficiency of the rectus femoris as an extensor of the knee increases with the extension of the hip, so that extension of the hip set the stage for knee extension.

Passive extension is just slightly bigger than active movement and is around 5-10° in healthy subjects.

Static analysis can be used to estimate the compressive forces acting on tibiofemoral and patellofemoral joint. These forces increase with a deep flexion angle and also depend on whether the leg is in stance or swing phase of the gait cycle. The knee's joint reaction forces are greatest at maximum knee flexion during the single-leg stance period of the gait cycle. However maximum

knee flexion during the single-leg stance varies according to the undertaken activity. As a result the joint reaction forces produced in the knee compartment are very variable, and any analysis must take into account the exact loading conditions. [12] [13] [14]

1.3 Articular cartilage

Cartilage, together with bone tissue and other minor histological varieties, belongs to structural skeletal or connective tissues that have mechanical properties and important functions in electrolytes replacement.

Its anatomical structure is responsible for its functional characteristics; architecture, metabolism and composition of cartilage have to be known in order to understand not only its outstanding

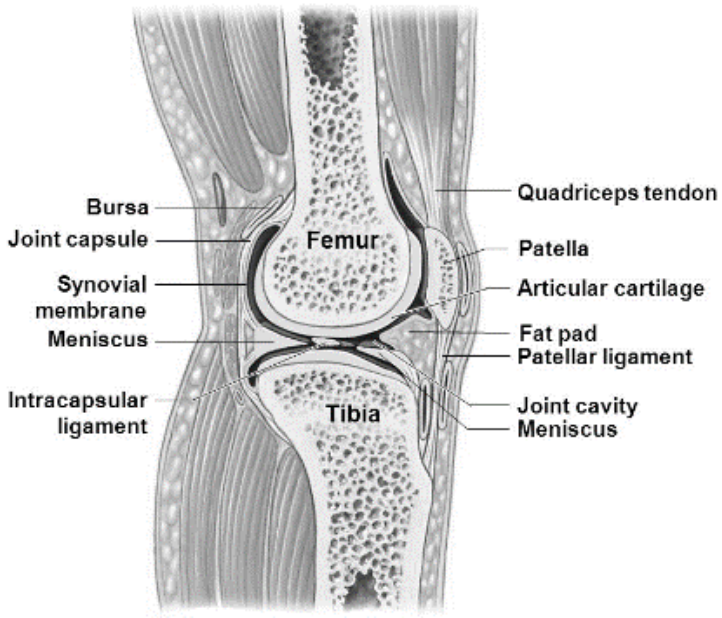


Fig. 4 Articular cartilage of the knee

biomechanical properties but also evolution and possible healing of its lesion.

1.3.1 Hystogenesis

Cartilage is a specialized, semi-rigid variant of connective tissue composed of cells called chondrocytes and an extensive highly specialized extracellular matrix. More than 95% of cartilage volume consists of extracellular matrix (ECM). ECM is solid and firm but also somewhat pliable, which accounts for its resilience. Since there is no vascular network within the cartilage the composition of the ECM is crucial to the survival of chondrocytes.

Chondrogenesis, the process of cartilage development, begins during the 5th week of embryonal life with the aggregation of chondroprogenitor mesenchymal cells to form a mass of rounded, closely apposed cells. These cells together give origin to the germinal center which forms a continuum with the surrounding mesenchyme.

Mesenchymal cells in these clusters start to secrete collagen and cartilage matrix differentiating into chondroblasts. At molecular level chondroprogenitor cells intensely express fibronectin, laminin and cartilage transcription factors such as omeoprotein-1 SOX9. In the early phases of cartilage differentiation production of collagen IIa is more prevalent, going on with the process, expression of collagen type IIb and aggrecan increase.

Secretion of extracellular material traps each chondroblast within the cartilaginous matrix thereby separating the chondroblasts from one another. Each chondroblast then undergoes one or two further mitotic divisions to form a small cluster of mature cells separated by a small amount of ECM. Mature cartilage cells, the chondrocytes, maintain the integrity of the cartilage matrix.

The mesenchyme which surrounds the cartilaginous layer condensates forming a compact layer of fibrous connective tissue: it is not well vascularized and is called perichondrium and it separates cartilage from the surrounding mesenchyme.

The perichondrium has important chondrogenic properties and covers mature cartilage everywhere except for the articular surfaces.

Cartilage is capable of two types of growth. The first type is interstitial growth which is the process that forms new cartilage within an existing cartilage mass. New cartilage produced by interstitial

growth arises from the division of chondrocytes within their lacunae, the daughter cells of the dividing chondrocytes produce new matrix and so the cells move apart one from the other and every cell occupies its own lacuna. The second type of growth is by apposition, which is the process that forms new cartilage at the surface of an existing cartilage. New cartilage cells produced during appositional growth are derived from the inner portion of the surrounding perichondrium. The cells resemble fibroblasts in form and function, producing the collagen component of the perichondrium.

In mammals the majority of the skeleton forms during development as cartilage, which is then replaced by bone. During the post-natal period cartilage remains in the areas between diaphysis and epiphysis of long bones allowing their growth in length (growth plate).

In adults cartilage is still present at the level of the articular surfaces and in a few other areas such as the ear, nose, larynx, trachea bronchi, costal cartilage intervertebral discs, pubic symphysis and menisci in the joints.

Neo-formation of cartilage takes place during repair processes secondary to fractures.

Most cartilage, unlike other connective tissues, is devoid of nerves and blood vessels. Consequently the exchange of metabolites between chondrocytes and surrounding tissues depends on diffusion through the ground substance; this limits the thickness to which cartilage may develop while maintaining viability of the innermost cells. [15] [16]

1.3.2 Components and biomechanical characteristics

Three types of cartilage that differ in appearance and mechanical properties are distinguished on the basis of the characteristics of their matrix: hyaline, elastic and fibrocartilage.

Of these three classes, hyaline cartilage (Fig. 5) is the most diffused in the human body. The matrix of the hyaline cartilage appears glassy in the living state: hence, the name hyaline [Greek:

Hyalos = glassy].

Hyaline cartilage is a complex living tissue full of chondrocytes located in spaces called lacunae. It provides a low friction surface, participates in lubrication of the synovial joints and distributes applied forces to the underlying bone.

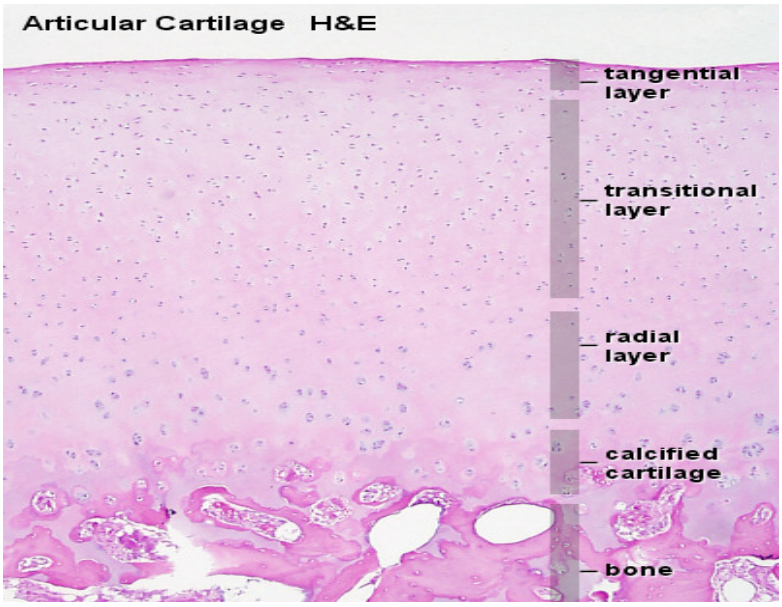


Fig. 5 Hystological aspects of hyaline articular cartilage

As already said in the embryo and fetus the skeleton is made by hyaline cartilage, which is then substituted by bone tissue during the ossification process. After birth, an area of cartilage remains and this is the so-called epiphyseal disc. It is located in between the epiphysis and diaphysis of long bones and is essential for their growing in length. This cartilage ossifies at the end of the growth process. Articular cartilage is a specific type of hyaline cartilage, it doesn't have perichondrium, it is a thin layer of tissue right above the subchondral bone and it is very smooth in order to allow the sliding of articular surfaces of the mobile joints.

Its biomechanical characteristics depend on its structure: few cells, highly specialized in protein synthesis, which are surrounded by a matrix rich in collagen fibers.

Chondrocytes are highly specialized and responsible for the unique features of cartilage; in normal conditions they have a poor mitotic activity, from the metabolic point of view they are responsible for the synthesis of macromolecules and the control of their distribution in the extracellular matrix.

Chondrocytes in the articular cartilage have a peculiar shape and disposition (Fig. 6), influenced by pressure loads that create mechanical, electrical and chemical signals that help direct their synthetic activity.

Modulating potential of the motor activity in the control of differentiation processes can be demonstrated by analyzing the evolution of periosteal scaffolds in the articular cartilage.

In particular, these scaffolds that are subject to biomechanical stimuli in vivo can undergo metaplasia, and thanks to the presence of totipotent stem cells in the osteogenic layer, can differentiate into hyaline cartilage.

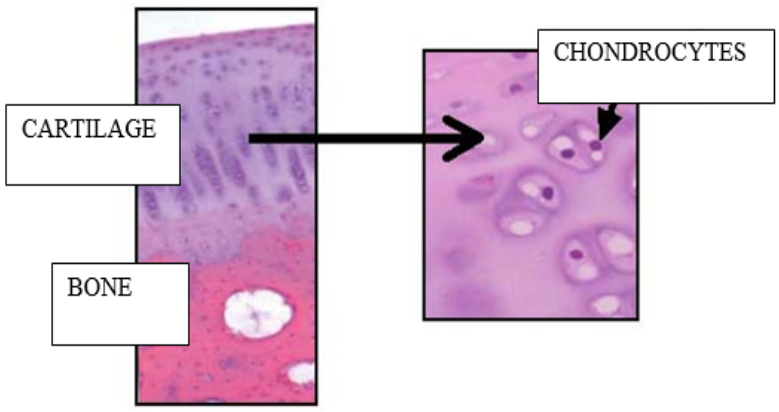


Fig. 6 Chondrocytes visualized by optic microscopy

With increasing intercellular substance, cartilaginous cells (chondroblasts, chondrocytes) move apart. They are enclosed in separated cavities in a newly synthesized matrix that are called lacunae. Lacunae can contain one or more chondrocytes (Fig.6).

In the deep zone of cartilage cells are grouped into clusters, the isogenous groups. When the chondrocytes are present in isogenous groups, they represent cells that have recently divided.

Cells are spherical and ovoid in shape and matrix is abundant. In the intermediate zone cells are rounded but are not grouped in clusters.

The superficial or tangential zone is the area that is more exposed to attrite and compression. Here the elements become flatter and the lacunae are close one to the other. Cells are more than the matrix. Chondrocytes in the superficial layer are ovoid with the major axis tangential to the free surface while in the intermediate layer the convexity is facing the superficial layer. In the radial layer chondrocytes are elongated and perpendicular to the subchondral bone. (Fig.7)

The distribution in adults and elderly is more irregular in the intermediate and tangential layer; in this age range another characteristic is matrix mineralization in the deepest part, the calcified zone.

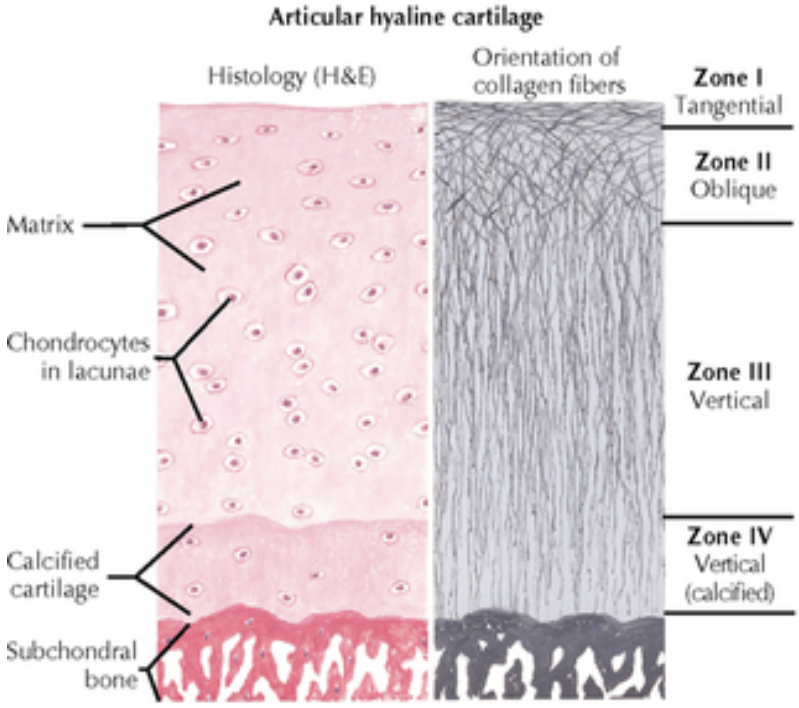


Fig. 7 Structure and composition of cartilage

The chondrocytes present in the cartilage of the epiphyseal plate are disposed in long parallel columns: this region of the bone is the so-called area of seriated cartilage that is substituted in time by the forming bone.

Chondroblasts contain a diplosome, numerous mitochondria, granular endoplasmic reticulum, free ribosomes and a big Golgi apparatus made by saccules, vesicles and vacuoles.

Glicogen is present in variable amount. Some vacuoles of the Golgi apparatus contain electrodense granules that stain with ruthenium red.

It is likely that this material is made by complexes of protein-glycosaminoglycan (or proteoglycan), elaborated by the ribosomes and the Golgi apparatus.

The appearance of chondrocyte cytoplasm varies according to their activity. Chondrocytes that are active in matrix production display areas of cytoplasmic basophilia, which are indicative of protein synthesis, and clear areas which indicate their large Golgi apparatus. In older, less active cells, the Golgi apparatus is smaller; clear areas of cytoplasm when present usually indicate sites of extracted lipid droplets and glycogen stores.

Hyaline cartilage matrix is highly hydrated to provide resilience and diffusion of small metabolites. Like other connective tissue matrices it is highly hydrated, from 60-80% of the net weight is intercellular water and much of this water is bound to the aggrecan-hyaluronan aggregates, imparting resilience to the cartilage. Some of the water is bound loosely enough to allow diffusion of small metabolites to and from the chondrocytes.

The high degree of hydration and the movement of water in the matrix allow the cartilage matrix to respond to varying pressure loads and contribute to cartilage's weight bearing capacity.

Collagen fibers and proteoglycans account respectively for 50% and 25-40% of cartilage dry weight. 14% is made of proteins (i.e. glycoproteins); sialoproteins 0,7% and lipids and other substances account for the rest. The elevated concentration of glycosaminoglycans is the main determinant of the high hydration of the cartilage matrix.

The two main components of extracellular matrix are proteoglycans and type II collagen fibers. Both of these molecules form a complex network, which allows the loading of the body weight for many decades. Type II collagen is typical of the articular cartilage. Collagen fibers in collagen are

thinner than those present in other tissues like bone or tendons. The main characteristic of a collagen fibril is the triple helical structure, where a single molecule is folded with two others. The collagen triple helix is formed by the union of three chains called alpha chains, which have a sequence rich in glycine and proline.

The proline is responsible for the left-handed spiral shape of the protein complex while hydroxyproline gives stability to the triple helix. This complex structure gives to type II collagen fibers an incredible resistance to traction forces. The other collagens in the ECM are thought to give extra stability to the type II fibrils, i.e. collagen IX that is not assembled into fibrils but forms covalent cross links between two or more fibrils of collagen type II.

The main cartilage proteoglycan is aggrecan, which is formed by a "core" protein linked to other molecules, the glycosaminoglycans (GAGs). The most represented GAGs are keratin sulphate and chondroitin sulphate. (Fig. 8)

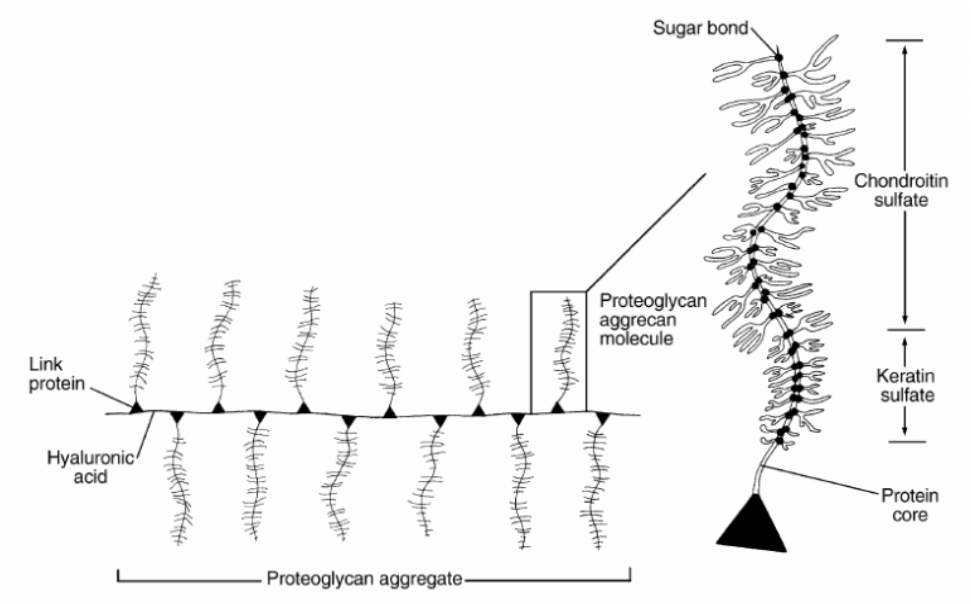


Fig. 8 Schematic structure of proteoglycans

The core protein is linked to a molecule of hyaluronic acid by other proteins that form the "linking complex".

A single bundle of hyaluronic acid is capable of linking several aggrecan molecules.

The biomechanical properties of articular cartilage have been studied with different models. The most suitable for the cartilaginous matrix seems to be the biphasic model proposed by Mow 30 years ago. In this model the response to various stresses is determined by an interaction between a solid phase (the matrix) and a liquid phase (the water).

The complex network of collagen II and proteoglycans provides two characteristics to the articular cartilage: resistance to a compressive stress and high elasticity. When a load is applied the water flows very slowly out of the tissue. This slow flow is due to the low porosity and to the negative charges present in the GAGs molecules, which exert a strong resistance to a volume reduction. In these conditions the main responsible for the load bearing is represented by the liquid: the uncompressible water sustains the compressive stresses protecting the solid components of the cartilage matrix, only partially involved in the biomechanical response. This is the so-called flow-dependent resistance to a compressive stress.

If the cartilage is damaged, such as in osteoarthritis, the water flows out very rapidly from the ECM involving significantly the solid component of the tissue in the biomechanical response. This may lead to a rapid deterioration of the whole cartilaginous tissue. [17]

On the other hand when a shear force is applied, no interstitial fluid occurs and the tissue generally deforms thanks to the randomly organized collagen fibers of the transitional zone. In this condition the matrix is directly involved in the biomechanical response and is therefore exposed to deterioration.

In order to reduce the shear stress, the cartilage tissue optimizes the superficial lubrication with the presence of a structure, which is perfectly organized from the biomechanical and morphological point of view due to the presence of the synovial fluid inside the joint.

Any type of damage, which causes an increase in the shear forces, like the superficial fibrillation, may lead to an increase in the involvement of the solid matrix in the biomechanical response and so to tissue deterioration. [18]

1.3.3 How articular cartilage changes with age

During life, all the cartilage components undergo many changes that alter their features.

Physiological degradation takes place during embryological development and during post-natal remodeling. There are specific signals such as IL-1 that stimulate chondrocytes to produce free radicals responsible for activation of metallo-proteinases.

Similar mechanisms occur in pathological conditions such as osteoarthritis.

Physiological repair of damaged cartilage takes place only in children; with cartilage aging there is a decrease in the number of cells responsible for repair: less cells and so less capability of generating new matrix.

Growth plate (Fig. 9) is characterized by a high cellularity, but cellular proliferation is inhibited in mature cartilage, probably because collagen fibers act as macromolecular barriers.

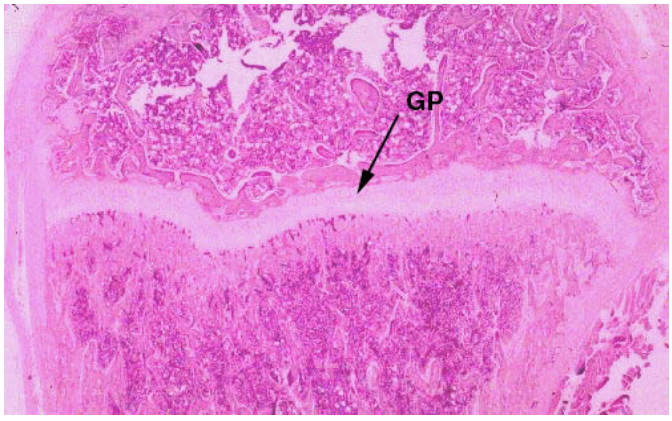


Fig. 9 Epiphyseal growth plate

In the matrix we can see differences in the distribution and composition of the components according to the age: in general, water is more abundant in the fetus, collagen fibers increase with age and relations between the different glycosaminoglycans change.

Collagen 3D structure is thought to be supportive for peptidoglycans, which are responsible for the resistance to mechanical stress.

When cartilage is compressed it undergoes deformations, and their reversibility is dependent on the capacity of peptidoglycans to bind water and by the fact that these molecules, expanded and hydrated, are limited in their movements because of the collagen net.

With the aging process peptidoglycans and water decrease and so cartilage is less responsive to mechanical stress and deformations can become permanent.

In the elderly cartilage undergoes a thinning process due to a decreased diffusion of nutrients among the ground substance. Over time the cartilage loses its transparency and becomes opaque and yellow; this is due to the decrease of peptidoglycans and an increase in proteins other than collagen. Cartilage is not vascularized and cells undergo atrophy.

Two degenerative phenomena are common during old age: calcification together with degeneration of cartilaginous cells and asbestiform transformation, matrix is invaded by many fibers (not collagen) that make the tissue very shiny, similar to asbestos.

When cartilage is damaged or destroyed, loss of substance is substituted by fibrous connective tissue coming from the perichondrium. In some cases it can undergo metaplasia and become cartilage.

1.3.4 Cartilage degradation

Cartilage degeneration is characterized by a massive proteolysis caused by collagenases with denaturation of collagen fibers. These processes begin at the surface and then migrate deeper. The superficial zone of hyaline cartilage consists of flattened chondrocytes and tightly packed collagen

II fibers oriented parallel to the articular surface. This layer is believed to provide resistance to shear forces and protect the deeper layers. The damage starts with collagen fibrillation at the top of the articular surface. In this stage, the histological proteoglycan specific stain diminishes as a result of the lack of proteoglycans. Chondrocytes proliferate in clusters and start to produce a large amount of proteoglycans, but they don't migrate nor fill the defect. These hypertrophic chondrocytes activate genes that are normally inactive and they codify the production of collagen I, III and X. Matrix composition changes, it calcifies and becomes more friable.

Altered synovial membrane produces cytokines that are able to increase catabolic functions and modify aggrecan synthesis causing a further loss of molecules by the matrix.

The residual aggrecan molecules take more space and retain more water, they cause edema and softening of cartilage. We can notice it also with arthroscopic procedures (I degree of Outerbridge classification). [19]

Cartilage degeneration continues and is divided into phases: thinning of the superficial layer, fissuration and erosion and if the damage progresses, fragments of the articular cartilage may be released into the joint and the subchondral bone may be exposed. As a result, the load is directly transmitted to the underlying subchondral bone, which responds by increasing its density and thickness.

CHAPTER 2

OSTEOCHONDRAL LESIONS

2.1 Epidemiology

Articular cartilage lesions represent a very frequent event, mainly among old people and athletes.

Degenerative pathology of cartilage is increasing in incidence and the main reasons are an increasing participation in sport activities and the associated high load bearing.

Full thickness lesions can be due to work-related trauma or sports. There are other factors that can increase the incidence of the cartilage lesions: age, previous fractures and surgeries, immobilization and drugs such as NSAIDs and corticosteroids.

The type of lesion usually changes according to the age of the patient: osteochondral fractures are more frequent during adolescence, full thickness lesions are rare before 30 years of age and partial lesions are common in the fourth decade. [20] Osteoarthritis is the most common cause of chondral lesions after age 40. Degenerative lesions are of different depths and shapes. Stiffening of subchondral bone results in less shock absorption and cartilage matrix breakdown [21].

A retrospective study [22] of 31.516 arthroscopic findings highlighted the presence of cartilaginous lesions in more or less 60% of cases, with an average of 2,7 lesions per patient. 41% of these patients had a III degree lesion, 19,2% a IV degree lesion, according to Outerbridge classifications.

We still don't know exactly the incidence of symptomatic and/or asymptomatic traumatic lesions of cartilage; we still refer to the data presented by Noyes [23] in 1980. He stated that the percentage of lesions in patients with acute hemarthrosis of the knee caused by work/sport trauma is around 5-10%.

All the joints of the skeletal system can present a chondral defect; the most involved areas are the knee, tibiotarsic and elbow. In the knee the principal localizations are the medial femoral condyle and the tibial plate (medial and lateral); it is less frequent to have lesions in the lateral condyle, the patella and the trochlea. Cartilaginous lesions can be isolated or associated with other pathologies of the joints; isolated lesions are rarer and are usually due to minor traumas.

According to Zamber [24] isolated lesions are present in 6,5% of the cases while in 61,5% of the cases they are associated to meniscal or ligamentous lesions and this is based on a prospective study considering 200 cases.

Curl [25] instead reported that the incidence of chondral lesions not associated to ligamentous or meniscal lesions is 36,6% in 31.516 arthroscopies.

In the vast majority of cases, there is association with ligamentous and/or meniscal lesions. These lesions can follow trauma and be independent from the damage to the cartilage, they can be the result of the same mechanism that caused the cartilage damage. Another possibility is that they can be the cause of the cartilage lesion altering articular stability and function.

Acute chondral lesions, rarely isolated, are more frequently associated to other articular damages than chronic chondral lesions, due to overuse. [26]

The unstable meniscal lesion, especially in the medial compartment, is very often associated to cartilage lesions. Even more common is the association between ACL rupture and cartilage damage, mainly in the medial compartment. When there is a rupture of the PCL, lesions in the patella-femoral area are also typical.

According to Murrel [27], the probability to have a chondral defect increase with the time passing from the ACL lesion and its reconstruction. Sometimes the cartilage lesion can come back after the reconstruction but in this case it is difficult to evaluate a possible association with the trauma or with the surgery itself.

Even the correlation between chondral and meniscal lesion is not very clear, it is true that an untreated meniscal lesion can lead to a chondral lesion but also an extensive meniscectomy can cause an early onset of arthrosis; there are many cases in literature reporting the onset of cartilage lesions after arthroscopy. [28]

Cartilage problems are still underestimated because of many reasons: the frequent association among different joint alterations, which share the same symptoms, and the lack of specific tests to evaluate cartilage state and the insufficient sensitivity of imaging methods.

2.2 Classification

Many classifications have been proposed in order to evaluate the gravity of lesions and to choose the best therapeutic option to re-establish a proper articular function.

Several pathologies can lead to chondropathies; an etio-pathological classification divides them in four main groups: osteochondrodistrophies, osteochondritis, osteonecrosis and arthro-synovitis.

Arthro-synovitis can be further divided into septic (due to bacteria, viruses, parasites) and sterile that can be traumatic, degenerative, metabolic or cancer-associated.

Cartilage lesions evolve through different stages, which characterizes morphological classifications [29]; this evolution is due to alterations of the relation between proteoglycans and cartilage, increase in the diameter of collagen fibers and the recurrence of traumatic events. The most used classification is the Outerbridge classification [30] that divides the lesions into four stages. (Fig. 10-11)

Fig. 10 Outerbridge Chondral Injuries classification

Grade I: Softening and swelling of cartilage

Grade II: Fragmentation and fissuring, less than 1.5 cm in diameter

Grade III: Fragmentation and fissuring, greater than 1,5 cm in diameter

Grade IV: Erosion of cartilage down to exposed subchondral bone

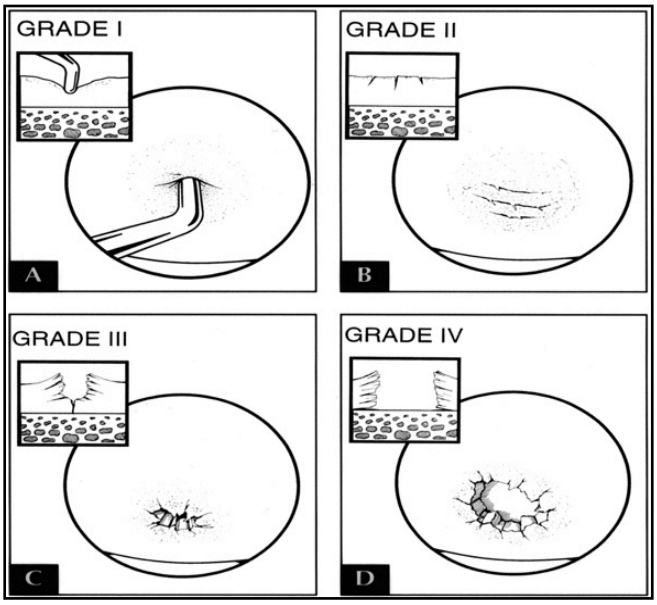


Fig. 11 Outerbridge arthroscopic classification of chondral injuries

Other authors take into consideration the morphologic classification developed by the International Cartilage Research Society (ICRS), that was published in 2000. It classifies lesions considering 4 grades of depth. (Fig. 12)

Grade 1a: Nearly Normal (soft indentation and/or superficial fissures and cracks; if lacerations or rupture it is grade 1b.

Grade 2: Abnormal (lesions extending down to <50% of cartilage depth).

Grade 3: Severely Abnormal (cartilage defects >50% of cartilage depth). Four subgroups:

3a) Defects that extend down to but not through the calcified layer;

3b) Defects that extend through the calcified layer;

3c) Defects that extend down to but not through the subchondral bone plate;

Grade 4: Severely abnormal (through the subchondral bone).

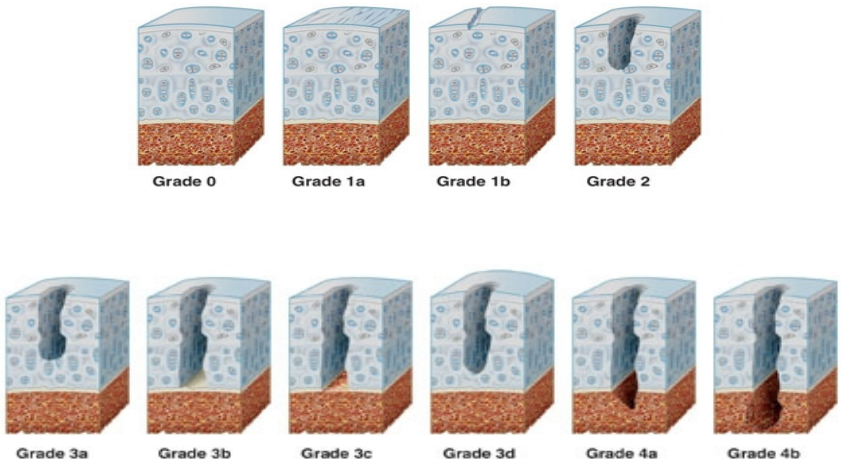


Fig. 12 ICRS classification of chondral injury

According to ICRS, only symptomatic lesions of grade 3 and 4 deserve a specific treatment (subchondral bone stimulation, osteochondral scaffolds or chondrocytes transplantation).

Osteochondritis, idiopathic pathologies involving bone and cartilage have another classification.

ICRS OCD I is a stable osteochondritis with continuous but softened area with intact cartilage; ICRS OCD II is stable with partial discontinuity; ICRS OCD III is characterized by in-situ lesions with complete discontinuity, while ICRS OCD IV is an empty cavity with dislocated or loose fragments. Apart from the severity of the lesion, we have to take into consideration other aspects: location and extension of the defect.

A precise subdivision into quadrants is useful for an accurate representation of the lesion. (Fig. 13) [31].

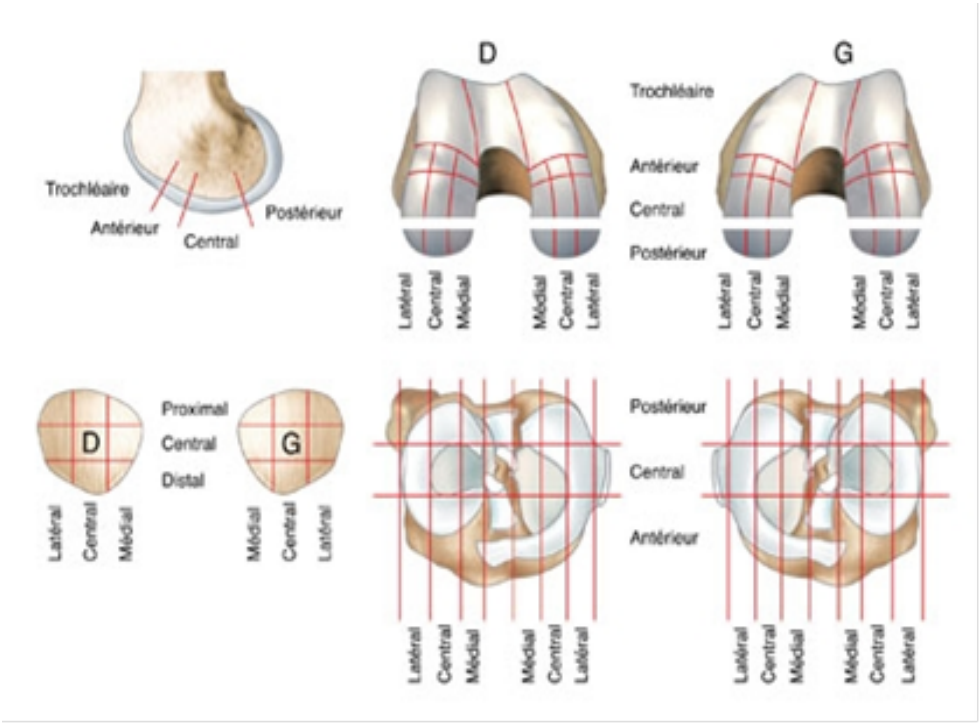


Fig. 13 Classification of cartilage lesions according to location and extension

2.2 Clinics and diagnosis

Chondral lesions can occur with a different range of symptoms, from being completely symptom-free to intense pain, limiting the daily activities.

The pain sensation can be due to the different structures in the joint: when there is cartilage loss, subchondral bone is subject to a higher pressure, which activates pain receptors of the peri-parietal nerve. There is an increased supply of venous blood to the subchondral bone which becomes congested, and this gives a further nervous stimulation; moreover metabolites and enzymes are released and they cause a painful synovitis, with capsular distension and even further pain. [32]

Pain can be present during or after physical activity and compromise it. It can also arise more subtly, as a mild complain which becomes so severe to limit the patient in his/her daily life activities. The pain can be dull, diffuse but also localized or stingy.

The location is variable: in case of patello-femoral injury, the pain would be anterior without instability; posterior femoral condyle lesion would result in pain associated to a sensation of instability [33].

Other symptoms commonly associated to this pathology are swelling after physical activity, intra-articular effusions, articular blocks and falls that can trick the orthopedic surgeon since mechanical symptoms are more associated to meniscal lesions.

Other aspecific symptoms are pain at palpation of the involved condyles, intra-articular noises and atrophy of the thigh muscles. Chondral lesions are divided into chronic, due to overuse of articular surface, and acute, usually associated with trauma. Symptoms like sudden pain, articular block, functional impotence and swelling are more typical of acute chondral lesions.

There are not pathognomonic manifestations for chondral pathology and so it is essential to evaluate carefully the patient. Starting from the history taking we have to elaborate a clinical suspect and direct our investigation in the most accurate way.

With history taking we have to investigate previous surgeries or trauma, pathological conditions that can induce cartilage degeneration, inflammation or structural alteration associated to an acute damage or overuse pathology, i.e. patello-femoral mal-tracking.

Other relevant data concern the onset modality, duration and predisposing factors for symptoms worsening, functional limitation, sensation of intra-articular loose body and articular blocks. An articular block of the knee can be due to a loose body in the joint, resulting from an OCD or a trauma that caused the detachment of the chondral fragment. The first thought is usually a bucket-handle meniscal lesion.

A proper visit should not be focused only on the involved joint; we have to evaluate the whole skeletal system for alterations that can cause problems distally or proximally because of the cinematic chains [26].

Physical examination investigates the patient in static and dynamic conditions.

In the first case the evaluation of the standing patient, standing on one or two feet, makes it possible to find postural alterations, pathological deviation from the axis.

During walking, some overload pathologies of cartilage that were missed in static conditions are evident, i.e. dynamic instability of lower limb joints.

With inspection we have to notice if there are any surgical scars, if muscular trophism is altered, if there is any swelling at the level of the joints. The range of motion (ROM) of the knee has to be evaluated, and it is normal between 0 and 150° (Fig. 14).

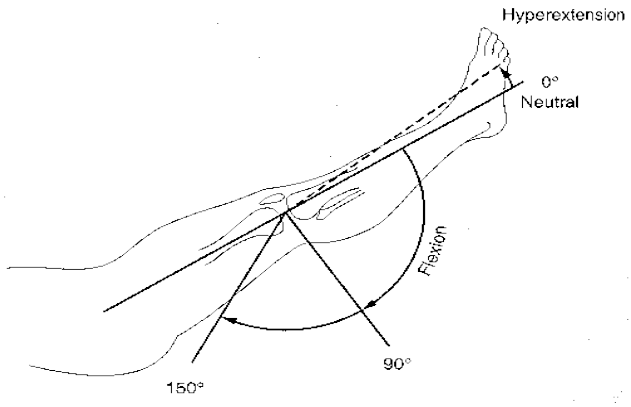


Fig. 14 ROM of the knee

With the patient relaxed and in supine position we have to perform palpation and see if he/she has pain; pain can be localized in the medial or lateral part of the joint, femoral condyles, patellar facets when we dislocate the patella.

We should also evaluate patellar mobility medially/laterally, and its position with respect to the quadriceps and patellar tendons. A patellar ballottement test is performed and we might detect

articular noises during flexion-extension, that may be a sign of articular degeneration. Unfortunately, there are not specific tests available so it is necessary to perform an imaging procedure to confirm or exclude our suspects [34].

The first exam, cheap, easy to perform, safe and widely available is conventional radiology.

With X-rays we cannot see cartilage directly but there are indirect signs that can direct us through the diagnosis.

MRI (Fig. 15) is the most specific examination to evaluate this kind of lesions; it is a repeatable and non-invasive test with multiplanar capacity, contrast is available to better study soft tissues, because of these reasons it is often preferred to CT scan.

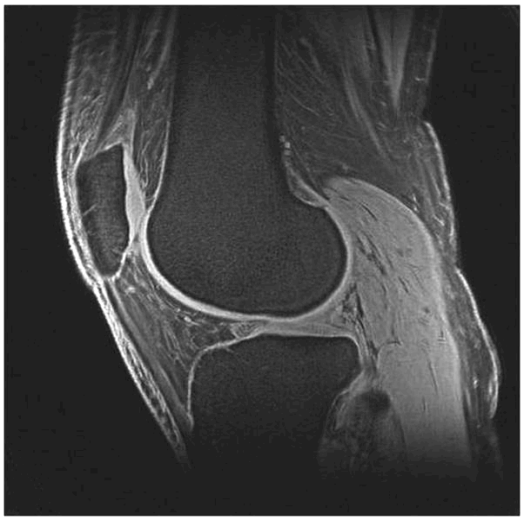


Fig. 15 Knee MRI

MRI allows the study of cartilage both morphologically, giving info about the lesions, thickness and volume and also from the biochemical point of view, so water content, proteoglycans, collagen, natrium, all of these are useful to characterize the health state of cartilage and to choose the best treatment for the found lesions.

However, MRI is not the solution to all questions. Many authors [35] have proven that sensitivity and reliability of this technique are not optimal; often, despite an accurate physical examination

together with modern techniques we can miss some lesions that we can discover only through arthroscopy.

Nowadays arthroscopy is the only truly reliable examination to diagnose and classify cartilage lesions. Some areas of the joint are not easy to access but can be studied in specific positions: posterior condyles can be seen in max flexion while it is possible to see the articular surface of the trochlea and the patella with extended or slightly flexed knee. To complete the study of the patient we can use biomechanical examinations and a dynamic evaluation can give us many useful information.

Kinesiologic studies can be used to evaluate movements during sports, work activities or everyday activities; if we correct wrong gestures and postures at this level we can remove risk factors and avoid cartilage lesions.

Biochemical markers are a new field in the diagnosis of cartilage lesions. With them we are able to characterize and quantify the chondral damage, even before the onset of symptoms.

They can be also useful to evaluate a current therapy, pharmacological or physical.

The most relevant markers are metallo-proteinases of synovial fluid like MMP3 (stromelysin) and MMP1 (collagenase), which are able to degrade intercellular matrix components.

Other authors [36] are also studying the synovial levels of MMP inhibitors and other cytokines in the blood. The main limitation to the use of these markers is the high cost.

In the future, their equilibrium would probably be at the base of treatment of chondral lesions and the use of inhibitors of harmful proteins could modify their natural history.

Nowadays, treatment of high-grade chondral/ostechondral lesions is essentially surgical, with reparative or reconstructive techniques.

CHAPTER 3

TREATMENT OF CHONDRAL LESIONS

3.1 Treatment principles

The increase in life expectation is associated with an increased joint overuse in subjects that are too young to undergo joint replacement. For this reason cartilage pathology is a current and very interesting problem for orthopedic surgeons. They are elaborating many different strategies of intervention but even if all of them share some positive results, no one can be considered ideal.

It has been known for a long time that cartilage has a poor regenerative capacity: in 1743 J. Hunter [37] wrote: “From Hippocrates to the present age it is universally allowed that ulcerated cartilage is a troublesome thing, and that once destroyed, is not repaired”.

Successive studies [38] have demonstrated that cartilage damage can be physiologically repaired within certain limits; the type of lesion, its entity, localization and age of the patient are the factors that influence the outcome.

The evolution of bigger lesions has been studied: they are filled by a fibrin clot and in the first 2-5 days there is migration of mesenchymal stem cells which later differentiate into chondrocytes.

In the first two months this newly formed tissue is similar to hyaline cartilage while with time it starts having characteristics typical of fibrous cartilage. Over time, this tissue degenerates into fibrocartilage; after three months there are evident signs of this degeneration, and after six-twelve months repair cartilage becomes irregular and eroded [39].

In the end, this resulting fibrous tissue has structural and functional features that are quite different and largely inferior to the original healthy hyaline tissue. This leads to an inevitable degeneration of the articular surface over time [40].

We can differentiate surgical techniques according to two main concepts: treatments without reconstruction of the defect and treatment with reconstruction.

Treatments without reconstruction are: articular lavage, articular debridement, shaving and bone marrow stimulating techniques such as subchondral perforations, abrasions and microfractures.

To reconstruct the defect instead we can use different approaches:

- Autologous graft like in osteochondral transplantation, mosaicplasty, perichondrium or periosteum transplantation and autologous chondrocytes transplantation;
- allograft or synthetic material like polymeric matrices and scaffolds.

Going back to the treatment for cartilage lesions, it is not possible to identify an ideal treatment.

Every technique has its pros and cons, and probability of success is related to the age of the patient, his/her level of physical activity, localization and also the size and depth of the lesion.

3.2 Treatments without defect reconstruction

3.2.1 Arthroscopic articular lavage

It is a simple and easy technique to perform but the disadvantage is that it improves the symptoms of the patient but it does not have any effect on the etiologic mechanisms of the disease.

Jackson et al. [41] have shown that 88% of patients who underwent “washing out” procedures had an improvement, but after 3 years just 68% of these patients were able to maintain the initial improvement.

Articular lavage is based on the removal of fragments of degenerated cartilage, inflammation mediators produced by the synovia and intra-articular catabolic products like proteases, hydrolases

and proteoglycans, responsible for inflammations. The clinical results are linked to the modulation of inflammation, thus leading to an improvement in the symptomatology and in the articular functioning. The cartilage lesion is not addressed by the articular lavage, so the benefits are usually short lasting. Despite some pros, this procedure is definitely not a solution for a very active person or for a professional athlete.

3.2.2 Articular Debridement

When articular debridement is added to the lavage, we have better and longer lasting results [33].

Hubbard (Hubbard, 1996) performed a study on patients with isolated cartilage lesions on the medial femoral condyle, graded III or IV in the Outerbridge classification: in this study it was documented a significant improvement in patients who underwent a articular debridement, whereas the same improvement was not seen in patient who underwent a simple articular lavage.

More than 50% of patients treated with debridement didn't have pain 5 years after the procedure.

Furthermore, Messner and Malatius [42], have carried out a study considering 28 young adults with a cartilage lesion bigger than 1 cm^2. They have noticed that 21 out of 28 have maintained a functional improvement 14 years after treatment.

Debridement technique consists in cleaning the articular cavity with mechanical removal of cartilage flaps and major fibrillations, excision of large meniscal tears, partial synoviectomy and removal of anterior osteophytes [43]. Its purpose is to remove mechanical impingement and formation of intra-articular loose bodies, reducing inflammatory state.

The use of cartilage debridement as an isolated procedure remains widely accepted for symptomatic isolated chondral lesions smaller than 2 cm^2 in less active patients [44].

Isolated debridement is a purely palliative procedure that does not lead to cartilage restoration, but it may successfully target the mechanical symptoms, such as clicking or catching. Since the origin of

cartilage-related pain is not well understood, its trend after debridement is unpredictable. The natural history of debrided small cartilage lesions remains unclear [45, 46].

3.2.3 Subchondral perforations

That is a marrow stimulation technique that tries to promote chondrogenesis and fill the cartilage defect. Normally, in other tissues of human body, the reparative process is based on bleeding, formation of fibrin clot, cellular mobilization, and production of mediators and growth factors.

However, as we have already outlined, cartilage is an avascular tissue and so it is not able to regenerate. A minimal spontaneous regeneration can take place at the borders of the defect, thanks to a transient chondrocytes proliferation. A bigger reparative response is present in lesions which penetrate the osteochondral layer, starting the process of formation of fibrocartilage.

In 1959, based on these assumptions, Pridie [47] proposed a technique to stimulate the spongy vascularized bone and activate chondrogenesis. This technique includes debridement of damaged articular cartilage and fibrous tissue involved in the defect. The aim of this procedure is to demarcate healthy cartilage so that the fibrin clot can bind easily to the borders of the defect.

The first phase of the treatment consists in cleaning the ground of the lesion with the elimination of the calcified cartilage through a curette. In this way the subchondral bone is exposed and it is surrounded by a perpendicular border of healthy and viable cartilage.

Second phase is perforation of the subchondral bone; the holes have to be very close one to the other but not too much to avoid damage.

The surgeon performs 3-4 holes 3-4 mm deep per cm^2 with an arthroscopic drill; after removal of the tourniquet, blood and fat come out of these holes, and due to the rough surface, cell adhesion is promoted and also formation of the fibrin clot. Drainages are not inserted because they can prevent clot formation; over time, pluripotent stem cells coming from the bone marrow differentiate, and

they give origin to the reparation tissue. Some studies performed on bioptic samples have highlighted a predominance of fibrous/fibro-cartilaginous tissue [48].

Unfortunately, these repair tissue does not have the same biomechanical characteristics of hyaline cartilage and it becomes unable to stand the many stresses present at the level of the knee, subsequently undergoing a gradual degeneration, and pain resumes due to the mechanical stimulation of the subchondral bone.

The negative aspect of this procedure is the necrotizing effect of power-driven perforations; furthermore, the cartilage-like tissue found in the early phases is substituted after 8 months-1 year by fibrocartilage which is weaker, softer and less resistant to mechanical stresses.

3.2.4 Abrasion arthroplasty

This technique was introduced by Johnson [49] in 1986: the surgeon uses a motorized burr to create multiple superficial dimples of about 1-3 mm of thickness. The abrasion of the sclerotic bone leads to bleeding and formation of a blood clot that attaches to and fills the defect of abraded areas and will transform in fibrocartilage by 4 to 6 months. This fibrocartilage is rich in fibroblasts and poor in proteoglycans and so very poor in quality and duration. In other studies by Friedman et al, Bert and Maschka, and Rand it was outlined that the patients with the greatest benefits were patients younger than 40 with satisfying results in 50% of cases at 3-5 years.

3.2.5 Microfractures

This technique was firstly introduced by Steadman [50] in 1986 and it is similar to and has the same indications of subchondral perforations. This is an arthroscopic technique: the first step consists in the accurate cleaning of the ground of the base of the lesion with removal of calcified cartilage and of a perpendicular layer of healthy cartilage.

Following the first step, the surgeon proceeds with penetration of the subchondral bone with a dedicated instrument (i.e. chondropick), less invasive with respect to perforations. Several small holes are performed in the bone to a depth of around 4-5 mm. This causes blood and bone marrow to seep out of the microfractures and create a fibrin cloth on the damaged area (Fig. 16).

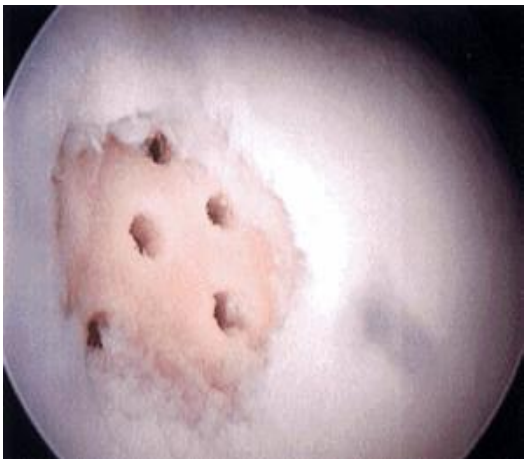


Fig. 16 Microfractures: arthroscopic view

The cells of the bone marrow are pluripotent mesenchymal stem cells that can differentiate into cartilaginous tissue, fibrous tissue or bone tissue according to the microenvironment and biophysical stimuli they are exposed to. Microfractures have a milder necrotizing compared to motorized drilling and the healing potential of the tissue is higher. Specially designed arthroscopic awls with different angulations can be used in order to reach also hidden areas, so that the resultant surface is more irregular and rough and the clot is better adherent.

Among young active patients who underwent microfractures, 75% of patients experienced an improvement in pain at 3-5 years from treatment; 20% experienced no improvement and 5% got worse. Evaluating daily life and working life activities performance, 67% of the patients had an improvement, in 25% of patients nothing changed and 13% of patients got worse. Professional athletes and patients with a physical job improved in 65% of cases [50].

This stimulation treatment shows many advantages: one-step surgery, easy, fast and cheap to perform. Unfortunately newly formed fibrocartilage has characteristics that are different from normal articular cartilage: it is less organized, more vascularized and has a different composition in water, proteoglycans, prevalence of type I collagen [48, 49].

This repair tissue is less resistant and less stable, and undergoes a progressive degenerative process, which is not fully understood yet. This brings to symptoms recurrence and a gradual increase in the size of the defect.

This procedure has been shown to provide positive outcomes in young patients (younger than 30 years old) with small, acute, traumatic, Outerbridge grade III and IV lesion with relevant functional demand and the need to come back to activity in a short time. On the other side, they are also useful for bigger lesions in patients with a reduced functional demand. Recent studies [51] have highlighted the value of continuous passive mobilization in the post-op while it was demonstrated that an early return to physical activity is a risk factor for worse results.

3.3 Treatments with defect reconstruction

3.3.1 Autologous single-plug osteochondral transplantation

This technique is used when there is an important loss of cartilaginous and bony substance.

It is an easy surgical procedure and it has the advantage of a single surgical step with the possibility of obtaining a good articular congruency. The disadvantage of this technique is that it cannot be done in arthroscopy and so the surgical trauma is quite significant for the patient and there is also an important morbidity of the donor site (Fig. 17). A single osteochondral plug from the trochlea or from the antero-superior part of the lateral femoral condyle is harvested by using osteotomes. Afterwards, the osteochondral autograft is positioned in lesion site properly prepared to receive the

plug. Yamashita et al [52] have highlighted that the transplanted graft maintains the viability of hyaline cartilage and has a fibrocartilage-like organization.

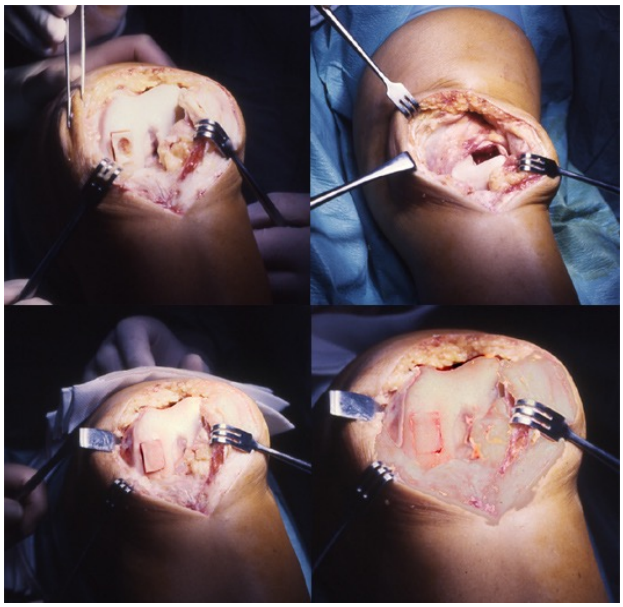


Fig. 17 Surgical Technique

3.3.2 Mosaicplasty

This technique was described by Hangody [54] in 1998. It uses small cylindrical osteochondral grafts, taken from a non-loading area of the antero-superior surface of the lateral femoral condyle (LFC), which are afterwards inserted at the level of the cartilaginous defect. (Fig. 18)

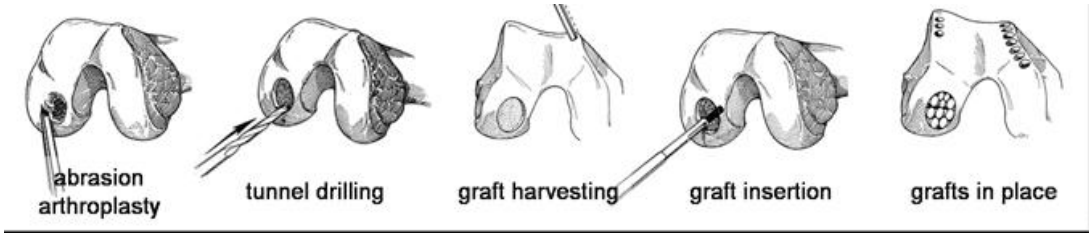


Fig. 18 Preparation of the lesion site, harvest of OC cylinders and implantation

This surgical technique can be done in a single surgical step, and it favors bone-to-bone healing of the grafts. In the first phase the lesion site is prepared with debridement and removal of granulation tissue (if present), and the next step is the mapping of the damaged surface.

An advantage of this technique is that it can be performed in arthroscopy, reducing this way the rate of open surgery complications (Fig. 19).

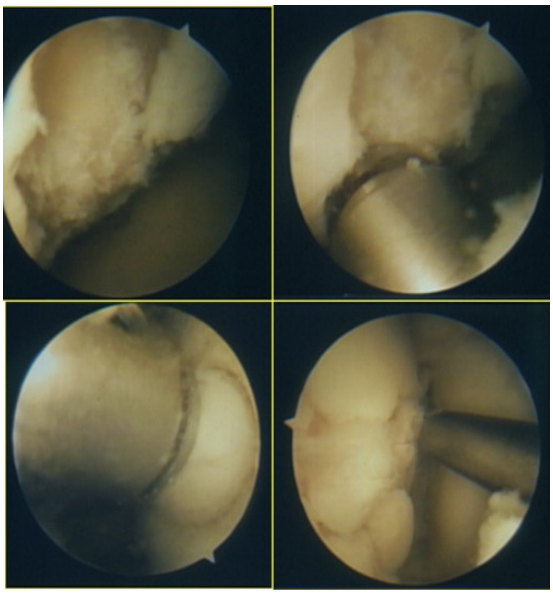


Fig. 19 Mosaicplasty, surgical technique: intraoperative arthroscopic view

The harvesting is usually performed in the less weight bearing areas: the external margin of the lateral femoral condyle, above the terminal sulcus or at the level of the roof and anterior margin of the intercondylar notch.

The graft harvester with a collared pin is introduced perpendicular to the donor site to obtain a circular structure. At more or less 16 mm of depth, the harvester is twisted abruptly 90° clockwise and counterclockwise with a parallel pull to remove the donor plug [29].

The recipient hole is created at a depth equal or 2 mm less than the donor graft just harvested, and extracted in the same manner as the donor core. It is important to maintain a perpendicular direction with the articular surface to create well-defined vertical walls in the recipient hole, which will

facilitate congruent plug placement. The donor grafts are inserted in these holes and fixed with "press-fit" technique. It is necessary to be careful with positioning to avoid damages to the cartilaginous surface, condition that can lead to worse result in the future.

Mosaicplasty has many advantages: harvesting of multiple osteochondral cylindrical grafts of different dimensions so that the lesion can be filled in a sufficient way (up to 70% of the entire surface) [54], and at the same time we can restore as much as possible the physiologic ray of curvature of the articular surface.

This kind of surgery has some important key points: it is essential to be very precise during harvesting and position of the plugs, early mobilization and gradual loading; ideal lesions for this treatment are traumatic one less than 2 cm^2 but full thickness, with well-defined margins surrounded by healthy hyaline cartilage [55]. The disadvantage of mosaicplasty is the fact that bigger lesions cannot be treated because they would require a too invasive harvesting with problems in the healing of the donor site.

3.3.3 Perichondrium and periosteal transplantation

This technique consists in filling the cartilaginous defects with perichondrium and periosteal grafts. Perichondrium seemed to have a big potential when used on animal models [56], inducing the growth of a cartilage-like tissue in the non-weight bearing areas, and of fibrocartilage in the weight bearing areas. In 1990 Homminga [57] used a perichondral autologous graft, taken from rib cartilage, to treat chondral lesions of the knee (grade III-IV). The grafts were cut to match the size of the defects and were positioned using human fibrin glue to stabilize them. In lesions sized between 2-3 cm^2 , perichondrium has given a significant improvement: arthroscopic evaluation at one year from surgery showed that in 90% of cases the defect was filled with tissue resembling cartilage and the improvement was evident also from the clinical point of view.

However, evaluations performed at 2 years showed a calcific hyper density in the majority of cases, thus suggesting a transplant failure. The graft underwent endochondral ossification and the formation of bone in the reparation site caused recurrence of symptoms [22].

Concerning the periosteum, it has osteogenic capacity, but it can also be used to promote cartilage formation in a chondrotrophic environment. It was showed that free periosteal grafts transplanted to the completely chondrectomized articular surfaces of patellae in experimental animals differentiated into cartilage [58]. The recipient site was prepared with debridement and periosteum was placed at the base of the defect.

This technique was used to treat full-thickness patellar defects through periosteal grafts harvested from the tibia, but even if the results seemed promising at the beginning, at long term evaluation all implants failed [22]. The rationale of this approach is similar to the one of marrow stimulation techniques, and is based on the attempt of bringing to the site of lesions stem cells that can give origin to new cartilage. Perichondrium and periosteum contain both fibrocytes progenitor cells and support cells that if stimulated can fill chondral defects. The best results have been obtained in young patients [59] in whom there is a bigger number of undifferentiated cells, while a reduction in chondrogenic potential was observed with aging.

The advantage of this technique is the single surgical step, even if there is the necessity of a double access, grafts are thin and difficult to manipulate, and the integration with subchondral tissue is not always perfect. A long rehabilitation is necessary: it was demonstrated that continuous passive mobilization in the post-op favors healing.

3.3.4 I generation autologous chondrocytes implantation (ACI)

The use of autologous chondrocytes implantation started in the early 80ies.

Many studies have demonstrated the possibility to stimulate chondrocytes reproduction in vitro [6], and animal studies showed the production of hyaline-like repair tissue when cultured chondrocytes were implanted beneath a periosteal patch [60].

3.3.4.1 Autologous chondrocyte transplantation with periosteal patch

The procedure involves the use of a chondrocytes solution (chondrocytes previously expanded in vitro) and a periosteal patch stabilized with stitches and fibrin glue to avoid the loss of the injected cells.

The surgical technique is divided in 2 steps (Fig. 20)

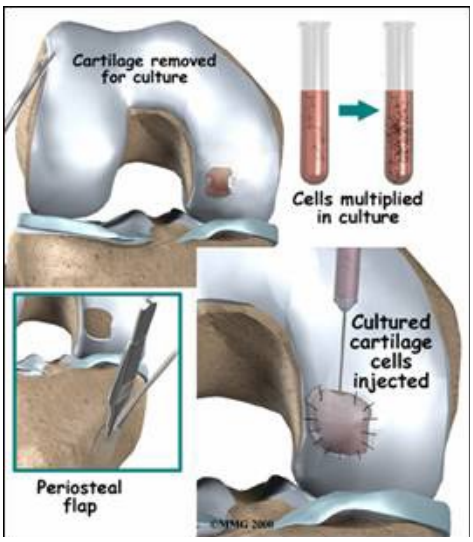


Fig. 20 Surgical technique for autologous chondrocyte transplantation with periosteal patch

The first step consists in an arthroscopic surgical procedure with the patient under general or spinal anesthesia and in a tourniquet-controlled bloodless field. The defect is examined and, if appropriate, biopsies of healthy cartilage (ca. 200 mg) are obtained from a non-weight bearing area. In the OR the bioptic material is put in sterile probes containing 0,9% NaCl and sent to the lab. The biopsies are prepared in the laboratory so that the chondrocytes are isolated and expanded in cell cultures; this process lasts approximately 4 weeks, during which the number of chondrocytes increases up to

10-12 times. Three days before the surgical step the cellular cultures undergo a quality assessment in which sterility and morphological characteristics are evaluated. If the cells have a viability of at least 85%, they can be moved to a sterile container and the orthopedic surgeon should perform the transplantation within 48 hours.

Therefore, the second operation consists of a traditional open surgery. Accurate debridement and stabilization of the cartilage edges are the fundamental steps of this procedure. Periosteum is harvested (from the proximal tibia or distal femur) and sutured to the defect with resorbable sutures (Fig. 21).

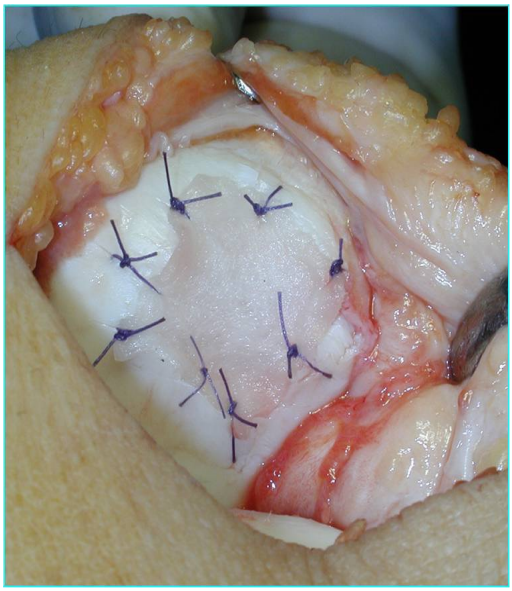


Fig. 21 Surgical technique for implantation of autologous chondrocytes: suture of a periosteal patch with resorbable stitches

Final sealing is achieved using fibrin-glue. It is important to create a watertight chamber underneath the periosteum, where the expanded cells are injected.

Most rehabilitation protocols use continued passive motion postoperatively and partial weight bearing for the first eight weeks. This surgical technique, followed by an adequate post-op rehabilitation, provides very satisfactory results up to long-term evaluation; in fact, the type of

tissue found in the bioptic samples is hyaline-like. Matrix is extended and homogeneous, round chondrocytes are present in the lacunae, and they are similar in shape and size to those of hyaline cartilage. Furthermore there is a predominance of proteoglycans and collagen type II, while typical aspects of fibrocartilage are not present.

There are anyway differences with normal healthy cartilage: cells are more numerous and are organized in a different way. Overall, this hyaline-like cartilage can deal with biomechanical demands of a patient with an active life style. The first clinical results were published in 1994 and they gave very positive outcomes, with bioptic examinations that confirm the presence of hyaline-like tissue.

In 1998, at the Annual Congress of American Academy of Orthopedic Surgeons, Peterson presented the results at 2-10 years in 219 patients treated with this technique, showing an improvement in 89% of patients with isolated lesions of the femoral condyles, in 74% of patients with a lesion of the femoral condyle associated with ACL injury, in 84% in case of OCD and in 69% in patellar chondral lesions.

Sgaglione [61] stated that autologous chondrocyte implantation is a safe, effective and reproducible procedure, which has to be considered a suitable option for treatment of chondral lesions bigger than 2 cm^2 in young patients who want to go back to a good level of physical activity.

Autologous chondrocytes transplantation represents an interesting therapeutic option but with some disadvantages: the liquid suspension of chondrocytes in culture is difficult to handle during the surgical procedure and the need of a periosteal patch that makes the procedure more difficult. The maintenance of the chondrocytes phenotype is difficult during a prolonged growth in culture: in these conditions chondrocytes tend to lose their ability to produce ground substance and type II collagen [64].

The suture of the patch is a long and accurate procedure, that can lead to post-op complications such as arthrofibrosis and infections.

Micheli et al [62] in 2001 has registered necessity of re-intervention for articular rigidity in 42% of cases. Hypertrophy of periosteal patch is also a frequent complication of this treatment and needs a re-intervention in the majority of cases [63, 64].

A further difficulty of this procedure concerns the formation of a negative pressure in the region beneath the periosteal patch, which avoid the leak of the liquid solution containing the chondrocytes during the first phases of mobilization of the limb.

3.3.4.2 Matrix assisted chondrocytes transplantation (MACT) – II Generation ACI

Second generation ACI uses tissue-engineering techniques to create a hyaline-like tissue in a tridimensional culture within a matrix. This procedure is based on the use of temporary and biodegradable polymeric matrices as growth site for viable cells in vitro. Chondrocytes kept in monolayer culture at low density eventually change from a polygonal or round to a flattened, amoeboid-like shape, and instead of producing type II collagen, they synthesize the genetically different type I collagen [65]. Conversely, it was demonstrated that the use of 3D scaffolds favors the retainment of chondrocytes' original phenotype [66]. Some essential features of these scaffolds are biocompatibility and biodegradability through safe biochemical processes and within adequate time spans. Solid scaffolds have a substrate to which cells can adhere, whereas gel scaffolds physically entrap the cells. Many different scaffolds have been used in the attempt to create new cartilage tissue, from natural to synthetic, with many different shapes and compositions.

The most used synthetic materials are polylactides like polylactic acid (PLA) and polyglycolic (PGA). Mechanical properties of synthetic biomaterials and their degradation are more easily modified with respect to natural fibers, but degradations of their components might be harmful for the native tissue and for implanted cells. Natural materials used to produce scaffolds are agarose, alginate, hyaluronic acid, gel, fibrin glue, all coming from collagen and acellular collagen matrix.

Matrices that have been commonly used in the clinical practice in Europe include collagen and hyaluronic acid. The use of 3D scaffolds for cellular cultures in open surgery allows a reduction in the time of the joint exposition because it is possible to avoid the periosteal patch harvesting.

Moreover, for some scaffolds, arthroscopic techniques for implantation have been developed.

The application of this approach has been well documented in many trials [67], with evaluation of the clinical outcome up to long term.

The use of Hyalograft-C® for treatment of chondral defects has been introduced in Europe in 1999.

This scaffold is a non-woven mesh of a hyaluronan benzyl-ester: HYAFF®. Hyaluronan is an important component of extracellular matrix [68]: it is a glycosaminoglycan, i.e. a linear polymer made by a dimer that is regularly repeated; it is a soluble molecule, whose molecular weight varies from 4.000 and 8.000 Da; when in water it creates a viscous solution. This polysaccharide is not just a "spacer": it has many functions in determining cartilage structure and function.

Hyaluronic acid is important in maintaining the structure of the tissue and its hydratation, and thanks to its hydrophilic properties it can influence cellular movements regulating osmotic pressure.

It actively interacts with proteoglycans and growth factors stimulating the production of ECM and acts as a scavenger of free radicals. Furthermore it was recently discovered that hyaluronic acid contains some receptors like CD-44, through which it can regulate cellular adhesion, growth and differentiation. Considering all its properties, it seemed an ideal candidate for tissue engineering constructs. Unfortunately, its rapid water solubility and the quick reabsorption make it not usable in purified form, so it has to be processes in a different form: HYAFF is derived from the total esterification of sodium hyaluronate with different alcohol and according to the esterification percentage we can obtain different polymers, different for duration and consistency.

The most used is HYAFF-11, entirely based on the benzylic ester of hyaluronic acid. It consists of a network of 20-lm-thick fibers with interstices of variable sizes, and has been demonstrated to be an optimal physical support to allow cell-cell contacts, cluster formation, and extracellular matrix

deposition. It has a weight of 120g/m^2 and thickness of 2 mm. Degradation mechanism of these biomaterials is very important; de-esterification in water causes the release of molecules that are not toxic [69].

This tridimensional structure presents interstitial spaces of different dimensions that act as a physical support for cells, allowing contact among them and clusters formation, maintaining phenotypical differentiation. The cells harvested from the patient are expanded and then seeded onto the scaffold to create the tissue-engineered product known as Hyalograft C. Seeded on the scaffold, the cells are able to re-differentiate and retain a chondrocytic phenotype even after a long period of in vitro expansion in monolayer culture [70]. Properties of HYAFF-11 to favor the growth of human chondrocytes and maintain the original phenotype have been demonstrated in vitro and the efficacy of the cell-scaffold construct was also proven by in vivo implantation in an animal model. Some studies has evaluated reparation tissue in chicks: chondrocytes attached to the support fibers, tend to aggregate and produce those molecules typical of hyaline cartilage: collagen type I and II and glycosaminoglycans. Another study based on Hyalograft-C transplantation with human cells in atymic mice has documented the formation of a tissue similar to hyaline cartilage: the implant was white, not vascularized and well attached to the articular surface. Hystological examination showed cells with round nuclei inside structures similar to lacunae and surrounded by abundant ECM whose composition was based on collagen and glycosaminoglycans [68]. According to the good results obtained in vitro and on animals, this technique seemed promising and study on humans started.

After some positive results, HYAFF started to be used for the treatment of symptomatic chondral lesions. (Fig. 22)

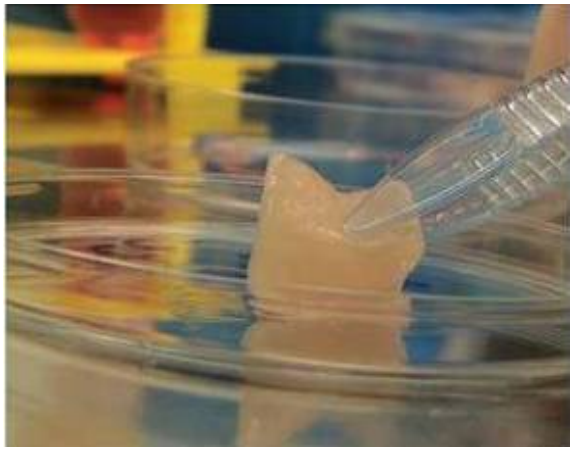


Fig. 22 Hyalograft-C

Cells harvested from the patient are expanded and then seeded on the HYAFF-11 scaffold to create Hyalograft-C®.

At the beginning, the patch was fixed at the site of the lesion with fibrin glue; later, it was seen that thanks to hydrophilic properties of the matrix, if the graft was well positioned, the tenso-active pression was enough to fix it.

Press-fit technique is enough because of the shape and the properties of the graft. Another advantage was that it could be inserted through an arthroscopic procedure with less morbidity for the patient, shorter surgical time, shorter stay in the hospital and less complications related to open surgery. Arthroscopic implantation technique was first developed to treat localized lesions of the medial or lateral femoral condyle. Nowadays it is used for almost all the lesion of the femoral condyles, even if very big. Lesions of the patellar cartilage and of the tibial plate are an exception and they need open surgery.

In patellar and trochlear lesions which are not reachable in arthroscopy, Hyalograft-C® is implanted with a small arthrotomic approach (medial or lateral para-patellar arthrotomy).

After the mapping, the lesion is prepared with a curette, the Hyalograft-C® patch is cut and implanted with press-fit technique through a specifically designed cannula

Sometimes, when the lesion is very big, it is possible to add fibrin glue in order to increase stability.

Medium-long term results showed good results at 2 years follow-up and the maintenance of stable values up to 7 years' evaluation [71].

This assumption is also reinforced by the fact that second-look arthroscopy [72] and histological [73] examination showed a normal appearance of the newly-formed cartilage.

In recent years, a study comparing microfractures and autologous chondrocytes transplantation was performed: 80 patients [74] with defects grade III-IV on the femoral condyle and the trochlea were included and divided in 2 treatment groups. Both groups showed a significant improvement at 5 years from surgery; however, the ACI group showed significantly better outcome in patient-reported subjective scores. Even more interesting was the return to sport activity: it was the same up to 2 years of follow up, but then it remained stable only in the ACI group, while it decreased in the microfractures group.

3.3.5 New "ONE-STEP" regenerative treatments

Recently new solutions have been proposed in order to overcome problems correlated to second generation ACI, that are high costs and the need of 2 surgical steps due to cellular culture.

Among the "one-step" techniques for cartilage reconstruction, the use of bone marrow concentrate as an augmentation to scaffold instead of chondrocytes have been recently tested.

The use of a kit to concentrate bone marrow-derived cells in the OR, collagen powder or hyaluronic acid membrane (as scaffolds for cell support) and platelet gel, led to the development of a one-step arthroscopic technique by Giannini et al. [76]. The evaluation of 48 patients treated for talar osteochondral lesions at 24 months of follow-up documented a significant clinical improvement and histologic and immunohistologic results obtained confirmed the presence of new cartilaginous

tissues with various degrees of tissue remodeling toward hyaline cartilage. These data suggest that the one-step technique is an alternative for cartilage repair, able to provide functional improvement and overcome the drawbacks of previous techniques.

Progresses made in material science, in cellular biology and nanotechnology allowed the creation of innovative TEC (tissue engineering constructs) for cartilage lesions repair. The ultimate goal is to obtain a complete integration of the TEC with the host tissue up to the complete remodeling of the implantation site. Musculoskeletal tissue, bone and cartilage are under extensive investigation in tissue engineering research. A number of biodegradable and bioresorbable materials, as well as scaffold designs, have been experimentally and/or clinically studied. Ideally, a scaffold should have the following characteristics: three-dimensional architecture; high porosity with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste; biocompatibility and a controllable degradation and resorption rate to match cell/tissue growth in vitro and in vivo; suitable surface chemistry for cell attachment, proliferation, and differentiation; and mechanical properties to match those of the tissues at the site of implantation.

Lately, the awareness of the involvement of the subchondral bone in many of these lesions, resulted in the need to develop cell-free treatment strategies focused on the entire osteochondral unit, and currently multiphasic scaffolds have been proposed that combine distinct but integrated layers corresponding to the cartilage and bone regions to regenerate both components of the osteochondral unit [77].

"Cell-free" chondral and osteochondral grafts have been developed with the aim to give specific regenerative signals to autologous mesenchymal cells coming from the bone marrow [78,79].

A first cell-free approach was proposed: a 1-step procedure combining subchondral microfracture with the fixation through a collagen I/III membrane that stabilizes the blood clot. The so called "AMIC® procedure" allows the treatment of big chondral defects (> 2 cm^2).

In the case of chondral lesions, especially the degenerative ones, subchondral bone is often involved: osteophytic changes can occur and also reabsorption of the underlying bone [80, 81]

Treatment should try to re-establish articular surface in the most anatomical way, trying to get back to physiological characteristics of the chondral tissue. The final result should be a repair tissue that resembles native articular cartilage. For this reason different scaffolds have been developed to treat more extended osteochondral defects.

Among the scaffolds currently adopted in clinics, the most relevant are three. The first is a bilayered cylindric implant equipped with a bone and cartilage phase composed of polylactide-glycolide copolymers, calcium sulfate, polyglycolic acid fibers, and surfactant (TruFit®: Smith & Nephew, Andover, MA). Preclinical studies have demonstrated the safety of the implant and the complete resorption of the scaffold with restoration of hyaline-like articular cartilage surfaces and subchondral bone in a high percentage of cases at 12 months. Although no systematic controlled studies are available on this technique, isolated reports have shown favorable results in the treatment after implantation of these osteochondral graft substitutes. However, MRI information at 12 months still demonstrated heterogeneous repair cartilage tissue and information on long-term durability is not available [82].

The second scaffold available on the market is Maioregen® (Fig. 23): it is a nanostructured biomimetic scaffold (Fin-Ceramica SpA, Faenza, Italy) with a porous 3-dimensional trilayered composite structure, mimicking the whole osteochondral anatomy: the cartilaginous type I collagen layer has a smooth surface, the intermediate tide-mark–like layer consists of a combination of type I collagen (60%) and hydroxyapatite (40%), and the lower layer consists of a mineralized blend of type I collagen (30%) and hydroxyapatite (70%) reproducing the subchondral bone. This scaffold was introduced into clinical practice because, after animal studies, it showed good results in terms of both cartilage and bone tissue formation. It provided similar macroscopic, histological, and radiographic results when implanting the scaffold loaded with autologous chondrocytes or the

scaffold alone. Therefore it has been suggested that the scaffold induces an in situ regeneration through homing of stem cells from the surrounding bone marrow [83]: based on the chemical features of the different layers of the scaffold, both subchondral bone and cartilage regeneration occur, thus restoring the osteochondral unit anatomy.

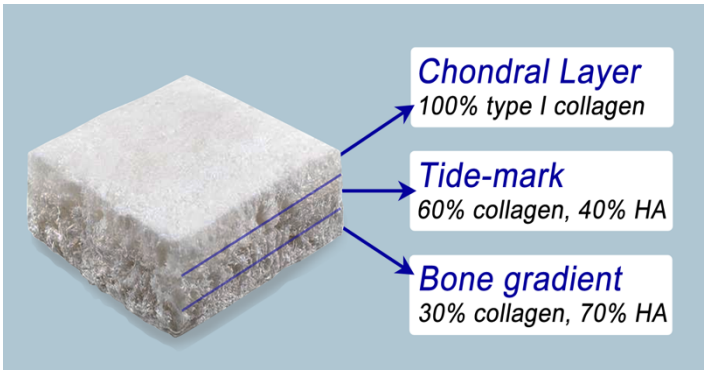


Fig. 23 Maioregen biomimetic scaffold

Clinical studies have showed that the implantation of this biomimetic scaffold to treat chondral and osteochondral knee defects proved to be effective in terms of clinical outcome at short follow-up time in a large patient population, even though controversial findings have been detected at MRI. [84].

The third scaffold is the Agili-C™ Implant: it is a bi-phasic, porous, resorbable scaffold for the treatment of articular chondral and osteochondral defects. It is the subject of the present study and will be better explained in the next chapter.

CHAPTER 4

AGILI-C™: A BIPHASIC ARAGONITE-BASED SCAFFOLD

4.1 Scaffold innovation

Currently there are a number of new technologies being developed for the treatment of osteochondral defects of weight bearing joints, including cell-based or acellular matrix-based technologies, biologic agents (such as mesenchymal stem cells) and other. Biomimetic scaffolds are being increasingly used and, considering the role of subchondral bone in the etiology of OA and articular cartilage lesions, there is a tendency toward the development of multiphasic scaffold able to promote regeneration of both the subchondral bone and cartilage layer.

Scaffold based technologies have demonstrated to provide an environment for cell proliferation and differentiation into proper lineages capable of repairing the osteochondral defect. Proprieties of the ideal scaffold are still a subject of study, with the purpose of increasing the healing capacities of cells and signaling factors to obtain a superior tissue quality and, therefore, better clinical outcomes. The potential to create a cell-free implant that is "smart" enough to provide the joint with the appropriate stimuli to induce orderly and durable tissue regeneration is attractive, and new biomaterials have been recently proposed to induce "in situ" regeneration after direct transplantation onto the defect site [85].

Tissue engineering aimed at creating 3D grafts by taking advantage of the patient's own stem cells and porous biomaterials as a template for tissue development. Such an engineered scaffold has several potential advantages, as the properties of the graft can be specifically tailored to introduce

structural, biological and biomechanical cues into the affected joints, that are necessary for a reproducible and durable repair [7]. To obtain better results in terms of tissue regeneration, the scaffold should mimic the biology, architecture and the structural properties of native tissues in order to facilitate cellular migration, attachment, proliferation and differentiation.

Furthermore, important functional properties include biocompatibility and biodegradability through safe biochemical routes in order to avoid long-term complications due to the persisting presence of non-autologous material.

In case of osteochondral lesions of the knee, surgical treatment is very challenging from a biological point of view, since two tissues are involved, bone and cartilage, with different ability to heal. For this reason, many research groups have focused on tissue engineering in order to obtain biomaterials that could restore the osteochondral structures.

A successful strategy to "engineer" osteochondral tissue is based on mimicking the natural contour of the articulating surface, achieving native mechanical properties and functional load-bearing ability, in order to lead to integration with the host cartilage and underlying subchondral bone.

The first scaffolds to be tested were biphasic composite scaffolds: poly-L-lactic acid/hydroxyapatite composite scaffolds were differentially seeded with fibroblasts transduced with an adenovirus expressing bone morphogenetic protein 7 in the ceramic phase and fully differentiated chondrocytes in the polymeric phase [86]. Biphasic and monolithic materials can be also obtained by mineralization of collagen nanocomposite and applying controlled freeze drying- These procedures are followed by chemical cross-linking, and also ionotropic gelification of alginate based materials has been tested [80] [87] .

In this chapter we describe the composition and features of a novel biphasic scaffold based on aragonite, i.e. the Agili-C^{TM} implant (Cartiheal Ltd, Israel)

4.2 Chemical-physical composition of Agili-C™

The development of the Agili-C™ implant was based on the innovative concept of using two well-known FDA-approved devices to create a scaffold for treating joint surface lesions.

It is a coral based scaffold, where the coralline skeletal material is composed of calcium carbonate in the crystalline form of aragonite. Corals are marine invertebrates from the Anthozoa class that include over 7,000 species with a wide variety of skeletal topologies, different morphologies and crystalline structures. Corals used for medical applications are limited to a select number of species: Porites, Acropora, Lobophyllia, Goniopora, Polyphyllia and Pocillopora [88].

Coral exoskeletons (aragonite) are remarkably similar to human bone, including their 3D structure and pore interconnections in the crystalline form of calcium carbonate (CaCO3). These features are thought to confer its osteo-conductive ability and make it a suitable material for bone repair.

This biomaterial provides a three-dimensional (3D) structure with mechanical properties and high inter-connected macroporosity (between 100 and 200 μm) required for vascular tissue ingrowth. [88]. The calcium carbonate structures are gradually resorbed and replaced by functional bone tissue. Coral derivatives are commonly used as a bone graft substitute and bone void fillers.

Several products to this purpose have been already FDA-cleared, for example, Pro-Osteon 200R (Biomet) and BoneMedik (Metabiomed). They are biocompatible bone substitutes based on natural coral. While coral is a good material for bone repair, as a stand-alone material, it cannot regenerate native hyaline cartilage. Shahgaldi et al. [45] performed implantations of coral plugs in the rabbit patellar groove, and although the quality of surface repair, indicated by 3D collagen structure and Safranin-O staining, was markedly better than that the one obtained for un-grafted empty defects, it did not regenerate normal articular hyaline cartilage. Therefore coral needed to be chemically and structurally modified in order to improve its cartilage regenerative potential, and this was done by the addition of hyaluronic acid. Hyaluronic acid (HA) is a high molecular weight unsulfated

glycosaminoglycan (GAG) present in all mammals and it is a critical molecule for the maintenance of the physicochemical characteristics of extracellular cartilage matrix (ECM). It has an important role in chondroprotection and chondrogenesis as it protects chondrocytes against apoptosis via CD44 [89] and I-CAM 1 [90]. It counteracts oxidative injuries in cultured human chondrocytes [91] and inhibits interleukin-1-evoked reactive oxygen species [92]. Hyaluronate also inhibits the Il-1b-stimulated production of matrix metalloproteinases [93]. HA bonded to a substrate exhibits stimulation of chondrogenic differentiation [90]. It influences cell motility, cell differentiation and cell development [94]. Finally, HA allows enhanced cell attachment and proliferation.

AgiliC ^{TM} scaffold consists of a porous, interconnected calcium carbonate (aragonite) derived from purified, inorganic coral exoskeleton (Figure 24): the lower part of the implant is composed of sole inorganic aragonite, while a square grid pattern of 2 mm deep-drilled channels is made in the top part of the scaffold, where it is impregnated with hyaluronic acid (Fig. 25 and Fig. 26).

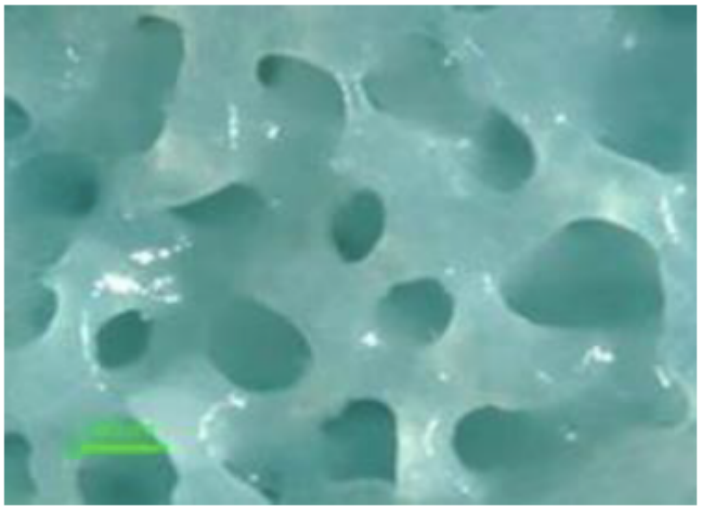


Fig. 24 Coral exoskeleton

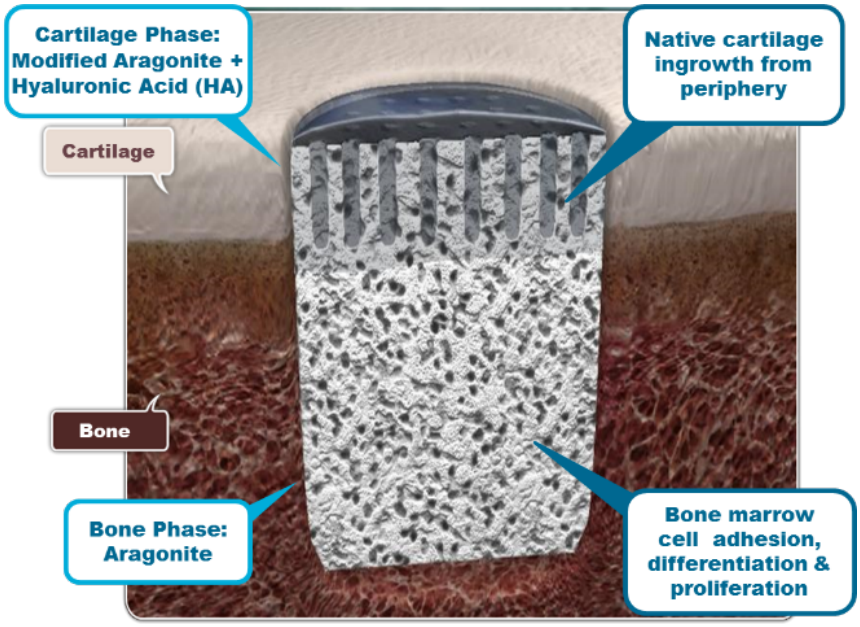
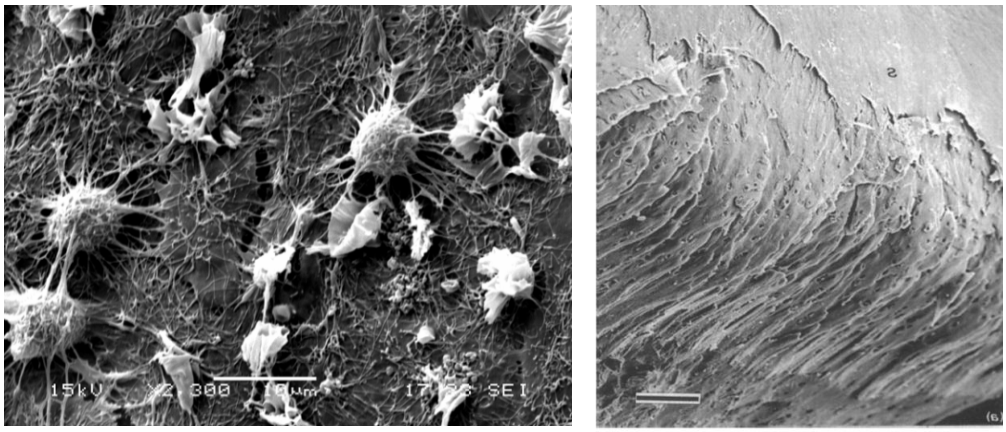


Fig. 25 Agili-C™ Structure and mechanism of action



Agili-C™ **Human Cartilage**

Fig. 26 Agili-C™ and native articular cartilage. Image obtained by electronic microscope

Histology performed by an independent laboratory in a series of preclinical studies on the goat model (with evaluation performed at 6 and 12 months' after implantation) confirmed the ability of Agili-C™ to regenerate hyaline cartilage, as demonstrated by the presence of collagen type II and aggrecan, and the lack of collagen type I in the repaired tissue, alongside the reconstruction of the

subchondral bone, with a gradual increase in tissue maturation over time (Fig. 27). Further in-vitro analysis [112] revealed the potential of the chondral phase of Agili-C™ implant to recruit autologous chondrocytes from the surrounding healthy cartilage: these chondrocytes migrate inside the scaffold and contribute to the deposition of the extracellular matrix (ECM) rich in collagen type II and aggrecan. Additionally, the formation of a layer populated by progenitor-like cells on the surface of the implant was documented. Based on the encouraging findings emerged from in-vitro [112] and animal trials, which also confirmed the safety and bio-compatibility of the Agili-C™ scaffold, a pilot clinical study on humans was performed [77] to confirm the safety of use and the potential to provide clinical improvement. The positive results from the pilot trial prompted a larger, multi-center observational study to be started, the results of which are here presented (Chapter 5).

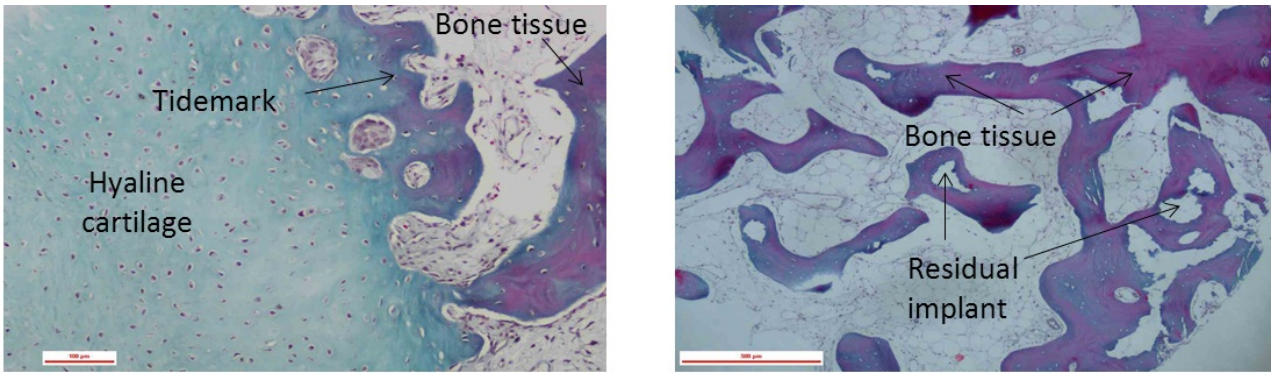


Fig. 25 Twelve months post-implantation: histology confirms hyaline cartilage and subchondral bone regeneration

4.3 Scaffold preparation and sterilization

The basic scaffold consists of coralline aragonite. Following a mechanical process, a square grid pattern of 1- to 2-mm-deep channels is drilled in the chondral phase of the scaffold, using Bungard CCD, a CNC drilling, a routing machine and an appropriate drill bit. This scaffold configuration was originally developed in the shape of cylinders (Fig. 28). It is 10 mm high with variable diameters available, from 7.5 to 20 mm, to match the lesion size.

A more recent tapered version of the implants, with an angle of 2 degrees along the longitudinal axis, has been designed to improve the press-fit implantation. After extensive purification processes, needed to treat and remove trapped particles, debris and organic remnants, the implants are sterilized by 25 kGy gamma radiation (Sor-Van Ltd., Israel). The implants used in this study were impregnated with HA (Arthrease, Bio-Technology General Ltd., Israel) homogenously in the chondral phase of the implant. The length of the material (12 mm) was chosen so that the distal part of the implant was bottomed and firmly embedded in cancellous bone beyond the subchondral bone plate, to guarantee optimal stability.

Fig. 28 Agili-C^{TM} implant (tapered version)

CHAPTER 5

PROSPECTIVE STUDY AT 2 YEARS' FOLLOW-UP

5.1 Introduction to the study

The peculiar characteristics of the articular cartilage of the knee, which is an avascular tissue, prevent its spontaneous healing once a defect is established. Many different techniques have been proposed in the last years with the aim to restore the continuity of chondral tissue; however, all these techniques present some advantages and disadvantages, and none of them can be identified as the ideal treatment. One of the ultimate emerging strategies for cartilage repair involves the implant of biomimetic biomaterial, able to induce ''in situ'' differentiation of the resident bone marrow stem cells [83,85], by providing the joint with the appropriate stimuli and produce orderly and durable tissue regeneration. When addressing defects of the articular surface where the subchondral bone is also affected, the challenge becomes even more difficult since bone and cartilage present intrinsic different features and regenerative potential. Poli-phasic tridimensional scaffolds have been conceived to promote concurrent regeneration of both the subchondral bone and cartilage in osteochondral defects of weight-bearing joints.

The aim of this multicentric prospective study is to describe the clinical and radiologic results at two years' follow-up after implanting Agili-C^{TM}, a cell-free aragonite-based biomimetic scaffold developed for the treatment of osteochondral defects of the knee.

5.2 Materials and methods

5.2.1 Patients recruitment

Ethical Approval

The present multicentric prospective clinical study was approved by the Hospital Ethic Committee and Internal Review Board of each Institution involved, and informed consent was obtained from each patient for study participation.

The following centers (with their inherent Leading Investigators) have been involved in the present clinical trial:

- Italy, Bologna - Prof. Elizaveta Kon and Prof. Maurilio Marcacci
- Belgium, Antwerp - Prof. Peter Verdonk
- Slovenia, Ljubljana – Prof. Matej Drobnič
- Romania, Timisoara - Prof. Jenel Marian Patrascu
- Serbia, Belgrade - Dr. Gordan Gavrilović
- Serbia, Novi-Sad - Prof. Dragan Savić, Dr. Oliver Dulić
- Poland, Kraków - Prof. Grzegorz Kwiatkowski
- Hungary, Budapest - Prof. Lazslo Hangody

Inclusion criteria

1) Patients 18 years or older, both females and males
2) Up to 3 treatable cartilage lesions, ICRS grades IIIa - IVb on the femoral condyles or the trochlea,
3) Total treatable area 1-7 cm^2
4) KOOS Pain score at baseline not less than 30 and not more than 65
5) Patients physically and mentally willing and able to comply with post- operative rehabilitation protocol and scheduled clinical and radiographic visits.

6) Informed consent signing

Exclusion criteria

1) Bony defect depth deeper than 8mm, according to imaging
2) Articular cartilage lesions in the tibia or the patella, ICRS grades IIIa to IVb
3) Any previous ligamentous repair or mal-alignment correction in the index knee within the last 3 months
4) Significant instability of the index knee according to IKDC Knee Examination Form 2000, Grade C (abnormal) or D (severely abnormal)
5) Lack of functional remaining meniscus
6) Meniscal transplantation in the past 6 months
7) Malalignment more than 5 degrees varus OR 5 degrees valgus according to standing X-ray
8) Any known tumor in the index knee
9) Any know history of infection in the index knee
10) Any known history of inflammatory arthropathy or crystal-deposition arthropathy
11) Any known systemic cartilage and/or bone disorder such as but not limited to chondrodysplasia or osteogenesis imperfecta
12) Body mass index >35
13) Osteoarthritis of the index knee graded as 4 according to the Kellgren- Lawrence scale
14) Chemotherapy treatment in the past 12 months
15) Any previous surgical cartilage treatment in the index knee within the last 6 months
16) History of allergic reaction or intolerance of materials containing calcium carbonate or hyaluronate
17) Patient who is pregnant or intends to become pregnant during the study

18) History of any significant systemic disease, such as but not limited to, HIV infection, hepatitis infection or HTLV infection; known coagulopathies, that might compromise the subject's welfare
19) Known substance abuse or alcohol abuse
20) Participation in other clinical trials within 30 days prior to the study or concurrent with the study
21) Known insulin dependent diabetes mellitus
22) Unable to undergo MRI or X-ray

Patients' demographics

126 patients were enrolled in the study among 8 different medical centers in Europe and consecutively treated: they were prospectively evaluated at 6, 12, 18 and 24 months of follow-up. Among this group of patients, 94 were males and 32 females, mean age was 32.67 ± 8.77 years.

All together the patients enrolled showed a medium BMI (Body Mass Index) of 25.6.

Looking at the concomitant presence of osteoarthritis in the index knee according to the Kellgren-Lawrence (KL) grade, 95 patients showed no or negligible signs of OA (KL grade 0-1) whereas a subgroup of 31 patients showed radiologic signs of osteoarthritis (KL grade 2-3). The KL classification is a radiographic classification tool based on X-Rays, with a 0 to 4 range, correlating with the severity of OA [96]. Defect sites were located as follows: 72 medial femoral condyle (MFC), 34 lateral femoral condyle (LFC) and 17 were defects at the level of the trochlea, 3 patients had multiple lesions in the MFC and LFC. The average size of the defect was 2 ± 1.3 cm^2.

Patients that before the symptoms onset were practicing sport at professional or competitive level were 36 (Tegner activity score equal to 10, 9 or 8), at amateur level 83 (Tegner activity score 7, 6, 5 or 4) and 6 of them were practicing sport just occasionally (Tegner activity score 3 or less). More than half of the patients enrolled for the study had already undergone previous surgery in the

affected knee: seventy-three patients (58%) were previously operated, against 53 (42%) who were operated for the first time (Table 1A). Among the 73 patients who were operated in the past, 22 had previous surgeries related to cartilage problems (Table 1B).

Site of the lesion	MFC	72 (57%)
	LFC	34 (27%)
	Trochlea	17 (14%)
	MFC + LFC	3 (2%)
N° of implants	1	102 (81%)
	2	22 (17.5%)
	3	1 (0.75%)
	4	1 (0.75%)
Onset of symptoms	acute	83 (66%)
	gradual	40 (32%)
	No answer	3 (2%)
Size of the defect	2 ± 1.3 cm^2	
Mean age	32.67 ± 8.77 yy	
Sex	Females	32 (25%)
	Males	94 (75%)
BMI	25.6 ± 3.63	
Previous surgery in the treated knee	yes	73 (58%)
	no	53 (42%)
Kellgren-Lawrence grade	0-1 (no OA)	95 (75%)
	> 2 (OA)	31 (25%)

Table 1A: Demographics of the patients included in the trial

Previous surgeries	(n 73)
ACL Reconstructions	11
ACL Reconstruction + debridement	1
Arthroscopic shaving	2
Debridement	7
Debridement + Hyalograft-C implant	1
Meniscectomies	23
Meniscectomies + ACL reconstruction	14
Meniscectomy + debridement	1
Meniscectomy + microfractures	1
Arthroscopic lavage	1
Microfractures	7
Subchondral drilling +/- meniscectomy	2
Procedures involving the patella	2

Table 1B: Detailed description of previous surgeries

5.2.2 Surgical Technique

The surgical technique is carried out with the patient lying in supine position in general or spinal anesthesia.

A pneumatic tourniquet is applied at the proximal thigh in order to block the vascular supply to the lower limb for a limited time span. According to the size and the location of the defect a mini arthrotomy is performed using medial or lateral parapatellar approach to expose the lesions.

The site of the lesion is then prepared using proprietary surgical toolset (Cartiheal Ltd, Israel).

A perpendicular aligner is positioned in the center of the lesion upon verification that it is perpendicular to the articular surface. The aligner is used to place a K-wire, which is used to correctly position a drill sleeve where a motorized drill is inserted to prepare the defect up to the established depth. A reamer is then inserted to ensure the correct depth is obtained and a shaper is introduced to finalize the lesion with the correct wall inclination.

A lodge 12-mm deep with perpendicular shoulders is created to allow press-fit fixation of the implant, which is 10 mm long (Fig.29 and Fig.30).

The shaper and the K-wire are then removed; the hole is appropriately cleaned with saline solution to wash out any debris.

The peripheral cartilage is regularized using a specific cartilage cutter or a scalpel to ensure smooth edges and to avoid invagination during implant insertion.

The Agili-C^{TM} implant is manually inserted into the hole, firmly pushed with the thumb and subsequently gently impacted to a position 2 mm below the surface of the articular cartilage through a silicone-covered tamper. When multiple Agili-C ^{TM} implants are used, it is important to keep a bone bridge of at least 5 mm between the implants to avoid impingement.

The stability of the implant is tested by cyclic bending of the knee while the graft is under direct vision, both before and after tourniquet removal. In the end, drainages are inserted and wounds are sutured with standard technique.

Fig. 29 Osteochondral lesion pre and post Agili-C^{TM} implantation

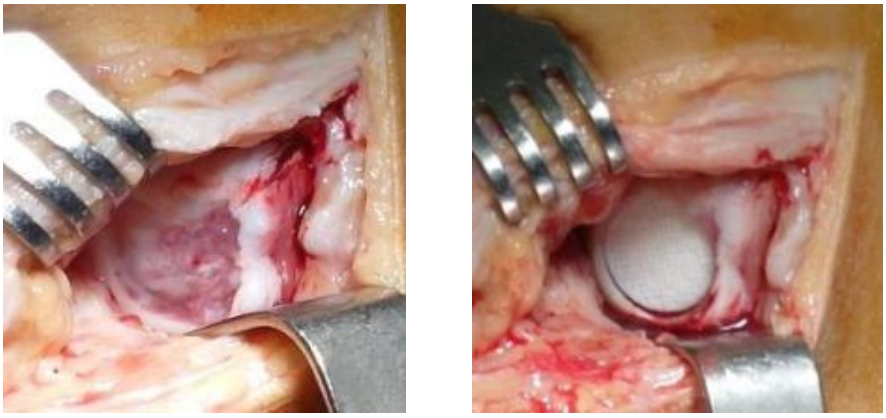


Fig. 30 Agili-C^{TM} implantation. Intra.-op pictures

5.2.3 Post-operative rehabilitation protocol

The rehabilitation program includes toe-touch weight bearing (with no significant amount of weight) using crutches for 4 weeks, with increasing partial weight bearing reaching full weight bearing after 6 weeks. During the first 48 hours cryotherapy in association with continuous-passive-motion (CPM) device (Fig. 31) are applied and carried on for 3 weeks, together with active assisted range of motion exercises.

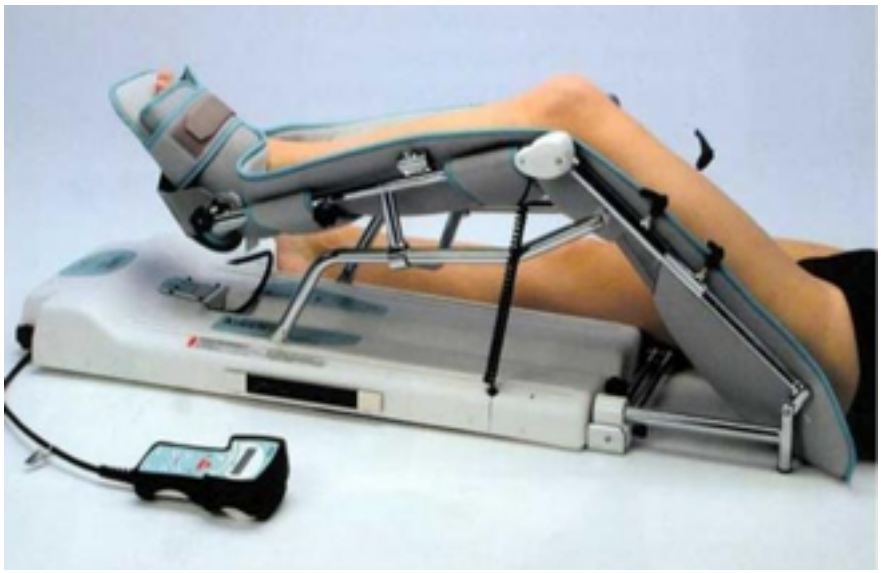


Fig. 31 Continuous Passive Motion machine

Quadriceps isometric sets and electro-stimulation are started immediately after surgery [77].

Stationary cycling is introduced at 4 weeks, when the patient reaches knee flexion of ca. 100°.

Hydrotherapy is advised immediately after suture removal.

Within the third month the patient should regain full active ROM and should introduce in its program proprioceptive/balance activities, walking and resistance.

Resistance muscle strengthening exercises can be started after the third month together with more demanding open kinetic chain (terminal leg extension) and closed kinetic chain (inner range quadriceps and modified leg press) exercises. Outdoor cycling activity and skiing are allowed not earlier than 6 months after the operation, when also agility skills relevant to patient's sport are re-introduced. Repetitive joint impact activities are allowed not before 1 year.

5.2.4 Patients evaluation

All the patients have been prospectively evaluated before the surgical procedure and during the follow-up visits at 6, 12, 18 and 24 months. During these visits, they have been clinically evaluated and interviewed to assess their symptomatology, actual physical status and knee functioning.

The clinical outcome of all patients was assessed using the Cartilage Standard Evaluation Form as proposed by the ICRS (International Cartilage Repair Society). The patients were therefore asked to complete the IKDC-subjective score questionnaire at every follow-up. Moreover, a functional test of the knee was performed at each follow-up time using the IKDC Knee Examination Form.

The final functional grade of the knee (normal, nearly normal, abnormal or severely abnormal) was rated according to the lowest ratings in effusion and passive motion deficit [97].

The Lysholm score [98] and the KOOS scale were administered preoperatively and at 6, 12, 18 and 24 months after surgery. The KOOS scale has five different subscales: Pain, other Symptoms, Function in daily living (ADL), Function in Sport and Recreation (Sport/Rec), and knee-related Quality of Life (QOL). The five patient-relevant subscales of KOOS are scored separately and each of the five scores is calculated as the sum of the items included. Scores are transformed to a 0–100 scale, with zero representing extreme knee problems and 100 representing no knee problems. [99]

The sport activity level was analysed with the Tegner score [100] and compared with pre-operative and pre-injury values. This is a numerical scale in which a condition of invalidity caused by the knee pathology is associated with 0, while the highest score that is 10 corresponds to a professional sports activity. All the patients, except for those who underwent implant failure and subsequent removal underwent imaging evaluation at 6, 12, 18 and 24 months follow-up. Examinations were carried out with a 1.5 or 3T MRI following a specific protocol to obtain optimal imaging of cartilage repair tissue of femoral condyles including the trochlea. “Defect Fill” has been assessed at MRI following the indication of the MOCART Score, which was originally developed to evaluate tissue regeneration following autologous chondrocyte transplantation [74]. This score has not been validated for osteochondral scaffold, and does not include specific evaluation of the subchondral bone repair. Therefore, only the parameter related to the Defect fill (expressed through a score ranging from 0 to 20) was used in the present analysis, also considering that no radiologic score evaluating osteochondral repair has been currently developed. The evaluation was performed in

consensus by an orthopedic surgeon and a musculoskeletal radiologist both experienced in cartilage procedures, who blindly assessed and reviewed the images.

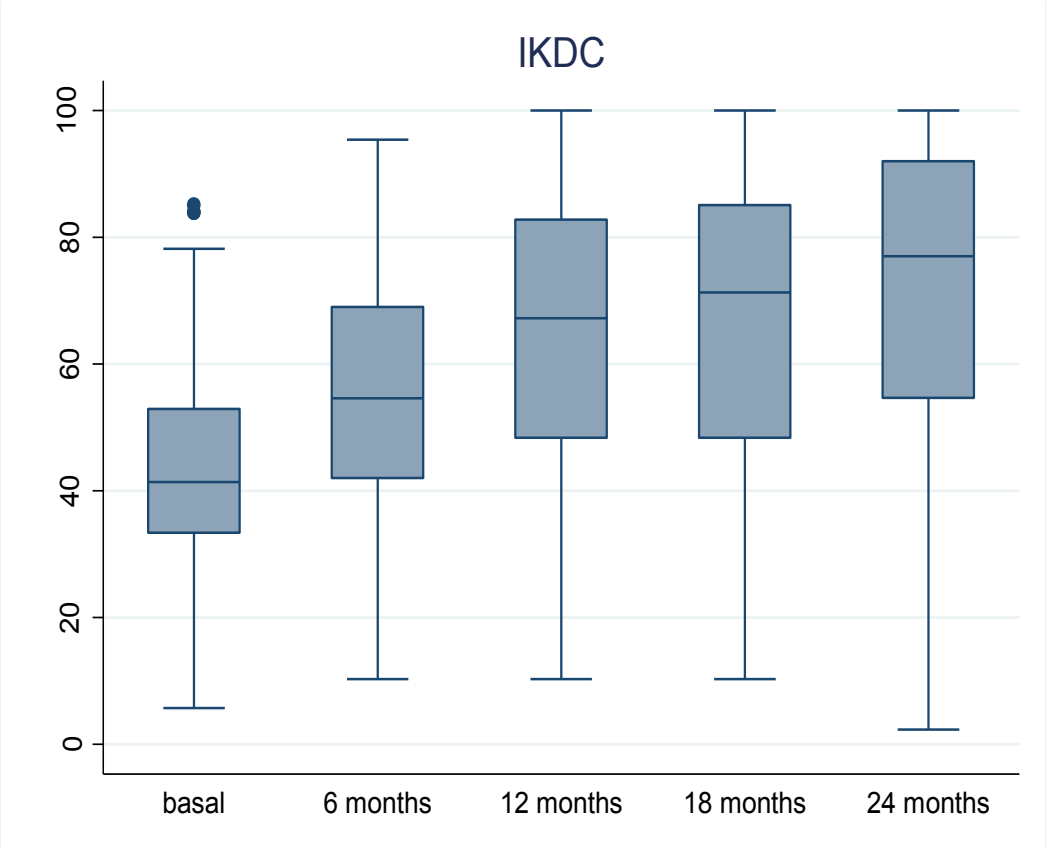
5.2.5 Statistical analysis

All continuous data were expressed as mean and standard deviation; categorical variables were expressed as frequency and percentages. The Wilcoxon non-parametric test was used to test the differences at different follow-up times. The Mann- Whitney nonparametric test was performed to assess between-group differences of continuous data. The Spearman rank correlation was used to assess correlation between rank and continuous data, and the Kendall tau ordinal correlation was used to assess correlation of ordinal data. Fisher's chi-square test was performed to investigate the relationships between dichotomous variables. Pearson's chi-square test evaluated by exact methods for small samples was performed to investigate the relationships between grouping variables. All nonparametric tests were evaluated by exact methods for small samples. For all tests, $P<.05$ was considered significant. All statistical analysis was performed with SPSS, version 19.0 (IBM, Armonk, New York).

5.3 Results

A statistically significant improvement in each of the clinical scores used was recorded from basal level to the 24 months' follow-up.

The IKDC subjective score markedly improved from the baseline evaluation (42.14 ± 16) to the 6 months (57.48 ± 19.46; $p < 0.0005$), with a further increase up to 12 months follow-up (65.94 ± 22; $p < 0.0005$), and then stable results were documented both at 18 months (68.67 ± 23.26) and 24 months (70.94 ± 24.69) follow-up (Graph 1).

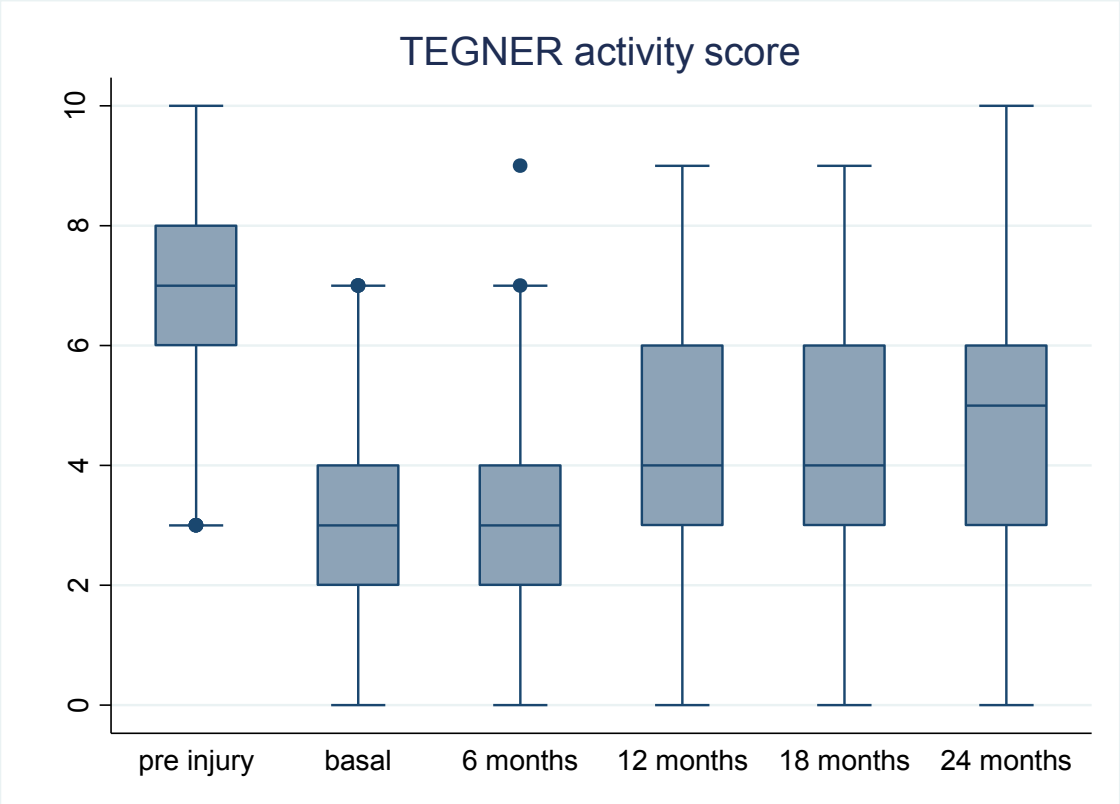


Graph 1 Subjective IKDC Score evaluation pre-op and at 6, 12, 18 and 24 months of follow-up

When considering the Tegner Score, the improvement at 6 months (0.23 ± 0.3) was not statistically significant with respect to the baseline evaluation ($p = 0.447$), whereas the other improvements at 12 (1.2 ± 0.3), 18 (1.4 ± 0.3) and 24 (1.36 ± 0.3) months of follow-up were significant with respect to the baseline ($p < 0.0005$).

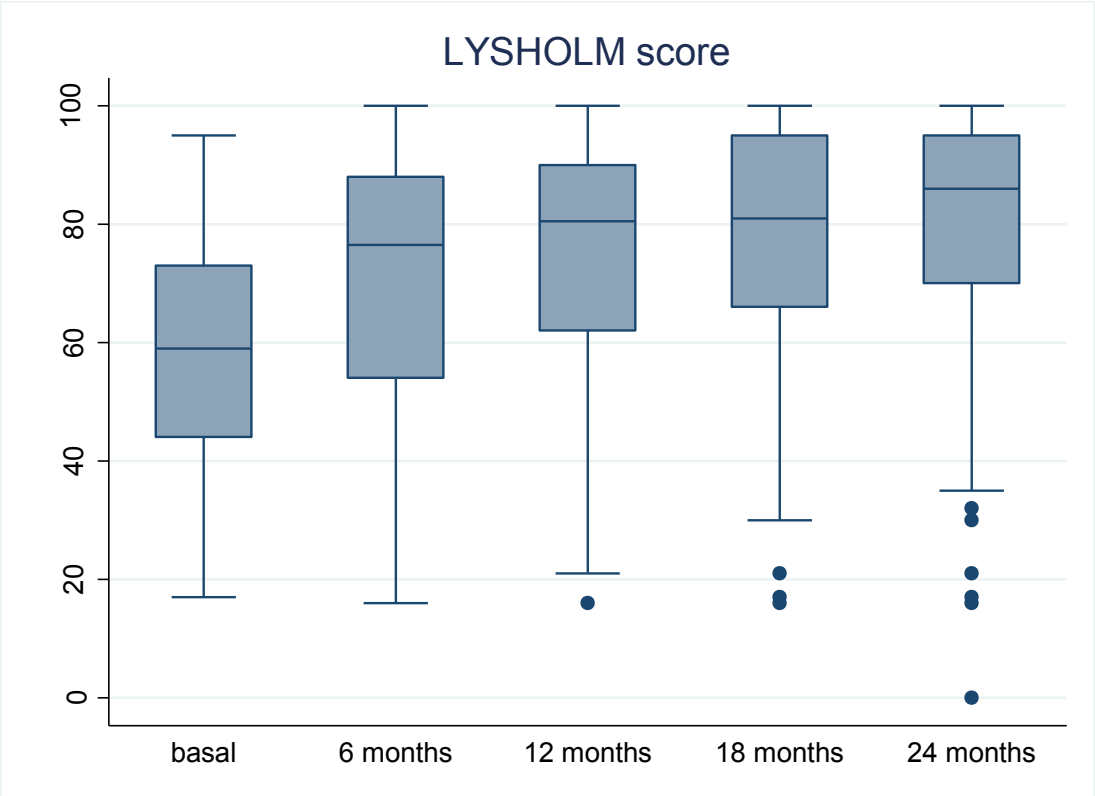
Further statistically significant improvements ($p < 0.0005$) were documented between 6 and 12 months of follow-up, 6 and 18 months, and between 6 and 24 months follow-up.

Despite the improvement, the Tegner score at 24 months remains significantly inferior to the pre-injury Tegner score ($p < 0.0005$; Graph 2), thus revealing that patients were not able to get back to the same pre-injury sport activity level at the 2 years' evaluation (Graph 2).



Graph 2: Tegner Score evaluation pre –injury, pre-operative, at 6, 12, 18 and 24 months f-up

With regard to the Lysholm score (Graph 3), patients reported a significant improvement from the pre-operative baseline evaluation to the 6 months' (12.94 ± 2.3, $p<0.0005$) and 12 months' (17.36 ± 2.3; $p<0.0005$) evaluations, with stable results at 18 months (18.31 ± 2.3) and 24 months (18.38 ± 2.3).



Graph 3: Lysholm Score evaluation pre-operative, at 6, 12, 18 and 24 months of follow-up

The KOOS scale has five separately scored subscales and significant improvements could be observed in all the subscales between the baseline evaluation and the final follow-up ($p < 0.0005$). (Table 2)

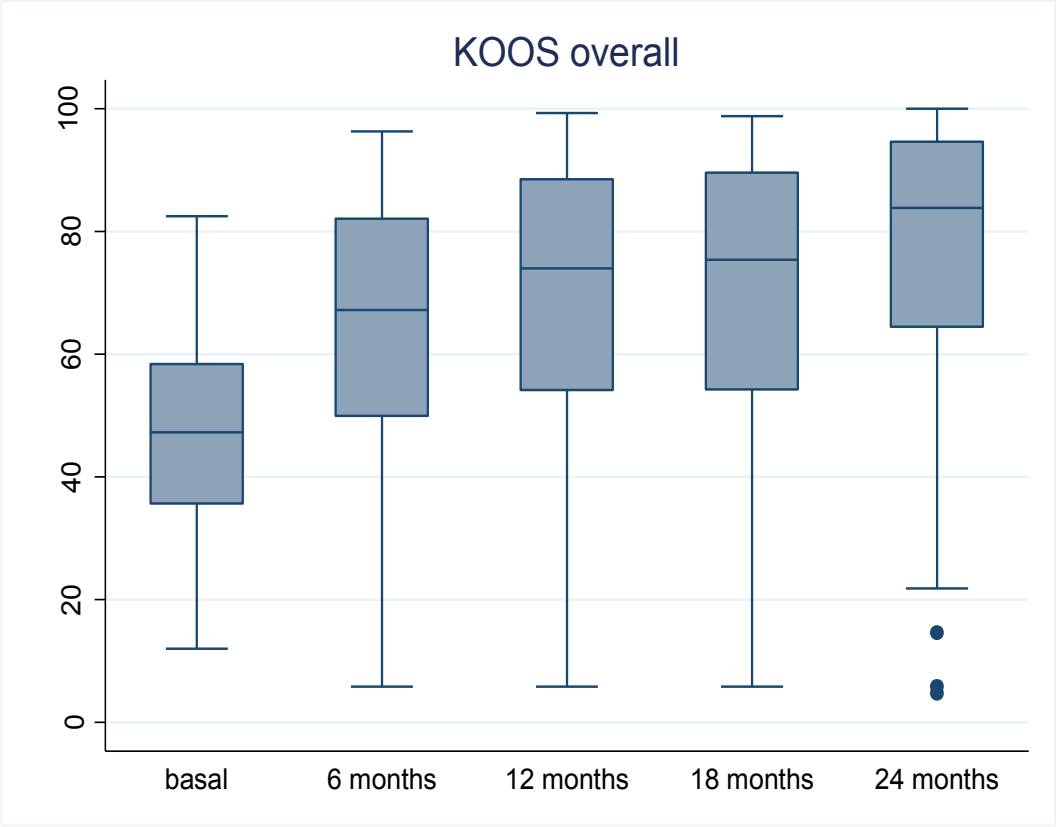
	FOLLOW-UP				
SUBSCALES	Baseline	6 months	12 months	18 months	24 months
KOOS Symptoms	59.7 ± 19.2	64.73 ± 21.77	78.34 ± 20.09	79 ± 21.44	80 ± 21.33
KOOS Pain	54.98 ± 15.5	77 ± 19.87	79.8 ± 20.43	81.46 ± 21.1	82.3 ± 21.66
KOOS ADL	63.3 ± 19.6	82.97 ± 18.86	85 ± 20.88	86.7 ± 19.76	87 ± 20.77
KOOS Sport	29.56 ± 21.44	49.7 ± 30.98	60.6 ± 31.2	64.17 ± 31.43	69.13 ± 31.43
KOOS QOL	25.32 ± 15.94	45.7 ± 26.94	52.45 ± 28.51	57.46 ± 28.85	61.23 ± 31.32
OVERALL	46.58 ± 15.04	66 ± 20.43	71.24 ± 21.78	73.75 ± 22.45	75.93 ± 23.68

Table 2 KOOS subscales variations: baseline vs 6, 2, 18 and 24 months of f-up

Overall KOOS trend (which is derived from the average score of each scale) is reported in Table 3 (showing all the significant difference among the various time-points), and Graph 4.

	Contrast ± SE	P value
6 m vs basal	17.06 ± 2.23	**P < 0.0005**
12 m vs basal	21.74 ± 2.23	**P < 0.0005**
18 m vs basal	23.03 ± 2.23	**P < 0.0005**
24 m vs basal	24.88 ± 2.23	**P < 0.0005**
12 m vs 6 m	4.67 ± 2.23	**P < 0.0005**
18 m vs 6 m	5.97 ± 2.23	**P < 0.0005**
24 m vs 6 m	7.81 ± 2.23	**P < 0.0005**
18 m vs 12 m	1.29 ± 2.23	P = 0.485
24 m vs 12 m	3.14 ± 2.23	P = 0.191
24 m vs 18 m	1.85 ± 2.23	P = 0.541

Table 3 Variations of KOOS overall (comparison among various f-up)



Graph 4 KOOS overall evaluation pre-operative, at 6, 12, 18 and 24 months of follow up

121 patients were evaluated with high resolution MRI at 6 months, 116 at 12 months, 114 at 18 months and 113 at 24 months. Defect Fill for each implantation site was measured as previously described. Statistically significant improvements can be seen between the 6 months MRI scan, 12 months (7.1 ± 2.54; $p = 0.006$), 18 months (12.32 ± 2.56; $p < 0.0005$) and 24 months evaluations (14.48 ± 2.55; $p < 0.0005$) and again between the 12-18 months interval (5.22 ± 2.54; $p = 0.04$) and 12-24 months interval (7.38 ± 2.54; $p = 0.004$).

Eleven patients (8.7%), who underwent implant removal within the two years follow-up, were considered failed. Reasons for implant removal were the following:

- 6 patients: infection
- 3 patients: loosening of the implant due to mal-positioning;

- 2 patients: lack of scaffold integration with persisting symptoms;

Further analysis was performed to evaluate the parameters that might have influenced the clinical outcome: sex, number and location of implants, previous surgery, onset of symptoms (acute vs gradual), and ICRS grade of the lesion (3 or 4) did not significantly influence the clinical outcome in this series.

The size of the defect was shown to influence in a statistically significant way three subscales of the KOOS score (Pain, ADL and QoL, $p = 0.015$, $p = 0.027$, $p = 0.049$ respectively) and, as a consequence, the overall KOOS score ($p = 0.023$). The subjective IKDC score is also affected by the size of the lesion and changes significantly according to it ($p = 0.022$), and a further significant influence of the defect size was on the Lysholm score ($p = 0.026$).

Age has been demonstrated to be an important parameter when considering some KOOS subscales: Pain ($p = 0.015$), ADL (0.004), Sport ($p = 0.009$), and also the overall KOOS ($p = 0.029$). The increase in the age of the patients at the time of implantation also negatively influenced other scores: subjective IKDC ($p = 0.005$), Lysholm ($p = 0.011$) and Tegner activity score ($p < 0.0005$).

The effect of BMI was also evaluated, and significant correlations were found with the following KOOS subscales: Pain ($p = 0.003$), ADL ($p = 0.001$ and Sport ($p = 0.015$), and overall KOOS ($p = 0.012$); subjective IKDC ($p = 0.004$); and Lysholm score ($p = 0.001$). In all cases there was a negative correlation between the BMI and the clinical questionnaire, i.e. the greater the BMI, the worst the scores.

All the patients enrolled for this study were evaluated also for their degree of osteoarthritis using the Kellgren-Lawrence score and a sub-analysis was performed dividing the patients into two subgroups: those having KL score from 0 to 1 (no or negligible signs of knee OA) and those having KL score from 2 to 3 (signs of moderate knee OA). No significant differences in any of the parameters considered were reported between the aforementioned groups of patient.

5.4 Discussion

The main findings of the present study is that Agili-C™ may represent a safe and effective treatment for grade III-IV chondral and osteochondral knee lesions, both in terms of clinical improvement and patient satisfaction.

The IKDC subjective and Tegner scores documented a significant improvement at 12 months with respect to the pre-operative level, with a further, although not significant, increase up to 24 months of follow-up, even if the post-operative Tegner score remained lower than the pre-injury one.

All the subscales of KOOS showed a significant growth between the baseline evaluation and the final follow-up at 24 months. Lysholm score also documented a statistically significant improvement up to 24 months with respect to the baseline.

The MRI evaluation results correlated with the clinical outcome, showing a gradual and significant increase over time up to the final 24 months' evaluation, thus suggesting a further trend towards improvement, to be confirmed at longer follow-up.

Secondary finding of the present study is that no significant differences in outcome were noticed among patients with and without signs of OA, thus supporting the assumption that an osteoarthritic environment does not impair the regenerative properties of the scaffold, at least in the short term.

Chondral lesions are a challenging and invalidating pathology with a high social impact, and the treatment of these defects still represents a great challenge for the orthopaedic surgeon. The biological characteristics of cartilage are enough to explain its low healing potential and therefore many treatment options have been introduced to deal with these lesions. In the last years, the subchondral bone has become object of increasing interest due to its peculiar role in the pathogenesis of chondral damage. It was demonstrated that it plays an important role even in superficial lesions initially limited to the articular cartilage layer: even focal chondral defects, if left untreated, may increase in size over time and result in concomitant changes in the underlying

subchondral bone plate [101]. Therefore, subchondral bone should be treated in order to have a correct restoration of the most superficial layers of the joint.

When approaching lesions of the articular surface, reparative or regenerative techniques, like microfractures, mosaicplasty and second-generation ACI, have already shown good results at short, medium and long term follow-up [60, 74, 102], but with some limitations. Microfractures have been proposed by Steadman et al. [50] and are the oldest and most commonly adopted technique to treat articular cartilage defects due to their ease of execution and low costs. Anyway, the repair tissue following microfractures is mainly fibrocartilage, with weaker biomechanical and biologic properties compared with hyaline cartilage, and previous studies have demonstrated that clinical outcomes tend to deteriorate over time, especially in those lesions where subchondral bone damage is already present at the moment of treatment [80,82].

With mosaicplasty, also known as osteochondral autograft, cylinders of intact articular cartilage together with the underlying subchondral bone are harvested from a non weight-bearing area of the knee (usually the trochlea) and implanted into the defect site. The graft provides a fully formed articular cartilage that has the potential to provide viable chondrocytes that can maintain the matrix [103]. The main disadvantage of this technique regards the donor site morbidity associated to autograft harvesting, which makes this technique not ideal for the treatment of large lesions [55]. Furthermore, it is very difficult to reproduce the natural geometry of the femoral condyles with grafts harvested from the trochlea, and clinical results have been shown to be worse when multiple plugs are used, thus reinforcing the concept that larger lesions have limited benefit with this technique.

Cell-based technologies, which include autologous chondrocyte implantation and matrix-induced autologous chondrocyte implantation are indicated for larger lesions, and have provided satisfactory outcomes even at long term evaluation, but they are affected by some disadvantages limiting their current application in clinical practice in many countries: first, the high cost related to cell

expansion and the strict GCP regulations impeding over laboratories, and secondly the need of two surgical steps (one for chondrocytes harvesting and the other for implantation) with the inherent higher morbidity for patients [104]. Furthermore, in case of deep osteochondral lesions, the cell-loaded matrix alone is insufficient to fill the defect and therefore a bone autograft should be placed at the bottom of the lesion before the implantation of the matrix (the so-called "sandwich technique").

Innovations in the biomaterials field are providing new fascinating surgical options to treat chondral and osteochondral lesions. The possibility of adopting biomimetic biomaterials to stimulate tissue regeneration has been confirmed by many in vitro [77] and animal studies [112], which showed the potential of these cell-free tri-dimensional scaffolds to be populated by autologous stem cells and "guide" them to differentiate in subchondral bone and cartilage tissue, based on the specific chemical and physical properties of the different layers of the scaffold itself. This way, the scaffold acts as an "enhancer" of tissue healing [83].

The main advantage of the scaffold-based approach is that it requires a single surgical step, thus avoiding the morbidity of multiple surgical steps typical of autologous chondrocyte transplantation. Furthermore, being cell-free, this treatment option is not affected by the high costs of cell-based techniques. In the last 15 years, many different biomaterials have been tested, but just a few osteochondral scaffolds have finally reached clinical application:

1) a bilayered cylindric implant equipped with a bone and cartilage phase, TruFit® (Smith & Nephew, Andover, MA), for which controversial results were shown: Dhollander et al. [105] recorded a failure rate of 20% (3 out of 15 patients) at 1-year follow-up and biopsies showed fibrous vascularized repair tissue. Moreover the comparison of a group of 35 patients treated with the implantation of this scaffold and 31 patients treated by mosaicplasty for similar defects, showed significantly higher outcomes for the latter ones [106]. Based on these

results from clinical trials, Trufit scaffold use has been discontinued in clinical practice and now it has been retired from the market;

2) Maioregen®, a three layered nanostructured biomimetic scaffold, which is still in clinical use, with good results documented up to long term evaluation [107]. A recent study compared this scaffold with microfractures (MFx) as an alternative surgical treatment, and it showed safety and overall positive clinical outcome provided by this collagen-hydroxyapatite biomaterial. While comparable results were found in the overall population, the osteochondral scaffold offered significantly better results compared to Mfx in the treatment of deep osteochondral lesions [107]. Despite the satisfactory clinical outcomes reported, the main problem related to the use of the Maioregen scaffold is the slow and limited subchondral bone healing, documented both at MRI and CT, which may play a role in limiting the maximum clinical results achievable by this scaffold;
3) The aragonite based scaffold Agili-C™ adopted in this study: the peculiar characteristics of coral and hyaluronic acid allowed to create a biphasic scaffold that chemically and morphologically mimics the structure of the cartilaginous ECM and the subchondral bone. Regeneration of both these tissues layer by the use of this scaffold has been proven in a previous animal trial [7] and encouraging findings have been already reported in pilot studies on small groups of patients [77].

Overall the results documented on the patients of the present study can be considered encouraging, with a failure rate comparable to that of the Maioregen scaffold at short term evaluation [84]. Even looking at the short-term results of matrix-assisted autologous chondrocytes transplantation (with various different membranes adopted by different authors), the aragonite scaffold seems to be similarly effective [5,74]. Anyway, longer follow-up evaluation is needed to confirm the effectiveness of the Agili-C™ implant.

With regard to surgical technique, the press-fit implantation is easy and reproducible thanks to a specifically developed tool-kit, but it is essential to accurately prepare the area of the lesion in order to obtain an adequate implantation site. In particular it is fundamental to drill perpendicularly to the joint surface and to reach the established depth with the reamer (12 mm) to ensure a proper positioning of the scaffold slightly below the level of the surrounding healthy cartilage. If the surgical technique is not followed properly, the implant can mobilize or break. The breakage of the implant can lead to the release of aragonite crystals in the joint, with a high inflammatory potential that can alter the joint homeostasis, also enhancing the risk of infection, which was the most common cause for implant failure and removal in the present cohort of patient.

One of the most interesting findings of the present trial is that the regenerative capacity of the Agili-C™ scaffold was also observed in patients affected by focal osteochondral defects in an osteoarthritic compartment (Kellgren-Lawrence grade II or III): a clinical improvement was documented, comparable to that obtained in patients without osteoarthritic changes. Based on this finding, the presence of OA should not be regarded as a contra-indication to cartilage repair procedures, even if, as showed by previous experiences with autologous chondrocyte transplantation, higher failure rate may be expected in the long term due to the progression of joint degeneration [111].

The present study suffers some flaws: first of all the lack of a control group and the short follow-up term, which warrants for a longer evaluation of the present cohort of patients to confirm the findings of the present analysis. Furthermore, another limitation is the radiographic evaluation of the performance of the scaffold, which was limited to the "Defect Fill" parameter of the MOCART Score. Unfortunately, up to the present time, no MRI score has been validated to evaluate the regeneration following osteochondral scaffold repair, and the MOCART Score itself was originally developed to assess tissue regeneration following autologous chondrocyte transplantation: therefore it lacks a proper evaluation tool for subchondral bone healing, which is a fundamental aspect in

osteochondral scaffold-based techniques. In light of this, we decided to use the most relevant parameter of the MOCART Score that could be effectively applied also for the present evaluation.

Despite these limitations, the clinical and radiologic results at short term in this large cohort of patients were encouraging and therefore a randomized multicenter clinical trial comparing the efficacy of Agili-C^{TM} versus microfractures has been started to assess whether this biomaterial-based treatment is able to provide superior results compared to the standard approach.

5.5 Conclusion

Agili-C^{TM} scaffold for the treatment of ICRS grade III-IV defects of the knee provides encouraging clinical and radiologic outcomes at short-term evaluation. Overall failure rate was 8.7%, comparable to similar scaffold-based procedures available for clinical use. Anyway, the present results should be confirmed at longer-term evaluation and further randomized studies are needed to compare the performance of this new treatment strategy with respect to other consolidated cartilage procedures.

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APPENDIX

Evaluation questionnaires for patients

2000 IKDC SUBJECTIVE KNEE EVALUATION FORM

Your Full Name_____

Today's Date: _____/_____/_____ (Day / Month / Year) Date of Injury: _____/_____/_____ (Day / Month / Year)

SYMPTOMS*:
*Grade symptoms at the highest activity level at which you think you could function without significant symptoms, even if you are not actually performing activities at this level.

1. What is the highest level of activity that you can perform without significant knee pain?

   4❑Very strenuous activities like jumping or pivoting as in basketball or soccer
   3❑Strenuous activities like heavy physical work, skiing or tennis
   2❑Moderate activities like moderate physical work, running or jogging
   1❑Light activities like walking, housework or yard work
   0❑Unable to perform any of the above activities due to knee pain

2. During the past 4 weeks, or since your injury, how often have you had pain?

Never	0 ❑	1 ❑	2 ❑	3 ❑	4 ❑	5 ❑	6 ❑	7 ❑	8 ❑	9 ❑	10 ❑	Constant

3. If you have pain, how severe is it?

No pain	0 ❑	1 ❑	2 ❑	3 ❑	4 ❑	5 ❑	6 ❑	7 ❑	8 ❑	9 ❑	10 ❑	Worst pain imaginable

4. During the past 4 weeks, or since your injury, how stiff or swollen was your knee?

   4❑Not at all
   3❑Mildly
   2❑Moderately
   1❑Very
   0❑Extremely

5. What is the highest level of activity you can perform without significant swelling in your knee?

   4❑Very strenuous activities like jumping or pivoting as in basketball or soccer
   3❑Strenuous activities like heavy physical work, skiing or tennis
   2❑Moderate activities like moderate physical work, running or jogging
   1❑Light activities like walking, housework, or yard work
   0❑Unable to perform any of the above activities due to knee swelling

6. During the past 4 weeks, or since your injury, did your knee lock or catch?

   0❑Yes 1❑No

7. What is the highest level of activity you can perform without significant giving way in your knee?
   4❑Very strenuous activities like jumping or pivoting as in basketball or soccer
   3❑Strenuous activities like heavy physical work, skiing or tennis
   2❑Moderate activities like moderate physical work, running or jogging
   1❑Light activities like walking, housework or yard work
   0❑Unable to perform any of the above activities due to giving way of the knee

Page 2 – 2000 IKDC SUBJECTIVE KNEE EVALUATION FORM

SPORTS ACTIVITIES:

8. What is the highest level of activity you can participate in on a regular basis?

4❑Very strenuous activities like jumping or pivoting as in basketball or soccer
3❑Strenuous activities like heavy physical work, skiing or tennis
2❑Moderate activities like moderate physical work, running or jogging
1❑Light activities like walking, housework or yard work
0❑Unable to perform any of the above activities due to knee

9. How does your knee affect your ability to:

		Not difficult at all	Minimally difficult	Moderately Difficult	Extremely difficult	Unable to do
a.	Go up stairs	4❑	3❑	2❑	1❑	0❑
b.	Go down stairs	4❑	3❑	2❑	1❑	0❑
c.	Kneel on the front of your knee	4❑	3❑	2❑	1❑	0❑
d.	Squat	4❑	3❑	2❑	1❑	0❑
e.	Sit with your knee bent	4❑	3❑	2❑	1❑	0❑
f.	Rise from a chair	4❑	3❑	2❑	1❑	0❑
g.	Run straight ahead	4❑	3❑	2❑	1❑	0❑
h.	Jump and land on your involved leg	4❑	3❑	2❑	1❑	0❑
i.	Stop and start quickly	4❑	3❑	2❑	1❑	0❑

FUNCTION:

10. How would you rate the function of your knee on a scale of 0 to 10 with 10 being normal, excellent function and 0 being the inability to perform any of your usual daily activities which may include sports?

FUNCTION PRIOR TO YOUR KNEE INJURY:

	0	1	2	3	4	5	6	7	8	9	10	
Couldn't perform daily activities	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	No limitation in daily activities

CURRENT FUNCTION OF YOUR KNEE:

	0	1	2	3	4	5	6	7	8	9	10	
Can't perform daily activities	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	No limitation in daily activities

Knee Injury and Osteoarthritis Outcome Score (KOOS)

Pain

P1 How often is your knee painful?	☐ Never	☐ Monthly	☐ Weekly	☐ Daily	☐ Always

What degree of pain have you experienced the last week when…?

P2 Twisting/pivoting on your knee	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
P3 Straightening knee fully	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
P4 Bending knee fully	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
P5 Walking on flat surface	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
P6 Going up or down stairs	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
P7 At night while in bed	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
P8 Sitting or lying	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
P9 Standing upright	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme

Symptoms

Sy1 How severe is your knee stiffness after first wakening in the morning?	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
Sy2 How severe is your knee stiffness after sitting, lying, or resting later in the day?	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
Sy3 Do you have swelling in your knee?	☐ Never	☐ Rarely	☐ Sometimes	☐ Often	☐ Always
Sy4 Do you feel grinding, hear clicking or any other type of noise when your knee moves?	☐ Never	☐ Rarely	☐ Sometimes	☐ Often	☐ Always
Sy5 Does your knee catch or hang up when moving?	☐ Never	☐ Rarely	☐ Sometimes	☐ Often	☐ Always
Sy6 Can you straighten your knee fully?	☐ Always	☐ Often	☐ Sometimes	☐ Rarely	☐ Never
Sy7 Can you bend your knee fully?	☐ Always	☐ Often	☐ Sometimes	☐ Rarely	☐ Never

Activities of daily living

What difficulty have you experienced the last week…?

A1 Descending	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A2 Ascending stairs	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A3 Rising from sitting	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A4 Standing	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A5 Bending to floor/picking up an object	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A6 Walking on flat surface	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A7 Getting in/out of car	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A8 Going shopping	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A9 Putting on socks/stockings	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A10 Rising from bed	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A11 Taking off socks/stockings	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A12 Lying in bed (turning over, maintaining knee position)	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A13 Getting in/out of bath	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A14 Sitting	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A15 Getting on/off toilet	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A16 Heavy domestic duties (shovelling, scrubbing floors, etc)	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
A17 Light domestic duties (cooking, dusting, etc)	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme

Sport and recreation function

What difficulty have you experienced the last week…?

Sp1 Squatting	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
Sp2 Running	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
Sp3 Jumping	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
Sp4 Turning/twisting on your injured knee	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme
Sp5 Kneeling	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme

Knee-related quality of life

Q1 How often are you aware of your knee problems?	☐ Never	☐ Monthly	☐ Weekly	☐ Daily	☐ Always
Q2 Have you modified your lifestyle to avoid potentially damaging activities to your knee?	☐ Not at all	☐ Mildly	☐ Moderately	☐ Severely	☐ Totally
Q3 How troubled are you with lack of confidence in your knee?	☐ Not at all	☐ Mildly	☐ Moderately	☐ Severely	☐ Totally
Q4 In general, how much difficulty do you have with your knee?	☐ None	☐ Mild	☐ Moderate	☐ Severe	☐ Extreme

LYSHOLM KNEE SCORING SCALE

This questionnaire is designed to give your Physical Therapist information as to how your knee problems have affected your ability to manage in everyday life Please answer every section and mark only the ONE box which best applies to you at this moment.

Name: _____ **Date:** _____

SECTION 1 - LIMP
- ☐ I have no limp when I walk. (5)
- ☐ I have a slight or periodical limp when I walk. (3)
- ☐ I have a severe and constant limp when I walk. (0)

SECTION 2 - Using cane or crutches
- ☐ I do not use a cane or crutches. (5)
- ☐ I use a cane or crutches with some weight-bearing. (2)
- ☐ Putting weight on my hurt leg is impossible. (0)

SECTION 3 - Locking sensation in the knee
- ☐ I have no locking and no catching sensation in my knee. (15)
- ☐ I have catching sensation but no locking sensation in my knee. (10)
- ☐ My knee locks occasionally. (6)
- ☐ My knee locks frequently. (2)
- ☐ My knee feels locked at this moment.. (0)

SECTION 4 - Giving way sensation from the knee
- ☐ My knee gives way. (25)
- ☐ My knee rarely gives way, only during athletics or vigorous activity. (20)
- ☐ My knee frequently gives way during athletics or other vigorous activities. In turn I am unable to participate in these activities. (15)
- ☐ My knee frequently gives way during daily activities. (10)
- ☐ My knee often gives way during daily activities. (5)
- ☐ My knee gives way every step I take. (0)

SECTION 5 – PAIN
- ☐ I have no pain in my knee. (25)
- ☐ I have intermittent or slight pain in my knee during vigorous activities. (20)
- ☐ I have marked pain in my knee during vigorous activities. (15)
- ☐ I have marked pain in my knee during or after walking more than 1 mile. (10)
- ☐ I have marked pain in my knee during or after walking less than 1 mile. (5)
- ☐ I have constant pain in my knee. (0)

SECTION 6 – SWELLING
- ☐ I have swelling in my knee. (10)
- ☐ I have swelling in my knee on1y after vigorous activities. (6)
- ☐ I have swelling in my knee after ordinary activities. (2)
- ☐ I have swelling constantly in my knee. (0)

SECTION 7 – CLIMBING STAIRS
- ☐ I have no problems climbing stairs. (l0)
- ☐ I have slight problems climbing stairs. (6)
- ☐ I can climb stairs only one at a time. (2)
- ☐ Climbing stairs is impossible for me. (0)

SECTION 8 – SQUATTING
- ☐ I have no problems squatting. (5)
- ☐ I have slight problems squatting. (4)
- ☐ I cannot squat beyond a 90deg. Bend in my knee. (1)
- ☐ Squatting is impossible because of my knee. (0)

Total: _____/100

Instructions: Please place a mark on the line to indicate the amount of pain you have had in your knee(s) in the past 24 hours.

RIGHT KNEE
No pain at all ——————————————— **Worst pain possible**

LEFT KNEE
No pain at all ——————————————— **Worst pain possible**

TEGNER ACTIVITY LEVEL SCORE

Please indicate in the spaces below the HIGHEST level of activity that you participated in BEFORE YOUR INJURY and the highest level you are CURRENTLY able to participate in. Using the circles below, check space you wish to participate in, in the future.

BEFORE INJURY LEVEL: _____ CURRENT LEVEL: _____

Level 10	Competitive sports- soccer, football, rugby (national elite)	◯
Level 9	Competitive sports- soccer, football, rugby (lower divisions), ice hockey, wrestling, gymnastics, basketball	◯
Level 8	Competitive sports- racquetball or bandy, squash or badminton, track and field athletics (jumping, etc.), down-hill skiing	◯
Level 7	Competitive sports- racquetball or bandy, squash or badminton, track and field athletics (jumping, etc.), down-hill skiing	◯
Level 6	Recreational sports- tennis and badminton, handball, racquetball, down-hill skiing, jogging at least 5 times per week	◯
Level 5	Work- heavy labor (construction, etc.) Competitive sports- cycling, cross-country skiing, Recreational sports- jogging on uneven ground at least twice weekly	◯
Level 4	Work- Work- moderately heavy labor (e.g. truck driving, etc.)	◯
Level 3	Work- light labor (nursing, etc.)	◯
Level 2	Work- light labor Walking on uneven ground possible, but impossible to back pack or hike	◯
Level 1	Work- Work- sedentary (secretarial, etc.)	◯
Level 0	Sick leave or disability pension because of knee problems	◯

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Surgical History

Have you had any additional surgeries to your knee other than those performed by Dr. Sterett? YES / NO

IF YES:

What procedure(s) were performed?

When was the surgery performed?

When performed surgery?

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