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CARTILAGE REPAIR

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CARTILAGE REPAIR INTRODUCTION

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The repair of cartilaginous tissue represents one of the major challenges for orthopedic surgeons. In the last two decades we have witnessed an incredible increase in interest in this field due to the advent of cell-based strategies and tissue engineering procedures deriving from the collaboration of multi-disciplinary scientific teams. In spite of these novelties which have offered orthopedic surgeons a broader choice of treatment options for articular cartilage and fibrocartilaginous tissue repair, we have observed the permanence of the traditional techniques in this menu-à-la-carte which in fact remain the most extensively used since the time of the arthroscopy pioneers.

This may lead to the conclusion that the efforts made so far have produced limited benefits for our patients in terms of efficacy and durability of the new methods proposed. However, the general feeling is that we can now handle the problem of cartilage lesion with a broader range of tools and strategies. We are undoubtedly cautious and fully aware that deeper knowledge concerning the available methods is still necessary and that a continuous analysis of, and comparison between, the different methods and corresponding clinical trials are crucial for defining the perfect strategy to tackle this difficult clinical problem.

The series of articles gathered in this issue attempt to present the state of the art on this topic. Together with the traditional techniques, which indeed should not be abandoned, the novel approaches deriving from confirmed new techniques and the expectations from current and future methods and/or materials are illustrated. Furthermore one of the latest strategies using PRP is presented in this issue together with the scaffold-based methods for the repair of the cartilage-protecting tissue: the fibrocartilaginous meniscus.

The general impression is that cartilage repair remains a social problem and a great challenge for orthopedic surgeons and basic research scientists. The problem has not yet been solved, although new methods and strategies are available and under development. Cartilage repair is now often reached but real tissue regeneration and, therefore, clinical success and patient satisfaction still remain an uncertain result.

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CARTILAGE REPAIR. TREATMENT FLOW CHARTS

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The conservative treatment of chondral lesions has been based for many years on similar criteria. The surgical treatment however is in constant evolution. The different techniques can be classified according to the goal that the surgeon wants to reach:

- Palliative (debridement and washing): degenerative tissue removal.

- Reparative (chondroabrasions, perforations and microfractures): cartilage fibrous tissue.

- Reconstructive (osteochondral graft OATS, autologous chondrocyte implantation ACI): restoration of the articular surface with hyaline or hyaline-like cartilage.

Before any preoperative planning, patient evaluation plays a pivotal role (anamnesis, clinical and instrumental evaluation). In the algorithm of any joint disease, the approach to cartilage lesions has to be addressed in secondary steps. Although it is a "noble" tissue, it has few intrinsic self-reparative potential. The correction of any joint malalignment, instability and meniscal lesion, that may have induced and/or determined the functional overload which in turn led to the chondral lesion, must be approached before any cartilage surgical treatment. The most appropriate treatment to improve pain, function and satisfy the patient's needs, may be chosen only after comprehending the principles, indications and limits of any surgical technique.

VARIABLES THAT INFLUENCE THE CHOICE OF THE SURGICAL TECHNIQUE OR THE FINAL RESULT

Etiology

It is not easy to define the origin of a chondral lesion, whether acute or chronic. It is mandatory to reduce and synthesize when possible the osteochondral lesion especially in young patients (1). When this is not possible, the choice of treatment depends on the size and depth of the osteochondral lesion. In overload lesions the surgeon has to correct the factors that caused the chondral disease and then the cartilage lesion.

The algorithm is the following: corrective osteotomy in varus/valgus knee if the chondral lesion is lateral/medial respectively; extensor apparatus realignment in case of femoro-patellar lesions; central and/or peripheral ligamentous reconstruction depending on the site of the lesion; repair a meniscal lesion or reconstruction with a meniscal implant or mensiscal graft in case of partial meniscectomy (> 33% of tissue loss), or total meniscectomy respectively. The treatment of acute chondral lesions give better results compared to chronic and degenerative lesions (2). Prospective randomized control trials comparing the different

Key words: Cartilage, microfracture, osteochondral transplantation, autologous chondrocyte implantation.

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surgical techniques for degenerative chondral lesion has not been reported in literature up to now.

Previous surgeries

In case of previous treatment failure, data collection of hospitalization (such as medical record, imaging, description, photos and films of the previous surgey) is very useful to plan further treatment. As shown in several meta-analysis studies, the outcome of a surgical technique is not as positive if preceded by failure as the outcome of the same technique used as first choice (2-5). Therefore it is to be considered a mistake to choose as a first surgical step "a simple, fast and economic technique" when there is no specific indication for that particular technique. Depending on the size of the chondral defect, a reconstructive technique should be considered in the case of failure of a reparative technique (6-7).

Timing

Better results can be obtained if the difference in time between the beginning of the clinical signs/symptoms and the treatment does not exceed an average of 2 years. In particular: microfractures < 12-18 months; ACI I or II generation 24-36 months; OATS not defined (3-5).

Age

It is difficult to define a limit for the treatment of a chondral lesion. Independently of the surgical technique, long term follow-up studies reported worst results in elderly patients. Different studies have revealed that young patients (< 30 years) have better results compared to older patients (8-9). In particular microfractures determine the best results in patients < 40 years (10). Hangody recommends an age limit of 50 years for OATS (6). Peterson et al. (11) in a long term study on ACI I generation (follow-up 10/20 years) does not report a significant difference in the final result considering age as a potential variable (average 46.4 years, limit 65 years). However, several studies have shown better results in the "osteochondritis dissecans" (younger patients) compared to "isolated or multiple femur lesions" (39.7 *vs* 43.7 and 49.6 respectively) (11).

Site of Lesion

Before surgery several questions need to be addressed:

- Which joint site is involved?
- Is the lesion located in a central zone of the joint where there is overload or is it in the peripheral zone?
- Is it a single, multiple or kissing lesion?

Microfractures have shown better results in the treatment of a femoral – tibial lesions compared to patello-femoral lesions (12). A similar trend can be observed in the OATS: 92% femoral condyle; 87% tibial plate; 74% patella (6). Long term studies have not shown different results in ACI except for patients with large lesion (6.38 $cm^2 \pm 2.40$) involving both patellar articular facets (3, 11, 13). The loss of height of the median patellar crest after debridement must be considered a negative prognostic factor. Therefore, Niemeyer et al. (14) proposed the "double eye technique": maintain the correct cartilage height of the patellar crest by treating separately the lesions on both surfaces (3, 5-6, 11).

Grade of lesion

The choice of the treatment is based on the ICRS classification (International Cartilage Repair Society) (15). In the superficial lesion (I°-II°) a conservative or pharmacological treatment with supplement drugs based on glucosamine/chondroitinesulphates or/with viscosupplementation could be indicated. However, in literature there are no high level studies showing the efficacy of pharmacological treatment. A symptomatic II° lesion (size > 1.5 cm^2 with superficial fibrillation) can be treated with joint debridement (16). The III°-IV° symptomatic lesions have to be approached with surgery. In III° lesion involving the bony layer, the choice is determined by the size of the defect. In the case of a grade IV lesion, the type of surgery depends on the thickness of the defect. In literature there are no studies showing the limits to the thickness of osteochondral lesions treated with microfractures or OATS. A contraindication for microfractures is the absence of subchondral plate, a fundamental structure for blood clot anchorage. A relative contraindication for OATS is the excessive depth of the defect that may determine potential instability of the osteochondral plugs (6). Peterson et al. define a lesion deeper than 8 mm as the limit for ACI (11). For larger lesions the use of cancellous or cortical-cancellous bone grafts should be considered

(3, 11). Allografts represent an attractive method to manage large and deep osteochondral defects. However, the time and the percentage of the graft incorporation is inversely proportional to the amount of bone graft (2, 8). Therefore, the risk of graft collapse/fracture is determined by the quantity of bone graft. Several authors reported the worst results in cases of immune response, a problem related to the amount of bone graft (2, 8).

Lesion size

The treatment choice for a III°/IV° lesion depends on the size of the defect. The limit is 2-4 cm^2. In patients with a low functional demand, a defect < 4 cm^2 can be successfully treated with microfractures or OATS. In young patients with high functional demands or athletes the limit for microfracture or OATS in 2 cm^2 (2, 5-6, 9). ACI techniques have shown constant positive results for lesions > 2-4 cm^2 at long term follow-up (3, 8, 11). Allografts can be considered an alternative to treat large but not deep osteochondral defects, especially in the case of failure of previous surgeries (2, 8).

Type of lesion contention

The cartilage lesion may be delimited or not by healthy or malacic cartilage. In the first case all techniques can be used. If the lesion is uncontained or surrounded by degenerated tissue, not all surgical techniques can be used: the absence of a cartilage wall surrounding the defect would not facilitate the anchorage of the blood clot generated by microfractures and the contention of the cellular suspension used in the first generation ACI technique. Therefore, it may determine graft instability (in OATS procedure) and in particular in the mosaicplasty technique. This problem can be solved by using resorbable nails to directly fix the periostium to the bone, or using one of the second generation ACI techniques.

Return to sport activities and long term follow-up

The average time for return to sport activities at the same level before the injury, in the amateur athletes is as follows: OATS 6.5 months, microfractures 16 months, ACI 25 months (2-3, 5-6, 9, 11). In professional athletes the average time is lower: OATS 5.5 months, microfractures 7.8 months, ACI 14.2 months. The rate of athletes that return to the same previous sport activity level is the following: microfractures 59% (range 25%-100%), ACI 48% (range 27%-100%), OATS 93% (9). In long term studies there is no evidence of decrease

Table I. *Indications and variables influencing the final outcome.*

Technique	Indications	Suboptimal outcomes
Microfractures	- Age <40 years	- Age >40 years
	- Focal contained lesion	- Uncontained lesion
	- Femoral condyles	- Lesion >4 cm^2
		- Patellar lesions
OATS	- Femoral condyles < 2-4 cm^2	- Deep osteochondral lesions
		- Kissing lesions
ACI	- Lesions > 2cm^2	- Kissing lesions
		- Uncontained lesions (I generation)
Allograft	- Osteochondral lesions	- Kissing lesions
	- Lesions > 2-4 cm^2	- Osteoarthritis

Femoro tibial lesion

↓

Alignment

↓

Ligaments

↓

Meniscus

First treatment option

	0 – 1 cm^2	**1 – 2 cm^2**	**2 - 4 cm^2**	**> 4 cm^2**
Microfracture	++	++	+/-	--
OATS	++	++	+/-	--
ACI	--	+/-	++	++
Allograft	--	--	--	+/-

Second treatment option

	0 – 1 cm^2	**1 – 2 cm^2**	**2 - 4 cm^2**	**> 4 cm^2**
Microfracture	++	+/-	--	--
OATS	++	+/-	--	--
ACI	--	++	++	++
Allograft	--	--	+/-	+/-

Legenda:

-- Treatment not recommended

+/- Questionable treatment

++ Treatment of choice

Fig. 1. *Flow chart for femoro-tibial lesions.*

in results for ACI and OATS during follow-up. However, only short term studies have been reported for OATS. Microfractures have shown worse results at 3-5 years follow-up, in particular with lesions >2 cm^2 (2, 5). Arthroscopic ACI techniques showed a faster return to sport activities compared to open ACI techniques at follow-up >2 years (17). Positive prognostic factors, especially for ACI techniques, are: age <30 years, lesion size <2 cm^2, time between symptoms and surgery <18 months, no previous surgical treatment, high level sport activities before injury and after surgery (9).

CONCLUSIONS

Considering the above mentioned variables, two different algorithms are proposed for III°-IV° femoro-tibial (Fig. 1) and patello-femoral (Fig. 2) lesions. In both algorithms two treatment options are suggested: primary and secondary. The first option

Patello-femoral lesion

↓

Alignment

First treatment option

	0 – 1 cm^2	**1 – 2 cm^2**	**2 - 4 cm^2**	**> 4 cm^2**
FKT	++	++	++	++
Microfracture	++	+/-	--	--
OATS	++	+/-	--	--
ACI	--	+/-	++	++

Second treatment option

	0 – 1 cm^2	**1 – 2 cm^2**	**2 - 4 cm^2**	**> 4 cm^2**
FKT	++	++	++	++
Microfracture	+/-	--	--	--
OATS	++	+/-	--	--
ACI	+/-	++	++	++

Legenda:

-- Treatment not recommended

+/- Questionable treatment

++ Treatment of choice

Fig. 2. *Flow chart for patello-femoral lesions.*

is the treatment of choice. The second one is to be considered in case of failure/contraindication of the first choice. In Table I the principal indications and variables which may influence the result of each technique are summarized.

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TRADITIONAL TECHNIQUE FOR ARTICULAR CARTILAGE REPAIR: JOINT DEBRIDEMENT

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Joint debridement is the oldest surgical treatment for symptomatic knees due to cartilage related problems. It was initially performed through a wide-open arthrotomy, but for the last 4 decades the arthroscopic approach has become a mainstay. It is always combined with a joint lavage. The rationale for this treatment is removal of the unstable cartilage flaps from the lesion, together with free-flowing cartilage debris from the joint. When a degenerated joint is targeted other soft-tissues, such as partial resection of degenerated menisci, resection of hypertrophic synovial folds, or osteophytes may be addressed simultaneously. As the joint debridement does not aim to restore the articular surface, it is a solely palliative procedure. Patients with predominant mechanical joint symptoms can expect substantial improvement of their knee function, while the reduction of intra-articular pain is less predictable. There are currently two well defined target patient populations for the joint debridement surgery: young active persons with small localized cartilage lesions who expect quick recovery, and elderly population with early stages of joint osteoarthritis in whom conservative management had failed.

Joint debridement is a surgical procedure that aims to remove the unstable cartilage flaps and major fibrillations from the cartilage lesion together with free-flowing cartilage debris. It has always been combined with a lavage, i.e. joint wash-out (1). Terminology has often been confusing, as "joint debridement" and "lesion debridement" have been used interchangeably. The expression "lesion debridement" should be focused only to the cartilage lesion itself, while the term "joint debridement" should address all the diseased intra-articular structures, such as redundant synovia, degenerated menisci and ligaments, free-bodies, or osteophytes. (2) An acute cartilage lesion may demonstrate vertical walls and U-shape form initially; however, a typical chronic (osteo)chondral lesion has a cater-like appearance. There is slow depth progression from the uninjured margins toward the deepest central part. Parts of the chronic lesions, especially the ones with previously failed cartilage repair procedures, may be filled with degenerated cartilage, fibrosis, or mixture of both. The calcified cartilage zone in such lesions is mostly abraded and the base of the lesion consists of sclerotic osseous tissue (Fig. 1) (3). Besides the mechanical symptoms related to the lesion, joint debridement and lavage tend to remove also inflammatory mediators and chemical irritants from the injured or degenerated joint. Debridement does not aim to restore the articular surface or stop the degenerative process inherent in the disease; it is therefore a purely palliative procedure that tends to reduce intra-articular symptoms. (4-5)

Surgical technique of joint debridement

The first reported joint debridement procedure was performed by Burman in 1934 (6). Open debridement

Key words: Joint debridement, cartilage repair, cartilage lesion

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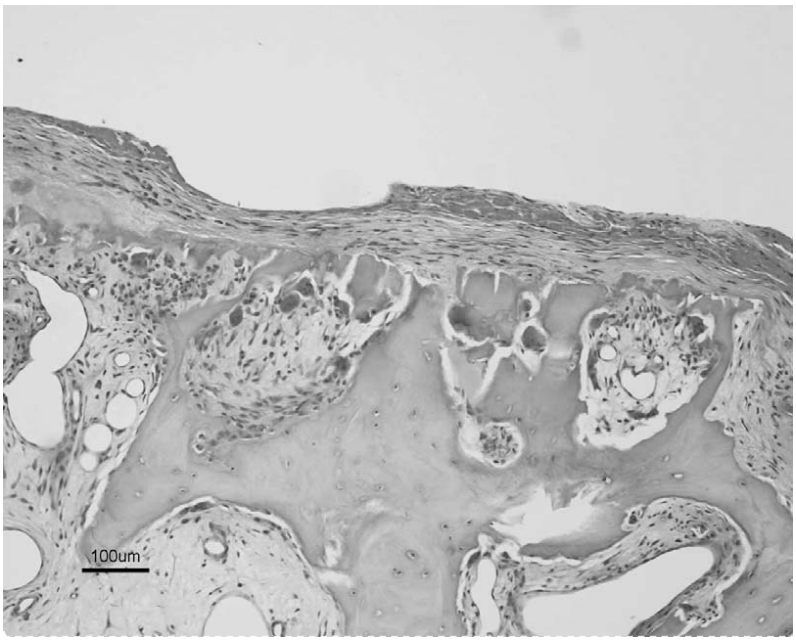

Fig. 1. *Histological sample removed from the central part of a chronic chondral lesion on the medial femoral condyle in a 29-year old male patient. The lesion is covered with fibrosis and remnants of degenerated cartilage. Tide-mark and calcified cartilage layer are absent. Note also the severe vascular invasion from the exposed subchondral bone, that has not resulted in an adequate repaired tissue formation (haematoxylin and eosin; horizontal bar distance 100µm, x 400).*

for osteoarthritic (OA) knee joint was popularized by Magnuson over 70 years ago (7). The arthroscopic joint debridement technique for symptomatic knee joints with mild to moderate OA has been promoted by numerous authors in the early 1980es, who reported similar results to those described by Magnuson. A symptom relief was encountered in 60% to 80% of patients with osteoarthritis, treated this way. (8-9) Arthroscopic joint debridement is typically performed by mechanical shaver devices ranging from low aggressive synovial resectors for soft-tissues to aggressive burs for osteophytes. Larger cartilage particles can be removed manually by arthroscopic punches, grasps, or scissors. The main disadvantages with this type of procedure are: collateral injuries to healthy cartilage, removal of healthy underlying cartilage (10-12) and potential lack of chondral surface smoothing. (13) The hydro-jet devices have not become widely accepted, due to their complexity, costs, and no clear clinical benefits over mechanical shaving devices, in spite of an initial positive response from the surgeons. (13) Similar destiny happened to the laser cartilage ablation. Although the articular surface of degenerate cartilage might appear smooth and congruent after such treatment, a serious damage to cartilage structure and chondrocytes occurs (14-15). A more recent alternative to mechanical shaving is represented by radiofrequency (RF) probes. These probes come as unipolar (one large skin electrode and one tip of the probe electrode) or bipolar (both electrodes on the tip of arthroscopic probe) devices. The bipolar probes were originally used as arthroscopic cutting and coagulation devices, but the surgeons soon realized that they also enable easy and effective smoothening of the rough articular surface. The application of RF devices on cartilage remains contradictory: it has been reported that high local temperatures associated with this method, bear the risk of cartilage destruction (16), but the clinical studies favor their outcome in comparison to mechanical shaving (17-19).

Surgical treatment of accompanying intra-articular problems

The principal concomitant intra-articular problems of the degenerated knee are meniscal tears, hypertrophic plicae, synovitis and osteophytes.

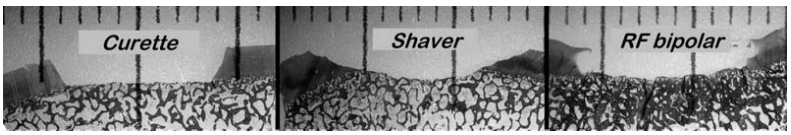

Fig. 2. *Typical histologic transections from an ex-vivo experiment on chondral lesion debridement in immature equine samples. (30) Note sharp and vertical lesion walls after arthroscopic curettage (left), crater-like lesion shape after mechanical shaving (center), and burned surrounding cartilage walls after bipolar radiofrequency ablation (right). Due to immature animal samples the calcified cartilage layer was absent and soft subchondral end-plate was injured at all times (trichrome Masson staining; distance between the long vertical lines 0.5 mm, x 6.3).*

Degenerative meniscal tears are predominately located in the posterior horns. They are typically multilayered and are not amendable for repair (20). Routinely all the unstable meniscal parts get resected by dedicated punches, scissors, or non-aggressive shavers. From the mechanical point of view, the torn meniscus does not provide any protection to the adjacent cartilage, therefore they may get resected if symptomatic (21). However, according to recent evidence, the degenerative meniscal tears should be initially treated conservatively unless the mechanical symptoms in the joint prevail (22). A hypertrophic medial parapatellar plica is a common finding in a diseased or post-traumatic knee. Such plicae may be symptomatic causing medial pain and tenderness adjacent to patella proximal to the joint line (23). Even though a plica may be seen as a pre-osteoarthritic condition causing cartilage wear from the medial condyle by rubbing and impingement, the criteria for which require resection and which do not are not clear (23). Most surgeons decide on plica removal according to several subjective criteria, such as: it interferes with visibility and maneuvering inside the joint, it feels hard on palpation and rubs over the condyle, it impinges between the patella and trochlea in extension, and it looks injured and swollen (24). Plicae can be either transected or entirely resected, but evidence favoring one approach over another is non-existant (24).

Synovitis is usually left untreated unless it interferes with visibility or causes mechanical problems. Synovitis following joint trauma or OA is a secondary problem, and it is expected to subside after the primary cause, the damaged or diseased cartilage, is treated. Osteophytes are standard companions of a degenerated joint and they take many years to form. If their size and/or location interfere with joint motion they can be removed by an aggressive shaver or a burr (25-26).

Cartilage lesion debridement prior to cartilage repair procedure

A separate issue is cartilage lesion debridement as the first step of a cartilage repair procedure. The goal of this debridement is to remove all diseased cartilage surrounding the lesion. This process targets all fissures and undermined cartilage, in addition to any fibrous tissue, degenerated cartilage, or sclerotic bone present in the base of the defect (27). The lateral walls of a debrided lesion need to be vertical and they should consist of healthy cartilage to ensure appropriate graft shouldering and secure attachment of marginal sutures for periosteum or membrane, in case they are needed (28). Also, to avoid bleeding from the subchondral sinusoids, debridement must not violate the subchondral end-plate (29). If bleeding in the lesion is encountered, placing compresses diluted with a combination of epinephrine and thrombin into the lesion can control hemostasis. Our recent *ex-vivo* study on cadaveric material, which studied different techniques of debridement prior ACI showed that the traditional open combination of scalpel and curettage provided the best quality debridement. Application of a mechanical shaver or RF-probes gave much poorer results for this particular purpose (30) (Fig. 2).

Rehabilitation after joint debridement surgery

The post-operative rehabilitation protocol after joint debridement is typically quick and non-demanding. Immediate full weight-bearing with active range-of-motion and isometric exercises are predominately allowed. Protective weight-bearing is advocated only in patients with severe

post-operative pain. Progressive stationary cycling is added as soon as the knee flexion allows. Initial proprioceptive training may be started around week 3. Patients typically return to their daily activities after 4-6 weeks (31). Young patients with an isolated cartilage lesion progress much faster than the older ones with diffuse joint degeneration which requires more extensive surgery. Nevertheless, joint pain and swelling are crucial factors influencing the intensity of rehabilitation protocol (31).

Evidence from clinical trials

Due to reports of recent studies with high medical evidence, the clinical interest in debridement of OA joints has been gradually declining. Authors have mostly demonstrated that arthroscopy for a degenerated knee provides no additional benefit to an optimized physical and medical therapy.

In a controlled trial, Moseley et al. (32) showed that there was no benefit when comparing arthroscopic lavage and debridement with placebo surgery. In 2007, Siparsky et al. carried out an evidence-based review of the literature on the arthroscopic treatment of knee OA and found limited support for its use (33). Dervin et al. (34) showed the importance of patient selection before knee arthroscopy. They showed that patients with evident lesions of the meniscus or cartilage flaps may benefit from surgery. Another study that compared arthroscopic debridement versus arthroscopic lavage confirms that, in well-selected middle-aged patients with knee arthritis, arthroscopic debridement may be valuable for providing transient relief of symptoms (35). Jackson et al. (36) showed that patients with less extensive arthritis as seen by radiography, less severe involvement of articular cartilage, and a younger age at the time of surgery, have a higher probability of improvement. In an observational cohort study, Fu et al. (37) compared autologous chondrocyte implantation versus debridement for treatment of full-thickness chondral defects of the knee. They concluded that patients who received autologous chondrocyte implantations obtained higher levels of knee function and had greater relief from pain and swelling at 3 years. A recent Cochrane review (1) of arthroscopic lavage and debridement for knee OA identified only three well-designed studies (32, 35, 38) and concluded from these that the procedure offers no benefits for OA caused by mechanical or inflammatory causes. On the basis of available evidence, arthroscopic lavage seems to provide only short-term relief for selected patients with mild radiographic OA and effusion. Arthroscopic debridement should not be used as routine treatment for knee OA, although patients with symptomatic meniscal tears and loose bodies with locking symptoms could benefit.

CONCLUSIONS

Joint debridement is a palliative surgical procedure that aims to remove the unstable cartilage flaps and major fibrillations from the cartilage lesion together with free-flowing cartilage debris. It can be used for either post-traumatic or degenerative intra-articular problems. Other intra-articular pathologies, such as meniscal tears, hypertrophic plicae, redundant synovia, and/or related osteophytes can be addressed simultaneously. Patients with predominant mechanical joint symptoms can expect substantial improvement of their knee function, while the reduction of intra-articular pain is less predictable. There are currently two well defined target patient populations for the joint debridement surgery: young active people with small localized cartilage lesions who expect quick recovery and elderly people with early stage knee osteoarthritis in whom conservative management has failed.

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CURRENT AND FUTURE SCAFFOLDS FOR ARTICULAR CARTILAGE REPAIR

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Cartilage tissue is difficult to treat, therefore several surgical approaches have been proposed over the years to treat chondral or osteochondral lesions. Autologous chondrocyte implantation (ACI) was the first clinical application of cartilage regeneration and was first performed 25 years ago for the treatment of isolated chondral lesions in the knee. The positive results of this treatment have to be weighed against several problems, both from biological and surgical points of view. Therefore treatments using biomimetic scaffolds were developed in an attempt to fulfill the requirements of cartilage regeneration processes. These scaffolds had substantial differences regarding the materials chosen, natural or synthetic, and their physical forms, but all aimed at overcoming the problems related to previous procedures. Scaffolds are a temporary three-dimensional structure of biodegradable polymers for the *in vitro* growth of living cells, and some more recently developed scaffolds are biphasic products that enable even large chondral or osteochondral articular defects to be treated. The surgical procedure is different depending on the scaffold used: some scaffolds require a two step-procedure, others a one-step procedure. This review describes the treatment of chondral and osteochondral knee lesions by using these scaffolds and shows the results and limits of this scaffold-based repair approach for the healing of the articular surface.

Several surgical approaches have been proposed over the years to treat chondral or osteochondral lesions, but the properties of healthy cartilage tissue are still unmatched by any available treatment (1-2).

Autologous chondrocyte implantation (ACI) was the first clinical application of cartilage regeneration and was first performed 25 years ago for the treatment of isolated chondral lesions in the knee (3). The positive results of this treatment have to be weighed against several problems, both biological and surgical. From a biological point of view, critical aspects are the maintenance of the chondrocyte phenotype during the prolonged monolayer culture and the risk of a not homogeneous distribution of the liquid cell suspension in the lesion site. From a surgical point of view, the standard ACI procedure presents various limitations related to the complexity and morbidity of the technique (4).

In an attempt to overcome the limitation of first generation approaches, scaffold-based treatments were developed. The rationale of using a scaffold is to have a temporary three-dimensional structure of biodegradable polymers for the *in vitro* growth of living cells and their subsequent implantation into the treatment area. Scaffolds aim to fulfill the requirements of cartilage regeneration processes, with substantially different strategies regarding the materials chosen, natural or synthetic, and their physical forms (fibers, meshes, gels) (4). Finally, there are some new biphasic products. The bilayer structure allows the entire osteochondral unit to be treated, which is important in particular in case of

Key Words: osteochondral defect, cartilage regeneration, scaffold, knee

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large chondral or osteochondral articular defects, because it reproduces the different biological and functional requirements for guiding the growth of both bone and cartilage tissues (5-6).

The aim of this review is to describe the treatment of chondral and osteochondral knee lesions by the use of scaffolds, and to show surgical options and results of this scaffold-based repair approach for healing the articular surface.

Two-Step Procedures

Matrix-assisted ACI techniques (MACI) have been used in clinical practice for a decade, and offer similar results to first generation ACI while overcoming most of its related drawbacks (4).

MACI: The first autologous chondrocyte transplantation using a porcine collagen type I/III membrane (Chondro-Gide, Geistlich Biomaterials, Switzerland) was performed in 1998. The surgical technique, as every Matrix-assisted Autologous Chondrocyte Transplantation (MACT) procedure, involves two surgical steps: harvesting articular cartilage from a non-weight-bearing area and, after culturing cells for 4 weeks and then seeding and culturing with autologous serum for the remaining 3 days on the rough side of the porcine collagen matrix, arthrotomic implantation of the bioengineered tissue into the defect. In 2006 Behrens et al. (7) treated localized cartilage defects using MACI® and obtained a good clinical outcome in 8 out of 11 patients 5 years after transplantation. In 2006 Ronga et al. (8) reported the successful treatment of a complex knee ligament, meniscal and chondral lesion in a 40-year-old sportsman at 2 years follow-up. Normal joint biomechanics was restored after two surgical steps. In 2009 Salzmann et al. (9) confirmed these good results in a comparative study: 9 patients achieved a significant clinical improvement such as that obtained in a matching group of patients treated with osteochondral autograft transplantation. Gigante et al. (10) focused on a specific patient population affected by patellar lesions: all 12 patients presented a significant improvement in all scales with 93% of satisfied patients. In 2010 Basad et al. (11) performed a randomized trial comparing MACI® and microfractures for the treatment of lesions > 4 cm^2: the evaluation at 2 years of both groups showed significantly higher and more stable results over time

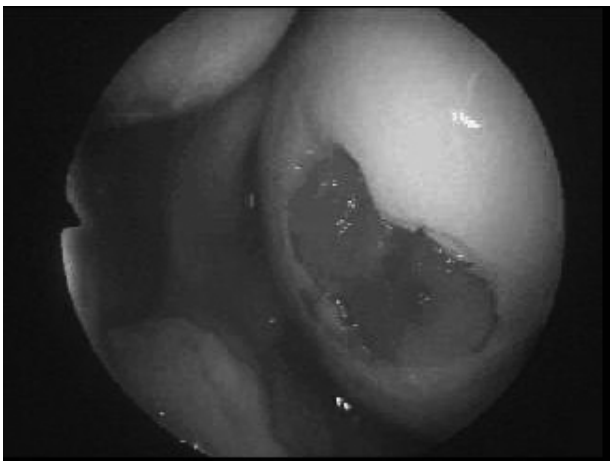

Fig. 1. *The hyaluronic acid-based bioengineered tissue (Fidia Advanced Biopolymers Laboratories, Padova, Italy) is positioned arthroscopically in the prepared area to cover the lesion.*

for MACI®. In 2011 Ebert et al. (12) also reported a clinical and functional improvement and positive MRI findings in 41 patients at 5 years of follow-up. In 2012 Macmull et al. (13) treated patients with symptomatic chondromalacia patellae: results were satisfactory and better than those obtained in a comparative ACI group. In 2011 Bauer et al. (14) combined MACI® and tibial osteotomy in patients with medial knee osteoarthritis and varus knee, and obtained good clinical and MRI results initially but at 5 years they worsened. Finally, in 2012 Ventura et al. (15) studied fifty-three patients with osteochondral lesions and documented good results at 2 and 5 years of follow-up, functional and pain improvement and complete integration of the graft with the surrounding native cartilage in 88% of the patients.

HYALOGRAFT® C: Hyaluronic acid is the main component of Hyalograft® C, introduced into clinical practice in 1999. This scaffold is made up of hyaluronic acid benzylic ester (HYAFF® 11, Fidia Advanced Biopolymers Laboratories, Padova, Italy) and consists of a network of 20-µm-thick fibers with interstices of variable sizes. The features of this device have allowed the development of an arthroscopic surgical technique (Figure 1). In 2005 Marcacci et al. (16) reported the clinical results of a multicenter study: at a 3-year mean evaluation 91.5% of patients improved, and cartilage repair was graded

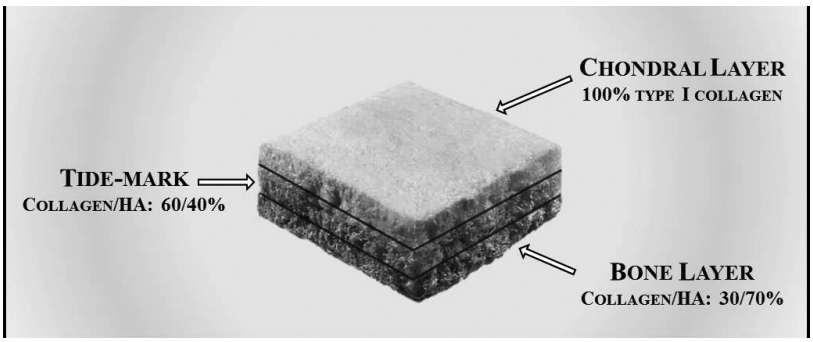

Fig. 2. *The osteochondral nanostructured biomimetic scaffold (Fin-Ceramica S.p.A., Faenza, Italy) has a porous 3D tri-layered composite structure, mimicking the entire osteochondral anatomy.*

arthroscopically as normal or nearly normal in most knees. Moreover, the majority of the second-look biopsies were judged as hyaline-like. In 2006 Nehrer et al.(17) confirmed the good short-term results, and in 2006 Gobbi et al. (18) reported a positive outcome at 2 years also treating 32 patellofemoral full-thickness chondral defects. The same authors subsequently evaluated this group of patients at 5 years (19) and showed a worsening with respect to the previous analysis, but still good clinical and histological results. In 2007 Marcacci et al. (20) and in 2009 Nehrer et al. (21) also performed a medium-term follow-up evaluation and confirmed the significant clinical improvement with stable results over time. Ferruzzi et al. (22) treated 50 patients affected by OCD and traumatic lesions and in 2008 showed consolidated clinical results at a minimum of 5 years' follow-up and a well-integrated cartilage tissue in most final MRI follow-ups. Moreover, they also compared patients treated with Hyalograft® C and patients treated with first generation ACI, and found a similar healing potential but fewer complications and shorter hospitalization in the MACT technique. In 2011 Kon et al. (23) reported durability of the good clinical results obtained and a correlation between imaging and clinical findings at 5 years' follow-up; in another following comparative study they also reported satisfactory results in 40 patients treated with Hyalograft® C at 5 years' follow-up, unlike the comparative microfracture group, where a deterioration was observed over-time. In 2010 Della Villa et al. (24) focused on assessing the post-operative phase and showed that intensive rehabilitation may allow a faster but safe return to competition and a positive influence on the clinical outcome at medium-term follow-up. Finally, in 2011 Filardo et al. (25) confirmed the good results obtained with Hyalograft® C at up to 7 years' follow-up, with overall good and stable results over time but a poorer outcome in degenerative lesions.

BIOSEED: Bioseed C® (BioTissue Technologies), composed of fibrin, polyglycolic/polylactic acid and polydioxanone is one of the most widely used synthetic scaffolds: it is a cartilage tissue-engineered graft that combines autologous chondrocytes, embedded in fibrin, with the tissue development-promoting properties of 2-mm thick porous gel-like matrix in an initially mechanically stable bioresorbable polymer scaffold, and has been applied in clinical practice since 2001. This biomaterial differs from the others in the fixation technique. After careful debridement of the defect to a rectangular shape, the graft is fitted and fixed by arming the corners with resorbable threads, anchored transosseously to each corner. in 2007 Ossendorf et al. (26) reported the clinical results of 40 knees affected by degenerative defects at 2 years' follow-up and showed a significant clinical improvement, besides good integration of the graft and formation of a cartilaginous repair tissue. In 2009 Kreuz et al. (27) confirmed these good results in 19 patients of the same group analyzed at 4 years. Finally, in 2010 both Erggelet et al. (28) and Zeifang et al. (29) found a significant improvement, similar to that achieved with the original ACI periosteum-cover technique, respectively in a retrospective comparative study and in a randomized clinical trial.

NEOCART: NeoCart (Histogenics Corporation,

Waltham, Massachusetts) is a 3-dimensional type I collagen scaffold seeded with autologous chondrocytes by a tissue-engineered protocol that includes treatment with a bioreactor. The resulting product is a viable proteoglycan- and glycosaminoglycan-rich tissue-like implant, which is surgically fixed to the damaged area with CT3 bioadhesive (Histogenics). In 2009 Crawford et al. (30) reported a good clinical outcome at 2 years' follow-up and good implant integration revealed by MRI.

NOVOCART: Novocart 3D (B. Braun-Tetec, Reutlingen, Germany) is a patch where autologous chondrocytes are embedded in a 3-D collagen-chondroitin sulfate scaffold. In 2012 Panagopoulos et al. (31) evaluated professional soldiers or athletes with large defects at a minimum of 2 years in a comparative study between classic ACI with periosteal flap and Novocart 3D: despite the overall improvement only 6/19 returned to previous athleticlevels. A trend towards better results for Novocart 3D was found, albeit without reaching statistical significance.

CARES: CaReS® (Ars Arthro®, Esslingen, Germany) is composed of autologous chondrocytes seeded on 3D type-I collagen gel. The cells are isolated, mixed with collagen gel, and after complete gelling and two weeks of culturing, the chondrocyte-loaded gel is available for transplantation. In 2010 Welsch et al. (32) evaluated a small group of patients treated with two bioregenerative approaches: 10 patients underwent CaReS® implantation and were compared with 10 homogeneous patients treated with Hyalograft C®. Although the clinical outcome at 2 years was comparable, MRI analysis showed a better surface of the repair tissue in the CaReS® group. In 2009 Wondrasch et al. (33) applied CaRes® or Hyalograft C® in 31 patients, and found an overall significant improvement at 2 years of follow-up. The author showed that early weight bearing was correlated with a higher prevalence of bone marrow edema after 6 months but this did not compromise the clinical outcome. More recently, in 2011 Schneider et al. (34) published the results of a multicenter study that evaluated patients from 12 to 60 months: overall good results were reported, with a continuous improvement and better results at the last follow-up regardless of lesion size, site and number of defects, whereas a greater improvement was found in the OCD group.

CARTIPATCH: Cartipatch® (TBF Banque de tissues, France) uses a vegetal origin hydrogel composed of agarose and alginate. This hydrogel is mixed with isolated autologous cell suspension and can be modulated at 37°C into complexly-shaped implants that solidify at approximately 25°C. Alginate provides matrix elasticity, making it easy to handle. In 2008 Selmi et al. (35) evaluated the treatment of chondral and osteochondral defects at a minimum follow-up of 2 years. Clinically, all 17 patients improved markedly, especially those with lesions larger than 3 cm^2, and good MRI findings, arthroscopic appearance, and predominantly hyaline cartilage in 62% of the biopsies was found.

ATELOCOLLAGEN GEL: Autologous chondrocytes cultured on atelocollagen gel have also been investigated. In 2007 Adachi et al.(36) reported a corticosteroid-induced osteonecrosis at both femoral condyles treated with atelocollagen gel (3% type I collagen; Koken, Tokyo, Japan) that was used as a scaffold for bone-marrow-expanded cells and cultured chondrocytes, respectively, to regenerate both osseous and chondral tissues. A synovial flap was sutured to cover the lesion and secure the osteochondral implants. MRI and clinical results showed a successful outcome at 2 years. In 2009 Toyama et al. (37) performed a multicenter study showing the usefulness of atelocollagen-associated chondrocyte implantation for the repair of chondral knee defects: both clinical and arthroscopic outcomes were positive, with a marked improvement and 92% of knees presenting normal or nearly normal arthroscopic appearance.

CHONDRON: Another gel-type autologous chondrocyte procedure (Chondron™, Sewon Cellontech Co. Ltd, Seoul, Korea) involves the injection of cultured chondrocytes mixed with fibrin (1:1) into the defect area previously prepared by debridement and multiple holes to favor graft purchase, and without the need for periosteum or other membrane covers. Fibrin gel can provide a three-dimensional scaffold with the advantages of technical simplicity and minimal invasiveness and seems to provide satisfactory results. Choi et al. (38) used Chondron for treatment of articular cartilage defects and in a multicenter study they evaluated

40 patients with more than 2 years of follow-up. In 2010 they showed the safety and effectiveness of this method. In 2010 Kim et al. (39) also, found a significant clinical improvement, as well as good MRI findings and a nearly normal arthroscopic appearance at 2 years of follow-up in a study of 30 patients.

One-Step Procedures

Some solutions to allow the implant of both scaffold and cells in one surgical step have been proposed recently to simply further the bioengineered approach.

CAIS: in 2011 Cole et al. (40) embedded healthy cartilage tissue harvested from an unaffected area of the injured joint into a 3-D polymeric reabsorbable scaffold (copolymer foam of 35 % polycaprolactone (PCL) and 65 % polyglycolic acid (PGA) reinforced with polydioxanone (PDO) mesh. The results of this cartilage autograft implantation system were reported in a randomized study that showed better subjective results at 2 years compared with microfractures. MRI evaluation revealed no differences in defect filling, tissue integration, or subchondral cysts, although more intralesional osteophyte formation was found in the microfracture group.

HYALOFAST + BONE MARROW CONCENTRATE + PRP: in 2010 Buda et al. (41) reported the use of a hyaluronic acid membrane (Hyalofast; Fidia Advanced Biopolymers, Abano Terme, Italy) filled with bone marrow concentrate and covered with a layer of platelet-rich fibrin. They achieved good clinical results, 80 % graft integration and 70 % defect fill according to MRI at 2 years of follow-up.

ALGINATE BEADS: in 2009 Almqvist et al (42) implanted mature human allogenic chondrocytes into a biodegradable alginate-based scaffold (Sigma, St Louis, Missouri, USA) in 21 patients. They observed a significant clinical improvement and no adverse reactions at 2 years of follow-up, although hyaline-like tissue was only found in a few patients.

Alternatively to the use of cell-based scaffolds, another treatment approach involves the implantation of cell free scaffolds for "in situ" cartilage regeneration by stimulating bone marrow stem-cell recruitment and differentiation.

AMIC®: Autologous Matrix-Induced Chondrogenesis combines microfracturing with the implantation of a porcine collagen type-I/III bilayer matrix to stabilize blood clotting. This proved to be a reasonable one-step treatment for cartilage defects. In 2010 Gille et al (43) reported satisfactory results in 87 % of patients evaluated at a mean follow-up of 37 months; MRI showed moderate-to-complete filling and a normal-to-hyperintense signal in most cases. In 2011 Dhollander et al (44) proposed an AMIC "plus" technique for the treatment of patellar lesions. This consists of the augmentation of AMIC® with platelet-rich plasma (PRP) gel. In a pilot study of 5 patients good clinical results were found at 2 years. However, there is not enough conclusive data to determine the effectiveness of this combined approach. In 2011 Schiavone Panni et al. (45) reported the use of another modified AMIC® technique (drilling + fibrin glue) in a study with 17 patients evaluated at a mean of 36 months: 76.5% of patients were satisfied or extremely satisfied and a 58.8 % reduction of defect area and subchondral bone edema at MRI was documented. Finally, in a retrospective study in 2011, Kusano et al. (46), described patients treated with AMIC for chondral and osteochondral defects. They reported an overall improvement in both groups, with the largest improvements being in the osteochondral subgroup at a mean follow-up of 28 months. Although MRI showed that tissue filling was present, it was generally incomplete and heterogeneous. In 2010 Pascarella et al. (47) combined a collagen patch (Chondro- Gide; Geistlich, Wolhusen, Switzerland) with 15-mm deep perforations made with a 2-mm Kirschner wire, to exploit the advantages of the Pridie technique, which might allow a greater number of MSCs to enrich the membrane: they reported good results at 2-years of follow-up.

For osteochondral articular defects different specific scaffolds have been developed.

Among these scaffolds only 2 for osteochondral regeneration are currently commercially available for clinical application.

TRUFIT: TruFit; Smith & Nephew, Andover, Massachusetts, USA is a bilayer porous polylactic-co-glycolic acid (PLGA)-calcium-solfate biopolymer. In 2011 Barber et al. (48) found that the plugs do not promote bone ingrowth, but rather lead to subchondral cyst formation in all cases.

Conversely, in 2010 Bedi et al. (49) reported that the mid-term MRI follow-up can significantly improve over time. In 2009 Carmont et al. (50) suggested that the plug appearance may significantly improve at further follow-up, and reported a significant clinical improvement in an 18-year-old footballer at 2 years.

MAIOREGEN: This osteochondral scaffold is a nanostructured 3-layer biomimetic scaffold (Maioregen; Fin-Ceramica S.p.A., Faenza, Italy) with a porous composite structure, mimicking the osteochondral anatomy. It is composed of a cartilaginous Type I collagen upper layer, an intermediate tidemark-like layer consisting of a combination of Type I collagen (60 %) and hydroxyapatite (40 %), and a lower layer composed of a mineralized blend of Type I collagen (30 %) and hydroxyapatite (70 %) to mimic the subchondral bone (Figure 2). In one surgical stage, an arthrotomic medial or lateral parapatellar approach is used to expose the damaged articular surface. Then the defect is prepared with chisels that are used to remove the sclerotic tissue and form a rectangular or square area 8 mm deep. Afterwards, the scaffold is cut exactly according to the size of the prepared lesion. Once cut to the right size the scaffold is press-fit implanted with the smooth layer on the surface and the rough layer facing the bottom area of the lesion. After promising preliminary results (51), longer term results have been recently reported. A case report on a 46-year-old man affected by multi-focal degenerative chondral lesions showed good results at 1 year, with return to previous level of athletic activity and restoration of the articular surface (52). In a pilot study on patients with chondral and osteochondral lesions, the authors confirmed the positive results obtained earlier, which consisted of a slower recovery in older, less active patients and poorer results in patellar lesions. However, at 2 years' follow-up, good results were reported in all patients by both clinical and MRI evaluations (53). Finally, good mid-term results were even reported with a combined biological (MaioRegen) and mechanical (external distracter) approach for the treatment of a complex knee lesion (54).

DISCUSSION

Cartilage regeneration is a challenging problem for surgeons in the operating room and basic scientists in the laboratory aimed at restoring a hyaline-like tissue with normal biomechanical characteristics.

Research in bioengineering has led to the development of new technologies and new surgical treatment options for cartilage lesions. The use of 3D structures for cell growth has been shown to promote the maintenance of a chondrocyte differentiated phenotype, while also simplifying the implant procedure.

To improve the available procedures, different strategies are being studied, mainly focusing on 2 aspects: simplifying the surgical technique and adopting more powerful agents to stimulate tissue regeneration. One-step cell-free procedures have been developed to avoid problems related to chondrocyte culture and expansion in scaffolds and also to reduce costs and surgical time. In fact, there is an increasing awareness that the role of scaffolds is not only to deliver cells, but to enhance tissue regeneration. For that reason, the use of cell-free scaffolds has been proposed and is gaining popularity. Finally, osteochondral scaffolds have been proposed to treat lesions where the subchondral layer is also involved in the pathologic process, and have shown interesting preliminary results. Although only studies reaching a minimum significant follow-up of 2 years have been reported, the present literature overview of scaffold-based treatment clearly underlines an increasing interest in the scientific community. However, most of the studies are just case series and more effort should be made to design and perform high level studies to support the preliminary findings of the first decade of cartilage scaffold-based treatment.

CONCLUSIONS

Regenerative scaffold-based procedures are emerging as a potential therapeutic option for the treatment of chondral and osteochondral lesions. In fact, this review shows a growing interest in new regenerative procedures and one-step scaffold-based strategies have been recently developed to further simplify and improve the results of the treatment of chondral or osteochondral lesions.

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CONFIRMED TECHNIQUES FOR ARTICULAR CARTILAGE REPAIR: THE HYALOGRAFT-C SYSTEM

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In the last ten years, after various preclinical trials and the development of innovative implantation techniques, a great deal of studies have been performed, focusing on the most varied categories of patients. Second generation matrix-assisted autologous chondrocyte transplantation using the hyaluronan scaffold Hyalograft® C arose as a very promising technique among all the cartilage repair procedures.

For the orthopedic surgeon cartilage lesions have always been a challenging problem. In recent decades several studies have been aimed at solving it and some open questions have finally been answered. We do know, for example, that an untreated lesion of the articular knee cartilage will almost certainly lead to osteoarthritis (OA) (1), which can only be effectively treated by joint replacement. Nevertheless it is still not known how to heal these defects or how to stop this degenerative process. Among the many treatments that have been proposed (2), one that has shown very promising results is Matrix-assisted Autologous Chondrocyte Transplantation (MACT). It can be briefly explained as an evolution of the original Autologous Chondrocyte Implantation (ACI) described by Brittberg and Peterson (3): instead of a monolayer culture of harvested chondrocytes injected under a periosteal flap fastened around the defect, the MACT technique uses a three-dimensional chondrocyte culture on a scaffold which is directly placed inside the cartilage lesion (4). Since 1999, a specific cell-based scaffold has been used and studied in our ward: the bioengineered scaffold Hyalograft® C.

Hyaluronan scaffold

Hyaluronic acid (HA), a naturally occurring and highly conserved glycosaminoglycan widely distributed in the body, has proven to be an ideal molecule for tissue engineering strategies in cartilage repair, given its impressive multi-functional activity in cartilage homeostasis (5). Through a conservative chemical modification, HYAFF® 11 (Fidia Advanced Biopolymers, Abano Terme, Italy), an esterified derivative of HA, is obtained, which may be processed into stable configurations to produce a variety of biodegradable structures with different physical forms and *in-vivo* residence times. Extensive biocompatibility studies have shown the safety of biomaterials containing HYAFF® 11 and their ability to be resorbed in the absence of an inflammatory response (6). Three-dimensional non-woven scaffolds based on HYAFF® 11 support the *in vitro* growth of highly viable chondrocytes and promote the expression of their original chondrogenic phenotype (7). Chondrocytes, previously expanded on plastic and seeded into the HYAFF® 11 scaffold produce a characteristic extracellular matrix rich in proteoglycans and express typical markers of

Key words: hyaluronan scaffold, cartilage repair, cartilage lesions

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hyaline cartilage, such as collagen II and aggrecan (8-9). When implanted in full-thickness defects of the femoral condyle in rabbits, chondrocytes cultured in the HYAFF® 11 scaffold regenerate a cartilage-like tissue (10-11). HYAFF® 11-based tissue-engineered cartilage was assessed in an *in vitro* and *in vivo* setting with respect to structure, biochemical composition and mechanical behavior showing functional integration of the construct with native bone and cartilage (12). Hyalograft® C (Fidia Advanced Biopolymers, Abano Terme, Italy), is the tissue-engineered graft composed of autologous chondrocytes grown on the three-dimensional HYAFF® 11 scaffold.

Hyalograft® C Implantation

The surgical technique for Hyalograft® C implantation has 2 stages: cartilage biopsy and graft implantation. The arthroscopic biopsy of healthy cartilage for cell culture allows the site of the lesion and cartilage quality to be assessed. During the first surgical operation, combined problems, including meniscal injury or anterior cruciate ligament insufficiency, are addressed surgically. A small biopsy of healthy articular cartilage is obtained from the superior femoral trochlea or femoral notch. Chondrocytes are extracted from this biopsy and expanded in culture for re-implantation on the HYAFF® construct. In more detail, a 100-mg cartilage biopsy is taken from a non-weight-bearing site of the articular surface (intercondylar notch) and sent to the processing center in a serum-free nutritional medium. The next day, the tissue is minced into smaller pieces and digested with 0.25% trypsin at 37°C for 15 minutes and then with 300 U/mL collagenase type II (Worthington, Lakewood, NJ) at 37°C for 4 hours in Ham's F-12 medium. The digested material is centrifuged at 1,000 rpm (180 g) for 10 minutes, and the pellet is re-suspended in Ham's F-12 containing 10% fetal calf serum (Sigma Chemical Co, St. Louis, MO), 1% penicillinstreptomycin, 1% L-glutamine, 1 ng/mL transforming growth factor 1, 1 ng/mL insulin, 1 ng/mL epidermal growth factor, and 10 ng/mL basic fibroblast growth factor (all growth factors were recombinant and of human sequence). Typically, from 200 mg of tissue 1 to 2 million cells are recovered. Cells are amplified in monolayer cultures up to 3 passages, and then seeded onto HYAFF® 11 scaffolds (2 x 2 cm). 8 x 10^6 cells are re-suspended in 0.4 mL of medium (as stated previously, but containing 50 µg/mL ascorbic acid), the cell suspension is pipetted onto the scaffold and the culture is kept at 37°C, 5% CO2 overnight. The next day, additional medium is added to submerge the cell construct completely; the medium is changed twice a week. Hyalograft® C chondrocyte three-dimensional cultures are ready for shipment after 3/4 weeks. The day of shipment the cell construct is washed exhaustively with phosphate-buffered saline and then sealed in a sterile plastic tray containing 4mL of nutritional medium. The expiration time of the product is 72 hours.

Open Technique

The open surgical technique includes a mini-arthrotomy, defect preparation, graft sizing, and successive implantation of the autologous chondrocyte culture graft. The exposure dimension depends on the size and the location of the defect: a medial parapatellar incision is required for defects of the medial compartment and a lateral parapatellar exposure is used for lateral locations. A complete visualization of the defect is necessary to perform an easy preparation of the defect site. The subchondral bone must be exposed to remove all damaged cartilage without damaging the subchondral layer. The subchondral bone has to be exposed avoiding any lesion, in order to maintain the hemostasis in the defect area. It is fundamental to leave a sharp rim of healthy cartilage all around the defect area. The defect is then measured and Hyalograft® C graft is prepared by matching the defect dimensions. The graft must completely fit inside the margins of the defect to ensure stability of the graft and avoid any possible mobilization. The graft is then applied into the defect, and its stability is evaluated after cyclic bending of the knee. The wound and skin are then closed in a standard manner.

Arthroscopic Technique

The arthroscopic implant (Fig. 1) was developed for medial or lateral condyle lesions. A tourniquet may be needed at this point to prevent bleeding. During the arthroscopic procedure, the lesion is visualized and debrided to a stable rim using a motorized shaver. All unstable cartilage flaps are removed; all fibrous tissue is also removed from the

base of the defect. The defect is mapped and sized using a delivery device varying in diameter (6.5-8.5 mm) with a sharp edge to achieve complete coverage of the defect. A flipped custom cannula is then inserted into the appropriate portal (anteromedial portal - medial femoral condyle; anterolateral portal - lateral femoral condyle). The flip allows the removal of the fat pad from the camera view field; this is especially helpful when the knee is placed in a high degree of flexion. A custom cannulated low profile drill (6.5-8.5 mm) is placed according to the location of the defect as previously noted. The drill is maintained in the desired position by a Kirschner guide wire (0.9 mm in diameter) that is fixed to bone. This reamer, which has a safety stop at 2 mm, was developed specifically to avoid deep penetration of the subchondral bone that must remain intact for successful graft implantation and function. Only the Kirschner wire passes through the subchondral plate. The low speed reaming of the lesion surface creates a circular area with well-defined margins for graft placement. This reaming step must be performed carefully to achieve stable and precise lesion contours. As such, the reaming step is repeated to prepare the entire defect surface. It is usually possible to prepare a large defect by changing the knee flexion angle and orientation of the cannula. After reaming, the joint is cleared of cartilage debris. The fluid inflow is then closed and the joint is dried using suction applied through the cannula. The sharp edged delivery system is placed in contact with the Hyalograft® C patch containing the autologous chondrocytes. The patch remains in the sheath of the delivery system; the patch is then transported through the cannula and placed in the prepared defect. A delivery tamp is pushed to plug the patch precisely into the defect. The procedure is repeated until the defect is filled. Multiple Hyalograft® C graft discs can be inserted to achieve full defect coverage and the stability of the grafts is evaluated by cyclic bending of the knee. The tourniquet is released, and the graft stability is evaluated again. Mobilization of the implanted patch was not observed in the present series.

Rehabilitation Protocol

Patients are discharged one day after the arthroscopic implantation procedure. In the first 2 weeks after surgery, continuous self-assisted passive motion is started from 0° to 90° from the second postoperative day, thus promoting joint nutrition and preventing adhesions. Stretching exercises and quadriceps contractions are allowed if tolerated. Foot touch weight-bearing activity is permitted, whereas complete weight-bearing activity is not allowed for the first 4 weeks. From the fourth to fifth week, weight-bearing activity is increased, beginning in the swimming pool, to recover the normal gait phases; muscle-strengthening exercise are allowed from the seventh week. Increased strength and functional exercises are then gradually allowed. Return to sport should not be attempted before 8 to 12 months.

Clinical Studies

In 2005 Marcacci et al. (13) reported the clinical results of a multicenter study on 141 patients evaluated at a minimum of 2 years' follow-up. At the mean 3-year evaluation 91.5% of patients improved, and cartilage repair was graded arthroscopically as normal or nearly normal in 96.4% of the knees. Better results were obtained in traumatic lesions and in patients affected by OCD. Moreover, the majority of the second-look biopsies were judged as hyaline-like, and a very limited complication rate was recorded. In the same period, Nehrer et al. (14) confirmed the good short-term results in a group of 36 patients with lesions of the femoral condyles followed-up to 3 years, noticing that patients older than 30 years reached more modest scores compared to younger patients. The same was observed in patients with multiple lesions. Conversely, with regards to the patellofemoral lesions, Gobbi et al. (15) performed a study on 32 patients with full-thickness chondral defects, and reported a positive outcome at 2 years. The same authors followed-up the same group of patients at 5 years (16) and showed a worsening with respect to the previous study, but still good clinical and histological results. Moreover, at this medium term follow-up they were able to report significantly better results in the IKDC objective score in the 9 trochlear lesions compared to the 21 patellae and the 4 knees with multiple defects. A medium-term follow-up evaluation was also performed by Marcacci et al. (17) and Nehrer et al. (18): they evaluated respectively 70 and 53 patients and confirmed the significant clinical improvement with stable results over time. They also suggested

an impact of the activity level of the patient on the final outcome (17). Another influencing factor on the clinical results at the one-year follow-up was the surgical approach (19): the arthroscopic group showed a faster improvement with respect to the patients who were treated by mini-arthrotomy. These results were not confirmed at a longer follow-up (19), or in other studies (18, 20): for example Ferruzzi et al. (20) treated 50 patients affected by OCD and traumatic lesions, with stable clinical results at a minimum of 5 years' follow-up and a well-integrated cartilage tissue in 93% of the patients at the final MRI follow-up. Moreover, they also compared them with a group of patients treated with first-generation ACI and showed a similar healing potential but fewer complications and a more rapid recovery when the arthroscopic MACT procedure was used. Concerning the overall complications of the ACI and MACT techniques, a large systematic review was performed by Harris et al. (21), who found that, in 82 studies up to 2010, out of 5,276 subjects treated with ACI/MACT techniques 305 failures occurred (5.8% subjects; mean time to failure 22 months). Failure rates after ACI with perioteal patch (PACI), collagen-membrane cover ACI (CACI), second generation, and all arthroscopic second-generation ACI (Hyalograft® C and similar scaffolds) were 7.7%, 1.5%, 3.3%, and 0.83%, respectively, and the failure rate of arthrotomy-based ACI was 6.1% vs 0.83% for all-arthroscopic ACI. The re-operation rate after PACI, CACI, and second-generation ACI was 36%, 40%, and 18%, respectively. Thus, they found that the second-generation ACI (MACT), where Hyalograft® C is the most commonly used scaffold, really does reduce the number of failures and complications of the first-generation ACI with the periosteal flap.

Some studies were performed to define the potential of Hyalograft® C compared to other cartilage repair techniques. Kon et al. (22) analyzed two groups of 40 patients: the first treated with Hyalograft® C, the second with microfracturing. They reported good stable results in the patients treated with Hyalograft® C at 5 years' follow-up, conversely to the comparative microfracture group, where a deterioration was observed over-time. These results were also confirmed in a demanding patient population of high-level soccer players evaluated at 7.5 years of follow-up: whereas microfractures allowed a faster recovery but presented a clinical deterioration over time, arthroscopic Hyalograft® C delayed the return to competition but offered more durable clinical results (23). Della Villa et al. (24) focused on the evaluation of the post-operative phase by evaluating highly competitive athletes: a cohort of 31 athletic patients was compared with a similar control cohort of 34 non-athletic patients. The athletic cohort followed a 4-phase intensive rehabilitation protocol. Eleven of the patients in this cohort were also treated with an isokinetic exercise program and on-field rehabilitation. The patients in the control cohort completed only phase 1 of rehabilitation. They found that an intensive rehabilitation allowed safely a faster return to competition and also positively influences the clinical outcome at medium-term follow-up. Athletes treated with the on-field rehabilitation and isokinetic exercise program also had a faster recovery and an earlier return to competition. Clar et al. (25) reported a more challenging treatment, using hyaluronic-based MACT as salvage procedure for a 14-cm^2 defect, due to a previous steroid-induced osteonecrosis, in a 17.5-year-old girl. Their aim was to avoid a total knee arthroplasty. After imaging had revealed vital bone remodeling, scaffold transplantation was performed, rehabilitation was started and, after 5.5 years, the patient showed continuous clinical improvement and was satisfied with the result. MRI follow-up showed a solid cartilage layer covering the medial condyle as a result of bone and chondral regeneration.

With regards to the imaging evaluation, a study focusing on MRI at 5 years' follow-up was performed by Kon et al. (26) on 40 patients. They reported the durability of the good clinical results obtained but only a partial correlation between imaging and clinical findings: the total MOCART score and the signal intensity of the repair tissue were statistically correlated to the IKDC subjective evaluation. Furthermore, the larger size of the treated cartilage lesions had a negative influence on the degree of defect repair and filling, the integration to the border zone and the subchondral lamina integrity, whereas more intensive sports activity had a positive influence on the signal intensity of the repair tissue, the repair tissue surface, and the clinical outcome.

Filardo et al. (27) focused their attention on

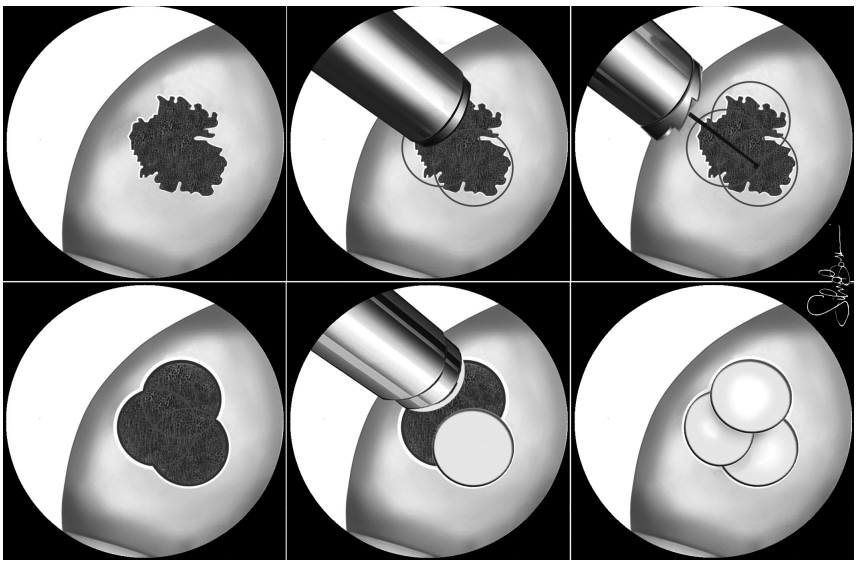

Fig. 1. *Schematic representation of arthroscopic technique: visualization of the damaged area; mapping of the lesion with a sharp trocar; a Kirschner wire guides a cannulated reamer according to the mapping previously performed; final preparation of the lesion; the delivered stamp is pushed to fit precisely the defect; patches are implanted inside the lesion.*

patients with a specific etiology: Osteochondritis Dissecans (OCD). For this pathology a modified technique of Hyalograft® C implantation has to be used, which in the first step, in addition to the cartilage biopsy, involves cancellous bone graft harvesting by a small incision at the metaphyseal area of the tibial head medial side and the creation of a bone window. The cortical flap is then closed, and the autologous cancellous bone chips are impacted arthroscopically into the base of the osteochondral defect in order to reach the level of the surrounding subchondral plate and restore the bony surface. Blood clotting promotes the stability of the implanted grafts that is assessed by knee flexion–extension movements. To perform the second step, it is necessary to wait at least 2 months to allow the formation of a stable subchondral bone area. In the above-mentioned study, thirty-four knees affected by symptomatic OCD grade III or IV on the ICRS (International Cartilage Repair Society) scale were treated and prospectively evaluated at 12-24 months of follow-up, and at a final mean 6±1 years of follow-up. A significant improvement in all scores was observed after the treatment. The IKDC subjective score improved from 38±13 to 81±20, and 91% of the knees were rated as normal or nearly normal in the objective IKDC at the final evaluation. EQ-VAS and Tegner scores showed a statistically significant linear trend of improvement over time increasing from 52±18 to 83±14 and from 2±1 to 5±3, respectively, at 6 years' follow-up. A better outcome was obtained in men, sport-active patients, and smaller lesions. In the same year, Filardo et al. (28) also reported the clinical results of sixty-two patients treated with Hyalograft® C for full-thickness cartilage defects of the femoral condyles and followed prospectively for 7 years. The IKDC subjective score increased from 39.6±15.0 to 73.6±18.8 at 12 months; a further slight improvement was observed at 24 months' follow-up (76.5±20.7), and then the results were stable and reached a final 7-year value of 77.3±21.5. Seven failures (11%) were reported, and excluding the failed cases to analyze separately the results obtained in the long-term responsive patients, a tendency (albeit not statistically significant) of further improvement of clinical outcome was observed at longer follow-ups: subjective IKDC reached a score of 82.7±17.5 and 82.4±16.6 at 6 and 7 years of follow-up, respectively. In another study Kon et al. (19) analyzed and compared results obtained using arthroscopic Hyalograft® C implantation or the mini-open approach (collagen-based MACT) for the treatment of cartilage lesions

in 61 patients over 40 years old with no clear signs of OA. Results were inferior with respect to those previously found for younger populations, and the failure rate was also higher, but a significant clinical improvement was found at 5 years. In fact, this group of patients also benefited in most cases from both cartilage regenerative procedures. Filardo et al. (29) analyzed instead a group of 58 patients affected by focal degenerative chondral lesions of the femoral condyles and trochlea treated by second-generation arthroscopic ACI. The mean age at surgery was 34.7 ± 9.1 years, and at 6 years follow-up they reported a significant and stable clinical improvement, but less than that of other lesion etiologies. The failure rate was 18.5%, which was markedly higher than in other patient categories, so they concluded that although this could be a promising approach for the treatment of degenerative chondral lesions, graft properties and mechanical and biochemical joint environment, have to be improved. Another issue concerns knees where an OA process has already started. Filardo et al. (30) analyzed forty-four patients affected by knee OA lesions where previous conservative treatments had failed but had refused or were not indicated for a prosthetic replacement, and underwent MACT as a salvage procedure. A statistically significant improvement was observed in all scores from the basal evaluation to the final follow-up; the IKDC subjective score improved from 38.0±15.8 to 67.0±18.3 at 2 years ($p<0.0005$), and a subsequent decrease to 57.8±20.6 at the final follow-up ($p=0.012$). During the study period treatment in 12 patients failed, thus making a cumulative failure rate of 27.3%. At the last evaluation half of the patients considered their condition not better than before the treatment and 39% would not repeat the treatment considering the results obtained. They concluded that tissue-engineered cartilage implantation is questionable for this indication, and the limits of this scaffold-based procedure have to be considered if it is used as salvage procedure for young patients affected by knee OA.

CONCLUSIONS

Second-generation matrix-assisted autologous chondrocyte transplantation using the hyaluronan scaffold Hyalograft® C has shown very promising results over the last ten years, but not all patients have been equally successfully treated. The available literature already shows that different patient categories can result in different outcomes after Hyalograft® C transplantation and research needs to identify specific patient categories that are less responsive to MACT. Identifying patient characteristics that predict clinical outcome might be helpful in the development of patient-specific treatment strategies. It will also help to provide better information and more realistic expectations for various indications.

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NOVEL CARTILAGE REPAIR STRATEGIES – THE AMIC TECHNIQUE

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Injuries to articular cartilage are one of the most challenging pathologies of musculoskeletal medicine due to the poor intrinsic healing capacities of damaged cartilage. Autologous Matrix Induced Chondrogenesis (AMIC) is an innovative treatment for localized full-thickness cartilage defects combining the well-known microfracturing with a collagen I/III scaffold. The current article reviews the treatment modalities utilized in cartilage repair procedures, focusing on the role of AMIC in clinical practice today and it´s way from "bench to bedside".

The limited intrinsic healing potential of damaged articular cartilage is a well-known problem in orthopedic surgery (1). Cartilage degeneration may be accompanied by pain, immobility, stiffness, loss of quality of life and can potentially lead to severe osteoarthritis in the long term. A plethora of emerging treatments and associated surgical techniques have been described to improve cartilage repair techniques. The treatment should aim at alleviating pain and restoring functionality in first place eventually leading to the formation of an entirely new articulating surface that essentially duplicates the original articular cartilage in its structure, composition and function.

Supporting the intrinsic repair mechanism is achieved by initiating the recruitment of chondrogenic progenitor cells (e.g. MSCs) from the bone marrow by penetration of the subchondral bone by drilling or microfracturing (2). Currently, microfracturing is the most common used cartilage repair procedure in cartilage defects (3) often resulting in fibrocartilaginous repair tissue. Chondrogenic progenitor cells migrate in the fibrin network of the blood clot (4). However, this fibrin clot is fragile and not mechanically stable to withstand tangential forces (5). Therefore, an implanted exogenous scaffold (e.g. a collagen matrix) is sought to improve the mechanical stability of the clot and may ideally provide a proper stimulus for chondrogenic differentiation and hence cartilage repair. Autologous Matrix-Induced Chondrogenesis (AMIC®) combines microfracturing with a collagen I/III matrix (Chondro-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland). The AMIC procedure provides 2 major advantages; on the one hand it is a one-step procedure with no need of cartilage harvesting potentially leading to donor site morbidity and on the other hand it is cost-effective with no need of in vitro cell expansion (6).

In this article we focus on the pre-clinical rationale of the AMIC technique and it´s surgical procedure, before summarizing first clinical results of this enhanced microfracturing technique.

Pre-clinical Rationale

Mesenchymal stem cells (MSCs) possess the ability to proliferate extensively in culture,

Key words: cartilage, AMIC, Chondro-Gide, knee, hip, ankle joint

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Fig. 1. ***(A - C)*** *Intraoperative findings of an open AMIC procedure (18 yrs. female, cartilage defect at the patella). Perforations into the subchondral bone with a sharp canula (Fig. 3A). Application of fibrin glue (Baxter-Immuno, Heidelberg, Germany) (Fig. 3B). The collagen I/III matrix (Geistlich Pharma AG, Wolhusen, Switzerland) is applied (Fig. 3C).*

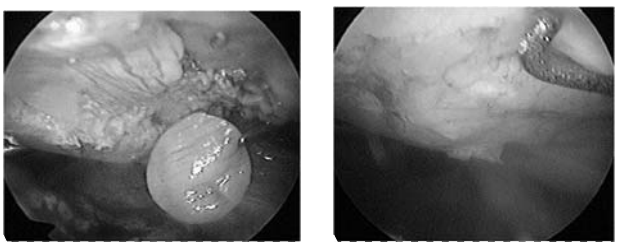

Fig. 2. ***(A – B)*** *Intraoperative findings of an all-arthroscopic AMIC technique (32 y.o. male, cartilage defect at the patella). After debridement of the cartilage defect numerous perforations of the subchondral lamina are performed. Circular overlapping patches of Chondro-Gide are placed in the defect area with Pean clamps (Fig. 2A). The matrix covered area is sealed with fibrin glue (Baxter-Immuno, Heidelberg, Germany) and stability of the implant is checked by arthroscopy (Fig. 2B).*

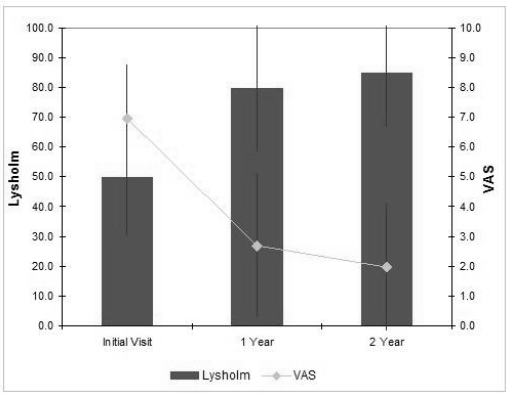

Fig. 3. *Results of the AMIC Registry: 1 and 2year follow of clinical outcome after AMIC evaluated by the Lysholm and VAS score (n=57). Scores are presented as medians.*

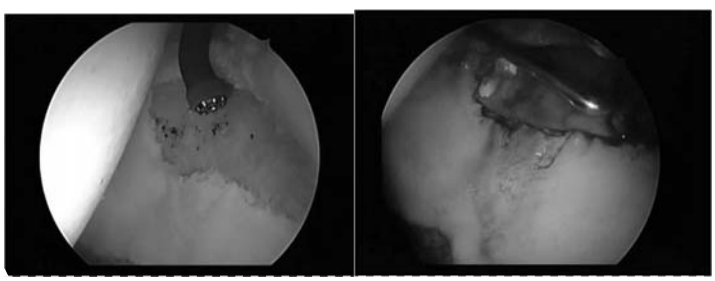

Fig. 4. ***(A-B)*** *Intraoperative findings of an all-arthroscopic AMIC technique at the hip (cartilage defect at the femoral head). After debridement of the cartilage defect (Fig. 4A) the matrix, which was trimmed to defect size, is placed in the cartilage defect (Fig. 4B).*

and chondrocytes derived from them have been observed to maintain a stable phenotype compared to chondrocytes derived from primary cultures. In addition, adult chondrocytes show a restricted proliferation capacity in culture resulting in a limited number of cells, almost insufficient for regenerative strategies (7).

The AMIC technique allows to access intrinsic cartilage repair resources of the bone marrow. Indeed, Kramer et al. were reproducibly able to detect the rapid appearance of human MS cells in the collagenous matrix (7).

Dickhut et al. analyzed whether a collagen type I/III carrier and fibrin glue (FG) combined to a biphasic construct supports chondrogenesis of MSCs *in vitro* allowing the local release of bioactive transforming growth factor-beta1 (TGF-b1) (8). In conclusion, this study demonstrated that a biphasic carrier made of these two biomaterials supports chondrogenic *in vitro* differentiation of human MSCs. Besides this it was shown that FG as a clinically applied biomaterial is suitable as a TGF-b delivery system.

In an animal model the potential effect of matrix assisted MSC transplantation for articular cartilage regeneration 8 weeks postoperatively was investigated by Jung et al (9). Cell distribution was more homogeneous in MSC compared to membrane-only group, where cells were found mainly near the subchondral zone. The authors concluded that autologous matrix assisted MSC transplantation significantly increased the histomorphological repair tissue quality during early articular cartilage defect repair and resulted in higher GAG/collagen type II-positive cross-sectional areas of the regenerated tissue.

In an ovine model with a follow-up period of 12 months, the average thickness of the repair tissue was significantly greater when a scaffold was used, especially a collagen I/III membrane (10). No differences were detected when comparing cell-free and cell-laden collagen membrane biomechanically and histologically.

In conclusion, *in vitro* studies show that the AMIC technique leads to the accessibility of the intrinsic cartilage repair resources represented by MS cells in bone marrow. Animal studies showed an enhanced defect filling and higher quality repair tissue when a collagen matrix was used.

Surgical technique

The AMIC technique was first developed for the knee joint. Therefore we will focus on the knee to describe the open and arthroscopic approach.

The open procedure is a standardized surgical technique and was described before (11). After exposure of the defect area (mini-arthrotomy), degenerative and attached cartilage is completely removed. Microfracturing is performed for instance via perforations of the subchondral bone with a sharp canula (Fig. 1A) and application of fibrin glue (Baxter-Immuno, Heidelberg) is done (Fig. 1B). The defect is covered with a collagen I/III matrix (Geistlich Pharma AG, Wolhusen, Switzerland) that was prior trimmed to fit to the cartilage lesion by adaption to an appropriate template(Fig. 1C). The knee joint is held in an extended position for 5 minutes before the joint is flexed 10 times to test the stability and position of the matrix.

In arthroscopic assisted AMIC, the implantation of the matrix is performed under dry, arthroscopic conditions, as previously described (12). Circles patches of Chondro-Gide are placed in the prepared defect area with Pean clamps (Fig. 2A). The patches overlap. According to the original technique, the porous surface of the matrix is facing the bone surface. Fibrin glue is applied and its excess is removed from the surrounding soft tissue, and left to set for 5 min. Then, 10 knee movements (consisting of flexion and extension) are performed to check the stability of the implanted matrix (Fig. 2B).

Clinical studies

AMIC has been established and performed in the knee since 2004. Over the past few years there is an increasing interest and experience for its application in the hip and ankle joints also. Below, we present the joint by joint clinical results to give an overview of AMIC in clinical practice today.

Knee joint

The AMIC technique was first described in 2005 by Behrens et al. and is at present widely used (13-14). So far, no data from a randomized controlled trial have been published investigating the performance of AMIC compared to other cartilage repair procedures. A couple of case series showed good to excellent results up to long-term follow-up.

In a study to evaluate the quality of the repair tissue obtained from biopsies harvested during second-look arthroscopy after arthroscopic AMIC augmented with bone marrow concentrate, 5 second-look core biopsies were harvested at 12 months. The clinical and histological data suggest that a nearly normal arthroscopic appearance and a satisfactory repair tissue was achieved, which was possibly still maturing at then (15).

In 1 of our series, 32 chondral lesions in 27 patients were treated with AMIC (11). These patients were

evaluated for up to 5 years after the intervention. The mean defect size of the chondral lesions was 4.2 cm^2. 87% of the patients studied were subjectively highly satisfied with the results after surgery. Significant improvement of all clinical scores was observed 12 months after AMIC, and further improvement was found up to 24 months postoperatively. MRI analysis showed moderate to complete filling with a normal to incidentally hyperintense signal in most cases. Results did not show a clinical impact of patient's age at the time of operation, body mass index and number of previous operations. In contrast, males showed significant higher values in the IKDC score compared to their female counterparts, although the reason remains unclear.

In a recent study we evaluated the data of the AMIC Registry, an internet-based tool to longitudinally track changes in function and symptoms by the Lysholm score and VAS (16).

The results of 57 cases with a follow-up period of 2 years were presented. The majority of patients were satisfied with the postoperative outcome, reporting a significant decrease of pain (mean VAS preop=7.0; 1year postop=2.7; 2years postop=2.0). Significant improvement of the mean Lysholm score was observed at 1 year after AMIC and further increased values were noted up to 2 years postoperatively (preop=50.1, 1year postop=79.9, 2year postop=85.2) (Fig 3).

Another retrospective evaluation of clinical and radiographic outcomes of patients treated with AMIC for chondral and osteochondral full-thickness cartilage defects of the knee was performed with a mean follow-up of 29 months (17). Significant improvements in clinical outcome scores (IKDC, Lysholm, Tegner, and VAS pain score) were noted. Patients were generally satisfied with their results. MRI evaluation showed that tissue filling was present but generally not complete or homogenous.

Emerging techniques have the potential to complement the "first generation" AMIC technique as described by Behrens et al. which is based on microfracture (13), for instance the addition of concentrated bone marrow from the iliac crest. A study of de Girolamo et al. suggests a difference in mesenchymal stem cells (MSCs) harvested after microfracturing to MSCs derived primarily from the iliac crest (18). It is observed that the MSCs from the microfractured areas demonstrate a different more irregular pattern compared to those harvested from the iliac crest.

In comparison to microfractures, drilling does not seem to have such a deleterious effect on the subchondral bone matrix as was formerly speculated but instead leads to better access to the bone-marrow stroma in the rabbit model (19). Following these new findings, the AMIC technique in the knee was modified and drilling instead of microfractures used to penetrate the subchondral layer (20-21). An arthroscopic approach of the AMIC technique was published by Piontek et al. (12). Compared to open surgery, the described arthroscopic technique may offer advantages including minimal soft tissue trauma and minimal blood loss.

In conclusion, AMIC in the knee has been reported to be an effective and safe method of treating symptomatic full-thickness chondral defects of the knee in appropriately selected cases.

Ankle joint

Chondral defects of the talus are common and remain a challenging therapeutic task to orthopedic surgeons. Several surgical techniques are available for treatment. Good early results are reported; however, literature is limited to case series and case reports and long-term outcome is unknown (22).

AMIC at the talus was first described in 2008; good clinical results were initially reported as an open procedure (23). In 2011 Simon et al. published the description of an arthroscopic AMIC technique having all the advantages of arthroscopy and with no need for osteotomy of the medial malleolus (24).

In a case series of 72 osteochondral lesions at the talus (Outerbridge III-IV) an increase of the AOFAS score improved from 47.3 to 88.3. The follow-up MRI demonstrated good cartilage regeneration without joint effusion (25).

Hip joint

Chondropathies of the acetabulum and the femoral head are a frequent cause of pain and functional limitation (26). AMIC was previously performed as an open procedure, raising the same issues associated with all arthrotomies, such as the risk of infection and a prolonged recovery time. These could be minimized by performing the

procedure arthroscopically (Fig. 4A-B). Compared with the knee, the hip has more soft tissue coverage and more bony constraint, making hip arthroscopy and open approaches more technically complex and invasive than those for the knee. AMIC as an open procedure by surgical hip dislocation has been described by Leunig et al. (27).

A fully arthroscopic approach of AMIC at the hip has been recently published (28). In a randomized case series both groups, AMIC and microfracture showed a significant increase of the HSS (Harris Hip Score) at 1year follow-up, but a deterioration of the HHS results was notable in the microfracture group, while in the AMIC group improvements could be maintained over time (3). No significant differences were seen in another series comparing arthroscopic AMIC and ACI in acetabular cartilage defects (29).

In another report, the potential etiology of the rare parafoveal femoral head lesions seen in 3 of 6 patients and their treatment with AMIC was described (30).

In conclusion, the AMIC technique is technically feasible for the hip joint in an open or all-arthroscopic procedure. Preliminary findings after AMIC for femoroacetabular cartilage lesions are promising, but further studies are necessary to elucidate this fact.

CONCLUSION

AMIC is an innovative treatment that made its way from “bench to bedside” and is today a well-established surgical procedure for articular cartilage defect therapy in the knee, hip and ankle joint. Clinical studies prove a significant decrease of pain and increase of the functional outcome scores after AMIC.

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PRP IN CARTILAGE PATHOLOGY

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Platelet-rich Plasma (PRP) is widely used to promote tissue regeneration through the *in-situ* administration of a milieu of growth factors that may contribute to the healing process of tissues with a low healing potential and, among these, the treatment of cartilage pathology is gaining increasing interest. However, besides its wide clinical application, it is not clear how much the use of PRP is supported by real scientific evidence. This review analyses the available evidence about the use of PRP to treat cartilage lesions. A search in the PubMed database was performed. Research criteria included: 1) papers in English, 2) papers on the clinical application of PRP for the treatment of cartilage degenerative pathology, 3) papers with a level of evidence of I to IV, and 4) papers reporting clinical results. Both conservative and surgical applications of PRP were considered for the review. Seventeen papers have been published mostly focusing on knee pathology, in particular as a conservative injective treatment. Osteochondral lesions of the talus have been the subject of 3 studies while 2 papers deal with applications in the hip via ultra-sound-guided injections. Overall clinical results were positive but the low quality of the papers combined with the great variability of procedures and PRP preparations make study comparison difficult and no conclusive indications can be drawn about the efficacy of PRP for the treatment of cartilage lesions.

Every day orthopaedic, rheumatology and sports medicine physicians address the complex issue of cartilage pathology, which is rapidly becoming more common (1-3) due to the entire population's growing involvement in sport activities, from the young to the middle-aged and even elderly individuals, prompted by the awareness of the importance of physical activity as a preventive medical approach. Besides the positive aspects of this widespread, healthy, active life-style, other medical problems are emerging: in particular, cartilage lesions are becoming one of the most important challenges for both basic researchers and clinicians. In fact, despite being able to sustain huge mechanical stresses, cartilage has limited healing potential for several reasons including its relative isolation from systemic regulation due to the lack of nerves and vessels when compared to other tissues. Furthermore, its complex histological structure, consisting of chondrocytes surrounded by matrix made of a specialized framework of collagen, aggrecans and fluid, determines an intrinsic vulnerability that, starting from small and focal lesions, can lead to an accelerated degenerative process culminating in osteoarthritis (OA), a chronic condition that is difficult to treat with conservative methods and often requires an invasive and sacrificing surgical approach such as arthroplasty. Several treatments, both conservative

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and surgical, have been proposed to address cartilage pathology (4-6) and the choice of treatment must be made according to the specific characteristics of the chondral lesion and also the possible presence of other comorbidities (meniscal status, axial alignment, other general medical conditions, etc.) (6). Among the available treatment options (4) a new trend has emerged over recent years, opening up a large and sometimes controversial research topic: the application of blood-derivatives for the treatment of cartilage defects and more generally to address joint degenerative pathology.

In particular, among blood-derived products, platelet-rich plasma (PRP) is gaining increasing attention as a promising, safe and cost-effective procedure to provide a concentrate milieu of growth factors for stimulating cartilage repair and reestablishing joint homeostasis.

PRP is an autologous concentrate of platelet-derived growth factors (GFs) and numerous other bioactive molecules, obtained directly from the peripheral venous blood of the patient. GFs are a group of polypeptides that play a fundamental role in the regulation of the growth and development of several tissues, as well as in the reparative processes, including cartilage. The biological rationale of PRP is that platelets contain storage pools of many GFs, including: platelet-derived growth factor (PDGF); transforming growth factor (TGF-β); platelet-derived epidermal growth factor (PDEGF); vascular endothelial growth factor (VEGF); insulin-like growth factor 1 (IGF-1); fibroblastic growth factor (FGF); and epidermal growth factor (EGF) (7-8). Besides GFs, alpha granules are a source of cytokines, chemokines, and many other mediators (9), all involved in complex biological mechanisms stimulating chemotaxis, cell proliferation and maturation, modulating inflammatory molecules and attracting leukocytes (9). Platelets also store dense granules, rich in ADP, ATP, calcium ions, histamine, serotonin and dopamine, which play a complex role in tissue modulation and regeneration (10). Finally, platelets contain lisosomal granules which can secrete acid hydrolases, cathepsin D and E, elastases and lysozyme (11-12), and most likely other not yet well characterized molecules, the role of which in tissue healing should not be underestimated. Several *in vitro* and *in vivo* animal studies have shown the potential beneficial effect of PRP in promoting cellular anabolism and tissue regeneration (13-14).

This review focuses on the results obtained in the clinical application of PRP, by analysing the available evidence for its use in treating joints affected by cartilage pathology (Table I). In particular, a search in the PubMed database was performed using the following research criteria: 1) papers in English, 2) papers dealing with the clinical application of PRP for the treatment of cartilage pathology, 3) articles with a level of evidence of I to IV, and 4) papers reporting clinical results. Both conservative and surgical applications of PRP were considered for this review.

STUDIES ON THE OF CLINICAL APPLICATION OF PRP

Surgical management

Sanchez and his group (15) were the first to describe the surgical use of PRP to treat a cartilage lesion in a 12-year-old football player with a large (> 2 cm^2) articular knee cartilage avulsion. The procedure was performed arthroscopically: the bed of the lesion was debrided to remove the scar tissue, then the chondral fragment was repositioned *in situ* and fixed with 5 reabsorbable pins. A 2-mm Kirschner wire was used to drill a hole in the center of the fragment, which was then filled with 2 mL of PRP previously obtained from the autologous blood of the patient. PRP was also injected all around the interface between the replaced chondral fragment and the healthy surrounding tissue. The patient was instructed to walk without weight-bearing for the first 4 weeks and rehabilitation was started two weeks after surgery. The clinical outcome was excellent with full functional recovery and resumption of sport within 18 weeks. The patient returned to the same pre-injury level just a few weeks later. MRI analysis revealed an almost perfect integration of the chondral fragment within the surrounding cartilaginous tissue. The application of PRP might have contributed to the regeneration by a dual mechanism, consisting of both a biological stimulus linked to the release of platelet-derived GFs and a physical process due to the formation of a gel able to help fragment fixation.

Another surgical application of PRP was tested by Dhollander et al. (16), who treated 5

symptomatic osteochondral defects of the patella with microfracturing followed by the application of a collagen I/III scaffold membrane implanted and sutured into the lesion site. PRP was then administered beneath the membrane at the interface with the microfractured subchondral bone. Evaluation was performed using KOOS, Tegner, Kujala, and VAS for pain scores, whereas MRI evaluation was performed using an original and modified MOCART score. The final follow-up was held 24 months after the surgical procedure. Clinical results at two years were satisfying both in terms of pain relief and functional improvement and even MRI evaluation showed good quality of the repair tissue, but a clear role of the platelet concentrate could not be shown.

A further study by Siclari et al. (17) showed the efficacy of a polyglycolic acid/hyaluronan scaffold immersed in PRP for treating full-thickness chondral defects of the knee. The purpose of the authors was to increase the regenerative potential after marrow stimulation, thus they performed Pridie perforations on the lesions site and then applied the scaffold with PRP in 52 patients, all treated arthroscopically and evaluated at 1 year follow-up using the KOOS score. A significant clinical improvement was observed in all KOOS subcategories at each follow-up and no major complications were observed; 10 second look arthroscopies and 5 biopsies were also performed, which revealed a homogeneous, well integrated hyaline-like repair tissue.

Talar osteochondral lesions have been treated by Giannini and his group (18-19): they described an innovative arthroscopic one-stage approach involving autologous mesenchymal stem cells (MSCs), PRP and, alternately, porcine collagen powder or HA membrane, used to create a scaffold directly applied into the lesion site after preparation of the defect. The first clinical trial (18) involved 48 patients affected by focal lesions (mean size = 2.1 cm^2) and evaluated at 6, 12, 18, and 24 months of follow-up with the AOFAS score. A significant increase in the score was found even at 6 months from the surgical procedure and was confirmed up to the final follow-up. The rate of return to high impact sport activity was satisfactory, since the majority of patients returned to sport at 11-month follow-up. A correlation was found between clinical outcome and lesion size, and previous surgery was shown to have a negative effect on outcome. Five second-look arthroscopies were performed at 1-year follow-up and in 2 cases biopsies were taken, which revealed, after histological and immunohistological analysis, the presence of new cartilage tissue with varying degrees of hyaline-like tissue remodeling. The overall findings suggested that this novel approach might stimulate tissue regeneration with interesting clinical efficacy, producing results comparable even to those of autologous chondrocyte implantation (ACI), but avoiding the double surgery time and the inherent stress for the patient. With regards to this, the same authors performed a further study (19) focusing on comparing the MSCs + PRP + scaffold technique with open and arthroscopic ACI. Eighty-one patients were included in this analysis, 10 of which were treated with open ACI, 46 with arthroscopic ACI, and 25 with bone marrow derived mesenchymal cell (BMDCs) "one-step" technique. Clinical results were compared up to 3 years of follow-up. AOFAS was the score adopted for clinical evaluation and radiographical analysis was also performed. The clinical improvement in each subgroup was significant and no inter-group difference was observed, thus confirming the possibility of matching the effectiveness of chondrocyte transplantation through a single step procedure. X-Rays showed no signs of OA progression and MRI revealed a good rate of defect filling and integration of the newly regenerated tissue within the surrounding tissue. Another aspect worth noting is the cost: in fact the authors pointed out that their novel one-step regenerative technique costs less than an half that of the traditional arthroscopic ACI.

After the encouraging results reported for talar osteochondral lesions, the same technique was applied to the knee (20): 20 patients with knee condylar osteochondral lesions were treated and evaluated for up to 24 months with IKDC and KOOS scores combined with MRI, and two biopsies were also taken. Besides the significant improvement in clinical scores, interesting correlations were found: combined surgery slowed down recovery although at final evaluation similar results were obtained with respect to those of patients without combined procedures; hyper-intense MRI signal of repair tissue was correlated with poorer clinical results. Histological evaluation showed the formation

Table I. *Synopsis of all the studies on PRP application in cartilage pathology*

AUTHORS, JOURNAL AND YEAR	LEVEL OF EVIDENCE	PATHOLOGY	APPLICATION	PROTOCOL	COMBINED TREATMENTS	CONTROL GROUP	PATIENTS	FOLLOW-UP	OUTCOME
SANCHEZ et al. Clin Exp Rheumatol 2008	Retrospective comparative study	Knee condropathy or OA	Conservative	3 weekly injections of PRP	No	Yes	30 PRP vs 30 HA	5 weeks	Better pain control and functional outcome in PRP group
SAMPSON et . al Am J Phys Med Rehabil 2010	Case series	Knee condropathy or OA	Conservative	3 injections of PRP one month apart	No	No	14 PRP	6 months	Clinical improvement at short term evaluation
WANG-SAEGUSA et al. Arch orthop Trauma Surg 2011	Case series	Knee condropathy or OA	Conservative	3 injections of PRP two weeks apart	No	No	261 PRP	6 months	Clinical improvement at short term evaluation
KON et al. Knee Surg Sport traumatol Arthrosc 2010	Case series	Knee condropathy or OA	Conservative	3 injections of PRP two weeks apart	No	No	100 PRP	24 months	Significant pain reduction and functional recovery. Time dependant effect of PRP injections with a mean beneficial effect of 9 months
KON et al. Arthroscopy 2011	Comparative trial	Knee condropathy or OA	Conservative	3 weekly injections of PRP	No	Yes	50 PRP vs 50 LWHA vs 50 HWHA	12 months	Best results for PRP in chondropathy group, no statistical difference between treatment for higher degree of cartilage degeneration
FILARDO et al. Knee Surg Sport Traumatol Arthrosc 2011	Comparative trial	Knee condropathy or OA	Conservative	3 weekly injection of PRP	No	Yes	72 leukocite rich PRP vs 72 leukocyte free PRP	12 months	Comparable clinical results with higher post-injective pain in leukocyte rich PRP group
NAPOLITANO et al. Blood Trasfus 2012	Case series	Knee condropathy or OA	Conservative	3 injections of PRP	No	No	27 PRP	6 months	Statistical improvement in pain and function
GOBBI et al. Sports Health 2012	Case series	Knee condropathy or OA	Conservative	2 monthly injections of PRP	No	No	50 PRP	12 months	Statistical improvement in pain and function
SPAKOVA et al. Am J Phys Med Rehabil 2012	Prospective trial	Knee condropathy or OA	Conservative	3 injections of PRP	No	Yes	60 PRP vs 60 HA	6 months	Superior results in PRP group at short term evaluation
SANCHEZ et al. Arthroscopy 2012	Randomized trial	Knee condropathy or OA	Conservative	3 weekly injections of PRP	No	Yes	79 PRP vs 74 HA	6 months	Higher percentage of responders in PRP group
CERZA et al. Am J Sport Med 2012	Randomized trial	Knee condropathy or OA	Conservative	4 weekly injections of ACP	No	Yes	60 ACP vs 60 HA	6 months	Superior clinical outcome for PRP in all groups of treatment
SANCHEZ et al. Rheumatology 2012	Case series	Hip OA	Conservative	3 weekly injections of PRP	No	No	40 PRP	12 months	Significant pain reduction and functional improvement
BATTAGLIA et al. Clin Exp Rheumatol 2011	Case series	Hip OA	Conservative	3 weekly injections of PRP	No	No	20 PRP	12 months	Clinical improvement but worsening over time
MEI-DAN et al. Am J Sports Med 2012	Quasi randomized trial	Osteochondral talar lesions	Conservative	3 injections of PRP 14 days apart each other	No	Yes (HA)	15 PRP vs 15 HA	7 months	Statistically better clinical outcome in PRP group
SANCHEZ et al. Med Sci Sports Exerc. 2003	Case report	Knee osteochondral fragment avulsion	Surgical	Intra-op	Fragment fixation + in situ administration of PRP	No	1	10 months	Full recovery and return to high level sport practice
GIANNINI et al. Clin Orthop Relat Res 2009	Case series	Osteochondral talar lesions	Surgical	Intra-op	Scaffold made of: MSCs + PRP + HA membrane (or collagen powder)	No	48	24 months	Significant improvement in all clinical parameters
GIANNINI et al. Injury 2010	Comparative study	Osteochondral talar lesions	Surgical	Intra-op	MSCs + PRP + HA membrane (or collagen powder)	Yes (historical controls)	81 (25 MSCs scaffold vs 10 open ACI vs 46 arthroscopic ACI)	24 months	Results comparable to those of arthroscopic ACI with lower costs

BUDA et al. J Bone Joint Surg Am 2010	Case series	Knee osteochondral lesions	Surgical	Intra-op	MSCs + PRP + HA membrane (or collagen powder)	Yes	20	24 months	Significant clinical improvement and interesting MRI findings
DHOLLANDER et al. Knee Surg Sport Traumatol Arthrosc 2011	Case series	Patellar osteochondral lesions	Surgical	Intra-op	Microfractures+ collagen based scaffold + PRP	No	5	24 months	Significant clinical improvement and interesting MRI findings
SICLARI et al. Clin Orthop Relat Res 2012	Case series	Knee osteochondral lesions	Surgical	Intra-op	Pridie Perforations + polyglicolic/HA scaffold + PRP	No	52	12 months	Significant clinical improvement and hyaline-like cartilage aspect at biopsies

of hyaline-like cartilage rich in type-II collagen throughout the entire thickness of the biopsies.

Conservative management

In 2008 Sanchez et al. first reported the injective application of a platelet concentrate (PRGF), in a retrospective observational study on 60 patients (21), 30 treated with intra-articular injections of PRGF in the knee and 30 with injections of hyaluronic acid (HA). Patients from both groups underwent 3 injections one week apart and were evaluated basally and at 5 weeks of follow-up using the WOMAC score, with particular regard to the "stiffness", "pain", and "function" subscales. The results were encouraging, even though the short follow-up is a weak point of the study. In particular, the therapy with PRGF showed better efficacy in pain control compared to HA.

In 2010, Sampson et al. published a study (22) on 14 patients (12 men and 2 women) with primary or secondary knee OA who received 3 PRP injections 1 month apart. Inclusion criteria were clinical and radiographical signs of OA in patients with previous unsuccessful conservative management. Evaluation was carried out for up to 52 weeks using the "Brittberg-Peterson Visual Analog Pain, Activities and expectation Score", VAS for pain, and KOOS Score. Cartilage thickness of the trochlea and both femoral condyles was measured via ultrasonography to assess any changes between pre and post treatment. Concerning the clinical outcome, the authors found a statistically significant improvement in the scores examined, with a reduction in pain both at rest and during physical activity. At one year follow-up, eight patients were completely satisfied with the treatment. No significant differences were observed in cartilage thickness after the injective treatment.

In 2010 Wang-Saegusa et al. (23) published a prospective study on a large cohort of 261 patients treated for mono- or bilateral knee OA, symptomatic for more than 3 months. The patients received 3 injections of PRP 2 weeks apart, and clinical evaluation was conducted at 6 months of follow-up using the WOMAC score, VAS, Lequesne Index and SF-36. Statistical analysis revealed significant results with an increase in all the scores .

At the same time Kon et al. also published a prospective study (24) evaluating 91 patients (a total of 115 knees) treated with 3 injections of 5 mL PRP (one every 3 weeks). Inclusion criteria were: clinical history of knee pain or articular swelling lasting more than 4 months, radiographic or MRI signs of OA. Exclusion criteria were: axial deviation more than 5°, hematological, rheumatic or severe cardiovascular diseases, diabetes, immunodepression, chronic anti-coagulant or antiaggregant therapy, use of NSAIDs within 5 days before the blood sampling for PRP production, Hb values < 11 g/dl and platelets < 150.000 / mm^3. Patients underwent clinical evaluation at basal level and at 2, 6, and 12 months of follow-up through objective and subjective IKDC, and EQ-VAS (general health status evaluation) scores. No major complications were seen, except for a case of marked post-injective pain and swelling which resolved spontaneously after 2 weeks. Eighty percent of the patients treated expressed satisfaction with the treatment received. The clinical outcome showed a statistically significant improvement in all the variables considered just 2 months after the end of treatment. These results were later confirmed at 6 months of follow-up, whereas a tendency to worsen was reported at 6 to 12 months of follow-up. Despite the decrease reported after 1 year, the clinical scores at that time were still higher than the basal level. Concerning the factors influencing the clinical efficacy, young male patients were the

best responders to PRP application. Furthermore, also the grade of articular cartilage degeneration is correlated with the clinical outcome: patients with chondropathy alone without signs of OA experienced better and more lasting results compared to patients with early or severe OA. A later study by the same authors (25) evaluating the patients at 24 months of follow-up confirmed the trend that emerged after the 12-month follow-up. In fact, a further and more marked decrease in the clinical picture, resulting in functional worsening, was evident, thus confirming the time-dependency of intra-articular therapy with platelet-derived GFs. The authors estimated the median duration of the PRP effect to be 9 months, and the influence of age and grade of degeneration was again correlated with results at longer follow-up. In another multi-center study carried out by Kon et al. (26), the clinical effectiveness of PRP was compared to low molecular weight HA (LWHA) and high molecular weight HA (HWHA). For this purpose, 3 homogeneous groups of patients were respectively treated with 3 weekly injections of PRP, LWHA, or HWHA. Subjective IKDC and EQ-VAS for general health status were used for evaluation at 2 and 6 months of follow-up. The results showed a better performance for the PRP group at 6 months of follow-up in both scores. In particular, the subgroup analysis according to the grade of articular cartilage degeneration (chondropathy vs early vs severe OA) revealed that, in the chondropathy group, PRP gave better results than HA at 6 months of follow-up. In the early OA group the gap with HA was not as wide as that observed in the previous subgroup and in the severe OA subgroup, no difference in clinical outcome was observed between viscosupplementation and PRP therapy at 6 months. Another finding underlined in this study was that patients under 50 years old had a greater chance of benefitting from this biological approach, whereas in the case of older patients there was no better outcome with respect to HA. Finally, a comparative study between PRP with or without leukocytes used to treat 144 patients suffering from knee cartilage pathology has recently been published and shows comparable positive clinical effects with both treatments, with the PRP-leukocyte group reporting more swelling and pain immediately after the injection (27).

Knee degenerative pathology was also the focus of a study written by Napolitano et al. (28). They treated 27 patients, affected by either simple chondropathy or initial OA, with 3 weekly injections of 5 ml PRP, following them up for 6 months with the NRS scale for pain and the WOMAC score. Significant results were obtained after treatment without occurrence of adverse events. Similar results have also been reported by the group of Gobbi (29) who treated 50 patients with 2 monthly injections of PRP and evaluated them up to 1 year of follow-up, showing good results both in patients treated for the first time, as well as in patients who previously underwent cartilage surgery.

Recently, two randomized controlled trials have been published. The first one is authored by Sanchez et al. (30), who investigated the efficacy of single spinning leukocyte free PRP compared to HA in 153 patients evaluated with WOMAC score at 6 months of follow-up. Despite the interesting clinical outcome, only the percentage of responders (primary outcome measure) was statistically higher in PRP group.

The second randomized trial was conducted by Cerza et al. (31). They treated 120 patients, divided in two groups, the first one receiving 4 weekly injections of Autologous Conditioned Plasma (ACP) and the second one 4 injections of HA. Patients underwent a 24-week follow-up and the ACP group showed a significantly better performance than the HA group: the clinical gap between treatments increased over time in favor of ACP up to the final evaluation. Surprisingly, authors reported a significantly better clinical outcome in ACP group even in those patients affected by Kellgren-Lawrence grade 3 knee OA.

Besides its application in the knee, PRP has also been used to treat hip OA and talar osteochondral lesions. Recently two studies dealing with PRP injective treatment for hip degenerative pathology have been published. The first one, by Battaglia et al. (32), reported the results of PRP ultra-sound guided injective treatment in 20 patients affected by hip OA (Kellgren-Lawrence Score from I to III). They performed 3 intra-articular injections 2 weeks apart and followed-up the patients with the HHS and WOMAC scores for up to 1 year. The immediate clinical outcome was positive but worsened at 3 months up to the final evaluation, thus confirming the time-dependent effect of PRP administration.

Another study on this topic has been recently published by Sanchez et al. (33), who treated 40 patients affected by OA with 3 weekly ultrasound-guided injections of PRP. Evaluation was carried out for up to 6 months using the WOMAC, Harris, and VAS scores for pain. The results were overall satisfactory with a significant reduction in pain level at the first evaluation after 6 weeks, which was confirmed even at the final follow-up of 6 months. Functional recovery was encouraging as evaluated through a specific subscale of the WOMAC score. Eleven out of 40 patients did not have any beneficial effect after injective treatment: in these cases metal resurfacing was required.

Finally, with regards to osteochondral talar lesions, a prospective study by Mei-Dan et al. (34) compared the efficacy of HA and PRP in 30 patients (15 per group) not responsive to other previous conservative management. The patients were allocated to receive 3 weekly intra-articular injections of HA (2 ml each) or PRP (2 ml each) and were evaluated for up to 28 weeks. Investigators used AHFS, AOFAS, and VAS scores for pain, stiffness and function. Results were statistically significant and PRP proved to be more effective in controlling pain and re-establishing function.

DISCUSSION

A PubMed research for articles on PRP produced more reviews than clinical trials (35). This point is worthy of note because it shows that more words than facts have been produced on this particular topic, and the limits of the current literature supporting the use of PRP.

When talking about PRP we have to consider two fundamental questions which are major topics of the current scientific debate: what is PRP? And what is the real scientific evidence available at present? Both questions require careful analysis.

The first question is to define PRP: in general, it is regarded as a blood derivate with a higher concentration of platelets compared to basal level. Usually the concentration should be approximately 400% of the peripheral blood PLT count (36-37), whereas anything less than this concentration is PRP diluted with platelet poor plasma (PPP). However, in literature PRP concentrations have been reported to range widely, from 4 to 8 times those found in whole blood (38), and good results have also been reported with lower concentrations (39). It is clear that, based on the biological rationale of this therapy, platelet count is a fundamental aspect to be considered to establish the therapeutic range of platelet concentration, especially since it has been seen that, beyond a certain range, their effect might even be detrimental (40). Nevertheless, 11 out of 17 clinical trials published do not report the mean platelet concentration of the PRP used, which highlights a serious scientific bug that might delay the identification of the best features PRP should possess. These studies often do not even clearly state whether the type of PRP used is rich in leukocytes.

Furthermore, several different procedures have been described to obtain PRP, thus implying qualitative and quantitative differences among substances used in various pre-clinical and clinical studies. Some preparation methods, besides increasing the number of platelets, also allow leukocytes and monocytes to be concentrated whose therapeutic role is controversial, with some authors underlining their potential anti-bacterial function and others pointing out that the proteases and reactive oxygen products delivered by leukocytes might have a negative effect (38). Activation is another source of variability: some authors do not activate platelets whereas others are using autologous thrombin, calcium chloride, batroxobin, and even physical methods or biomaterials (38).

Finally, the applicative protocols can vary widely in terms of amount of substance, number of administrations and timing, therefore adding further variables to consider for study comparison.

To summarize, the current state of knowledge concerning PRP as a biological product is still full of questions and the clinical studies published to date have not made a satisfactory contribution to solving these key problems. If the goal is to try to standardize preparation, storage, activation methods and protocols, we are still very far from achieving it.

The second fundamental aspect to consider is the quality of current literature on PRP applications in cartilage pathology. It is evident that the majority of published papers do not provide high quality scientific evidence.

Firstly, reviewing the surgical application of

PRP, all the studies published are biased by the lack of a control group, which might show whether PRP administration can provide a better clinical outcome compared to the surgical procedure alone. This is the most relevant limitation, because at present it is impossible to establish to what extent the clinical results can be attributed to PRP. Furthermore, the mean level of evidence is poor: in fact we have only one case report and 5 case series. Considering the papers published by Giannini et al. (18-19) (on talar ostechondral lesions) and Buda et al. (20) (osteochondral knee lesions), there are so many biological variables to consider (bone marrow derived stem cells, PRP, hyaluronan membrane or collagen powder) that it seems quite difficult to determine the real role of PRP. Dhollander et al. (16) used PRP as a biological enhancer in only 5 patello-femoral lesions and, also in this case, the contribution of PRP cannot be assessed properly. The same conclusions can be drawn from Siclari et al.'s (17) experience.

Looking at conservative management, the majority of studies focus on the application of PRP in the knee.

Most studies involve case series, such as the one by Sanchez et al. (21), which is limited by the small number of patients and by the very short follow-up, the one by Sampson et al. (22) which has an even fewer patients, and the one by Wang-Saegusa et al. (23) which, despite the considerable number of subjects, is biased by the scarce homogeneity of patients treated.

The group led by Kon published a comparative trial among PRP, LWHA and HWHA (26). The results were interesting but important limitations have to be considered: the lack of randomization and a control group; the primary outcome scale (appropriate for the evaluation of cartilage lesions but probably less sensitive for OA and for the older group); the evaluation of patients treated in different centers; the low number of patients treated and the evaluation of the results only at short-term follow-up. The same authors were the first to compare two different methods of PRP preparation (double vs single spinning) in homogenous patient cohorts; they found no significant clinical difference but more post-injective pain and swelling for leukocyte rich PRP (27). Even in this context, the lack of randomization and the evaluation of patients treated in different centers by different physicians are significant weak points.

Looking at the two randomized trials available, some controversies emerged. In fact, even though Sanchez et al. (30) confirmed that PRP treatment produces better results in low grade articular degenerative pathology, only the primary outcome showed evidence of PRP superiority with respect to HA. Conversely, the study by Cerza et al. (31) revealed that ACP was able to determine a better clinical outcome even in case of severe OA. Platelet concentrate method, patient selection, or study design could be responsible for these differences, but their role has still to be demonstrated.

Concerning hip degenerative pathology, only two studies (32-33) have been published and both are characterized by a small cohort of patients, no randomization or control group, so although the safety of the procedure has been assessed, no conclusive indication can be given for the application of PRP in this particular disease.

Finally, a quasi-randomized trial published by Mei-Dan et al. (34) focused on the comparison of PRP and HA for the treatment of talar osteochondral lesions. The results were in favour of PRP but the small number of patients evaluated at short follow-up suggests that other trials are needed to confirm their indication.

In light of all these observations, the real efficacy of PRP has still to be proven in all fields of application and only randomized controlled trials with a large study population can achieve this challenging purpose. The standardization of data assessment for the clinical application of PRP is mandatory: authors should be careful when reporting their studies at least in regards to the PRP preparation method, the possible presence of leukocytes and their counts, PLT counts, activation method and the exact therapeutic protocol, including the concurrent administration of local anesthetic and/or other substances. Only in this way will it be possible to compare clinical trials and verify the efficacy of PRP and the optimal characteristics for clinical use.

CONCLUSIONS

The administration of growth factors is certainly a fascinating approach to treat cartilage pathology.

At present several studies are ongoing to establish which particular molecules can provide the best therapeutic effects and to determine the best application protocol. The use of PRP is a widely used method to supply platelet-derived growth factors in the articular environment. However, the literature currently available is not conclusive on the real efficacy of this approach. There are difficulties related to the treatment itself and the great number of variables concerning PRP production, storage and administration, which make study comparison very challenging. Besides these difficulties, the average quality of the published articles is low, due to the lack of randomized controlled double blind trials. Thus, no clear indication can be made in favor of PRP application for cartilage pathology compared to other traditional approaches. More biological studies are needed to identify the best formulation for PRP preparation and higher quality clinical trials should be performed to define the best application protocol and the real therapeutic potential of this biological treatment option.

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COLLAGEN MENISCUS IMPLANT. A PROSPECTIVE STUDY WITH A MINIMUM 10 YEARS FOLLOW-UP

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Defects of meniscal tissue, even partial ones, can cause degenerative knee changes. Hence scaffolds for meniscus regeneration have been proposed for partial meniscal defects in order to save the meniscus. The CMI-menaflex (Ivy Sport Medicine) is a collagen scaffold of bovine origin. Stone proposed the use of this implant in 1992 and it has been available for clinical use in the medial meniscus since 2000. The aim of this study was the clinical and magnetic resonance imaging (MRI) evaluation at long-term follow-up of the effectiveness and safety of the CMI. Twenty-eight patients received a CMI implant between 2001 and 2002 and participated in our previous study of clinical and MRI at medium term. These patients were called again for another visit and MRI. Twenty-six patients were available for the 10-year follow-up. All the patients had a clinical evaluation with the Lysholm score and the Tegner activity scale before surgery and 2, 5, and 10 years after. An MRI examination was also performed after 2, 5 years and in 15 cases after 10 years. The Lysholm and Tegner score improved significantly 2 years after surgery and remained essentially unchanged in the controls at 5 and 10 years. At the MRI evaluation the complex CMI-meniscus appeared present, but often smaller than the native meniscus. The signal matured over time, but rarely was completely similar to a normal meniscus. The cartilage surface of the medial compartment did not show degenerative changes up to 5 years after surgery, at 10-years follow-up a slight progression of joint degenerative disease was observed. No adverse reactions to the implant were reported. The CMI generally induced a significant clinical improvement that is stable over time at 10-year follow-up. The MRI examination showed that the complex CMI-regenerated tissue was reduced in size during the first 2 years but remained unchanged at the following controls. A progressive maturation of the signal was observed over time. The appearance of the chondral surface was maintained or slightly degenerated 10 years after surgery in most cases.

It is well known that deficiency of meniscal tissue can result in alteration of joint homeostasis and degenerative changes overtime (1-4). Improved understanding of the function of meniscal tissue has swayed the therapeutic approach towards conservative or reparative treatments. However, not all damaged menisci can be treated with minimum resection or repair, and large resections are sometimes inevitable or unavoidable. Thus, tissue engineering techniques attempting to achieve meniscus like tissue regeneration have recently been proposed. The use of a three-dimensional porous scaffold can facilitate the migration and proliferation of progenitor cells and vessels that induce the formation of a tissue similar to native meniscus (5-6). These scaffolds must be designed

Key words: meniscal tissue,meniscal cartilage, collagen scaffold

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with porous gaps of specific size and orientation and must have biomechanical characteristics and stiffness that promotes tissue regeneration and protect it from loading undergone by normal joint function. At the same time, catabolites of a scaffold should not damage surrounding articular cartilage or induce foreign body reactions and should be able to balance the joint loads in order to absolve pain (7-8). Different types of scaffolds are currently under investigation, but to date only two, CMI – Menaflex (Ivy, Sports Medicine, Montvale, New Jersey, USA) (collagen, glycosaminoglycans) and Actifit (Orteq, London, UK) (Policaprolactone-polyurethane), are employed. Both scaffolds are available solely for treating partial meniscus deficiencies.

The CMI-Menaflex scaffold/collagen implant, used most frequently, was proposed in 1992 (9) and has been available for clinical use since 2000. It is composed of type I collagen isolated and purified from bovine Achilles tendon with added glycosaminoglycans (GAGs), has a shape similar to the normal human meniscus, is arthroscopically implantable, and is biocompatible and bioresorbable. Ultrastructurally the scaffold is very porous. This facilitates induction of proliferation and differentiation of cellular elements within the scaffold, with consequent production of extracellular matrix to reproduce a meniscal like tissue while the scaffold is gradually absorbed. In vivo studies in both animal and human models confirmed that CMI encourages the proliferation of fibrochondrocytes and production of an extracellular matrix (9-10). In recent years, studies of clinical outcomes of collagen meniscus implants in the medium and long term follow-up show a significant clinical improvement and no progression of degenerative articular changes in most cases (11-15). The aim of this study is to evaluate clinical outcomes, the effectiveness and the safety of the implant at long-term follow-up.

METHODS AND MATERIALS

Twenty-eight patients received a CMI implant between 2001 and 2002 and participated in our previous study of clinical and magnetic resonance imaging (MRI) results at medium term (11). Patients were called again for another visit and MRI, with a minimum follow up of 10 years. All the patients gave their informed consent before intervention.

The indications for the CMI were irreparable medial meniscus tears with meniscus removal greater than 25% of total meniscus or presence of persistent pain after meniscectomy, according to the instructions for use of the CMI set forth by manufacturer. Knees were stable with neutral alignment or a ligament reconstruction and/or osteotomy was performed. Patients with Outerbridge grade IV chondral lesions, autoimmune diseases, infection, other systemic diseases, collagen of animal origin allergies, and over 60 years of age were not considered suitable for CMI implantation. Twenty-six patients were available for the 10-year follow-up and 15 underwent MRI. The average age of patients at surgery was 41.4 years (range 23-53). One patient suffered recurrence of painful symptoms and was treated with arthroscopic debridement in another clinic. Two patients were unavailable. Four subjects had a previous meniscectomy, the others had irreparable meniscal tear. Nine cases had anterior cruciate ligament (ACL) reconstruction and one case an (high tibial osteotomy) HTO. The average length of the implanted CMI was 4.5 cm (range 2.6-6.0 cm).

All the patients had a clinical evaluation with the Lysholm score and the Tegner activity scale before surgery and 2, 5, and 10 years after. An MRI examination was performed 2, 5 and 10 years after surgery with the criteria described by Genovese et al. (16). Also, the morphology and the MRI signal intensity of the complex CMI-regenerated tissue were evaluated with the Genovese score. Three patterns were identified and classified from 1 to 3, with higher scores reflecting patterns more closely resembling those of the normal meniscus. The CMI/residual meniscus complex was classified as grade 1 if the CMI was totally reabsorbed, as grade 2 when CMI appeared small with regular or irregular morphology, and as grade 3 if the shape and size of the CMI was identical to the ones of a normal meniscus. Regarding signal intensity, a markedly hyperintense CMI was considered grade 1, a slightly hyperintense CMI was grade 2, and if CMI signal intensity was isointense relative to the normal meniscus (no signal), CMI was graded as grade 3. The chondral surface was evaluated using the Yulish score (17). According to this author cartilage lesions are classified as grade 1 if cartilage presented with normal contour ± abnormal signal; grade 2 if superficial fraying and erosion or ulceration of less than 50% of thickness were demonstrated on MR arthrography; grade 3 in the presence of partial-thickness defect of more than 50% but less than 100%; grade 4 for full-thickness cartilage loss. Normal cartilage was classified as grade 0.

Surgical Technique

Arthroscopy of the knee joint was performed through standard anterolateral and anteromedial portals. The

removal of damaged or pathologic tissue was only to reach healthy tissue in the red–red or red–white zones. The meniscus rim was perforated in order to get a bleeding bed. The size of the defect was measured with a special arthroscopic device. The collagen meniscus implant was then trimmed to the appropriate dimensions and introduced into the joint through a cannula. The CMI was then fixed with size 2-0 non-absorbable sutures using an inside–out or all–inside technique. Vertical mattress sutures were used for the body of the implant and horizontal mattress sutures were used to secure the CMI to the anterior and posterior horn of the host meniscus.

Physical rehabilitation was started on the first post-operative day: patients' knees were placed in a brace locked in full extension for 6 weeks. The patients were allowed to remove the brace three to four times a day to perform assisted passive motion exercises with a range from 0° to 60° during the first 4 weeks, and with a range from 0° to 90° for the next 2 weeks. No weight bearing was allowed for the first 6 weeks. The knee brace was gradually discontinued after 8 weeks and return to full unrestricted activity was allowed at six months

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) (version 13.0; SPSS Inc., Chicago, IL). Normal distribution of the Lysholm data was assessed. The Lysholm score at preoperative evaluation at 2, 5, and 10 years after surgery was compared using the paired-samples *t*-test. The Tegner activity score at preoperative evaluation at 2, 5, and 10 years after surgery was compared using Wilcoxon signed rank test. Independent sample *t*-test and the Wilcoxon–Mann Whitney test were also performed to compare clinical outcomes in different groups of patients (primary meniscus lesion compared to previous meniscectomy; CMI implantation only compared to CMI implantation and other associated surgery). The level of significance was set at $p<0.05$.

RESULTS

At the time of final follow-up, no patient had signs of meniscus pathology such as catching, joint locking, or swelling. One patient had a new surgery, arthroscopic debridement, 6 years after CMI and was excluded from the study because the case was considered a failure. No complications related to the implant were reported in these 26 patients. The average Lysholm score improved significantly from 56.9 at the preoperative visit to 93.3 at the control visit 2 years after surgery, and remained unchanged at the following 5 and 10-year visits (93.2 and 93.5, respectively). The average Tegner score increased from a 2.6 preoperative value to 5.1 after 2 years and remained similar at 5 and 10 years follow-up visits (5.6 and 5 respectively) (Fig. 1). No differences were observed between patients operated on for knee pain after meniscectomy and those treated for acute meniscal injury, nor were there differences between the subjects who had other surgical procedures (ACL reconstruction and/or HTO) and those who received only the CMI. At the MRI examination the scaffold always appeared visible, but its size was smaller than a normal meniscus in 60.7% of patients at 2 years after surgery and in 70.4% and 72% of patients at 5 and 10 year examinations respectively. The MRI signal had continued to mature between 2 and 10 years after each implant, with progressively decreasing signal intensity, but it was not comparable to the low signal of a normal meniscus (Fig.2). The chondral surfaces

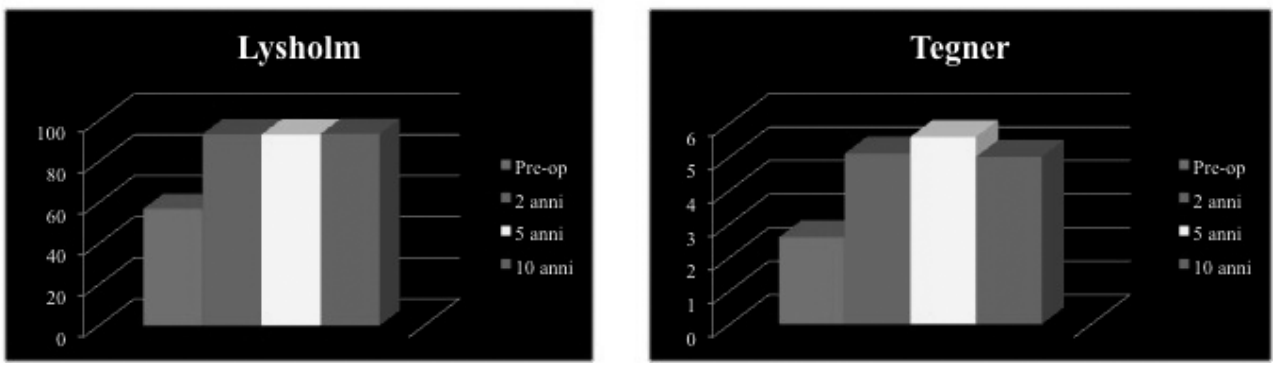

Fig 1. *Lysholm and Tegner scores improve significantly 2 years after surgery and the results remain unchanged at the following follow-up visits.*

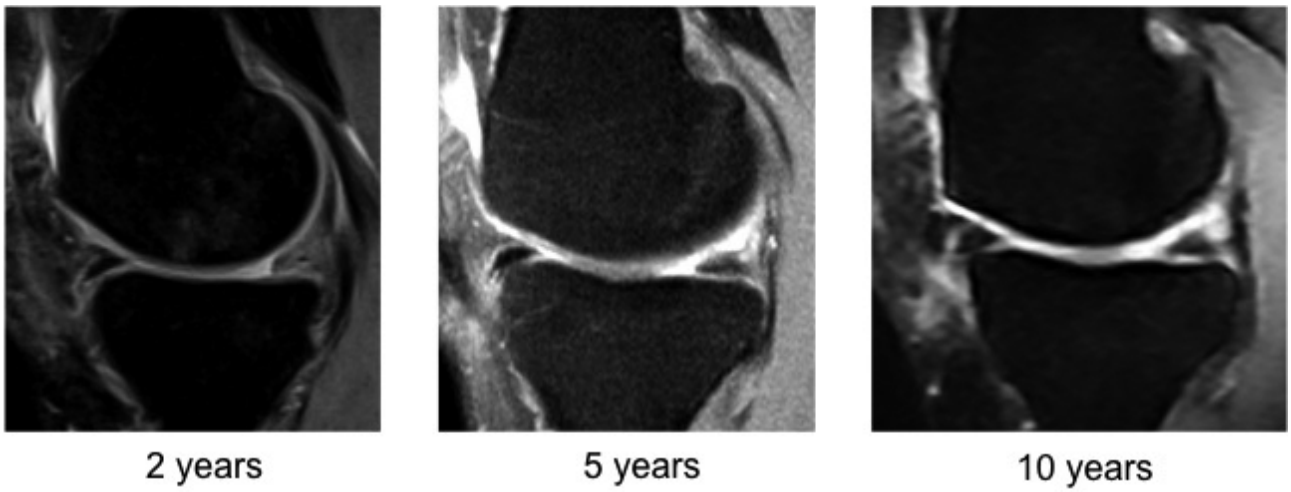

Fig 2. *The MRI signal matures progressively. It becomes more homogeneous and the signal intensity decreases progressively, but at 10 years after surgery it is generally not completely similar to a normal meniscus.*

of the medial compartment had not degenerated further since placement of the CMI during the first 5 years. In the following 5 years a light progression of degenerative changes was observed. In 4 cases of 15 who underwent MRI at the 10-year follow-up, the Yulish score was progressively worse. In 3 cases the score changed from grade 0 to grade 2 and from grade 0 to grade 3 in another.

DISCUSSION

The goal of the meniscal replacement is to restore the normal transmission of loads in order to avoid or reduce joint degenerative changes that have been documented after a meniscectomy (1-4). The use of porous scaffolds, in the case of partial meniscus defects, has been proposed for regeneration of the deficient meniscus tissue (6-7). Many different types of scaffolds are still being studied, but only the CMI-Menaflex collagen and the polyurethane Actifit appear to be safe and effective in restoring the function of the meniscus and are available for clinical use. In our study we wanted to test the clinical outcome, effectiveness, and safety of the collagen implant at long-term. Of the 34 patients treated with CMI between 2001 and 2002 for symptomatic deficency of the medial meniscus, 28 were rated using the scores of Lysholm, Tegner, and MRI at 2 and 5 years after surgical treatment (11). The clinical scores at follow-up were significantly improved compared to preoperative scores and patients showed good or excellent results after 5 years. The chondral surface of the medial compartment did not show progression of degenerative changes using MRI examination. The MRI signal continued to mature between 2 and 5 years with gradual reduction of hyperintensity, without, however, becoming similar to that of a normal meniscus. In most cases the CMI-regenerated tissue complex was reduced in size respect the native meniscus. These data were confirmed by Steadman et al. and Zaffagnini et al.(12-13). In a large randomized prospective study comparing patients treated with CMI to subjects treated with meniscectomy, Rodkey et al (18) presented clinical findings similar to ours, but only in patients with chronic injuries that had undergone previous meniscal surgeries. Their subjects treated with CMI for acute meniscal tears did not show significant improvement compared to the control group. In our prior study at medium term we also described the arthroscopic appearance of 8 implants performed at different times from surgery. In all these cases a biopsy of the regenerated tissue was also performed. Often the implant appeared smaller and irregular compared to the original, but the scaffold was always at least partly present. Histological examinations of the biopsies showed the presence of residues of the scaffold up to 3 years after implantation, while at 5

years the scaffold appeared completely reabsorbed. The regenerated tissue was increasingly mature over time. Histological examinations of biopsies taken 5 years from surgery revealed that density of connective tissue varied depending on area of the tissue being examined. The regenerated tissue contained more cells than a normal meniscus. The absence of phagocytes and macrophages confirmed the biocompatibility of the CMI. We also noticed some vascular formations, not normally present in meniscal tissue.

The clinical evaluation carried out 10 years after surgery demonstrated that clinical scores were virtually unchanged. The MRI performed at 10 years after surgery showed that the regenerated tissue maintained morphology similar to that seen at the 5 year examination, while the signal intensity had become continually less hyperintense. Although in most cases, signal intensity was not completely similar to a normal meniscus intensity at 10 years after surgery.

The MRI appearance of the articular surface was essentially unchanged from 2 to 5 years and had a modest deterioration in the assessment performed afterwards, but it is difficult to give a meaning to this issue, as our study lacked a control group. Zaffagnini et al. (14) compared a group of 18 patients operated on with CMI to a group of 18 patients treated with simple meniscectomy in a long-term prospective study. Clinical outcomes assessed with the VAS scale, Tegner and SF-36 showed a significant improvement at 10-year follow-up in patients with meniscal replacement compared to those treated with meniscectomy. Radiographic studies and MRI showed a significantly decreased narrowing of the medial compartment joint space and a better appearance of the articular cartilage in the group with CMI, although the latter data was not significant. In the present study, the MRI signal of regenerated tissue confirmed that tissue integrity was maintained between exams performed at 5 and 10 years. Also, in 2011 Monllau et al. (15) reported the results of CMI with a 10-year minimum follow-up. In this prospective study of 25 subjects, clinical and radiographic controls were compared to MRI at 1 and 10 years after surgical treatment. The authors confirmed significant clinical improvement assessed with Lysholm's scale and decreased pain using the VAS scale. The MRI study of the complex CMI-regenerated tissue showed a reduction in the size and intensity of the signal, as was also noted by Zaffagnini (14) and in our previous study. In the study by Monllau et al. (15), intermediate control visits are not reported and it is not possible to accurately define the progression of the scaffold's appearance as a function of elapsed time. Similar to Zaffagnini et al. (14), Monllau et al. (15) observed conservation of the medial joint space in subjects who had received the CMI using radiographic evaluations. Radiographic evaluations were not reported in our study, which was a limitation. We decided to not include these data in the evaluation because the radiographs were performed with different machines by different technicians and at different sites. The greatest weakness of this study is the lack of a control group.

CONCLUSION

Our study has shown that 10-year results of collagen meniscus implantation are satisfactory in patients with irreparable medial meniscus tears with meniscus removal greater than 25% of total meniscus or presence of persistent pain after meniscectomy. No severe adverse events were observed and clinical improvements recorded at 2 and 5 years after surgery were maintained at 10 years. The CMI surgical technique is less complex than the technique for meniscus transplantation, it avoids problems of implant sizing, and immunological reaction and disease transmission are unlikely. The CMI is useful to treat partial meniscus lesions because it is possible to maintain the residual healthy meniscus tissue. The original scaffold is reabsorbed over time, and the regenerated tissue is irregular and reduced in size compared to a normal meniscus. The new tissue matures over time, becoming similar to a normal meniscus, but even 10 years after implantation, the appearance of the regenerated tissue remains different from the normal meniscus.

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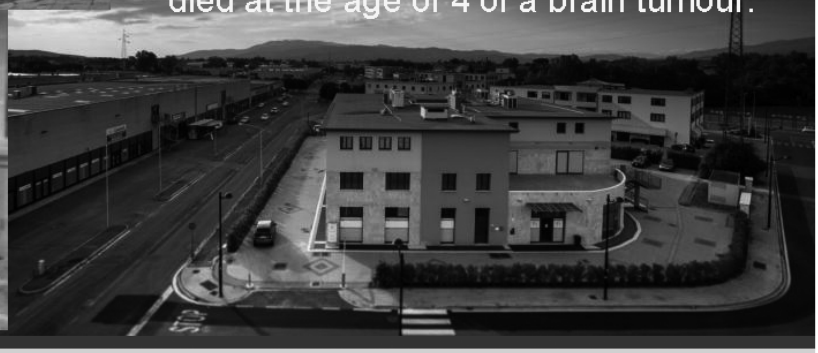
The Foundation was constituted on November 21, 2006 with the name of Nicola's Foundation ONLUS, in memory of Prof. Giuliano Cerulli's son Nicola, who died at the age of 4 of a brain tumour.



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The institutional aims of the Foundation

- To promote research and training for a health service focused on the individual's needs and therefore more humane and humanizing
- To promote scientific culture

To develop basic and applied research projects with the contribution of international experts

- To contribute to education and scientific progress in the bio-medical field in developing countries
- To support medical-scientific education
- To promote scientifically supportive activities and health campaigns

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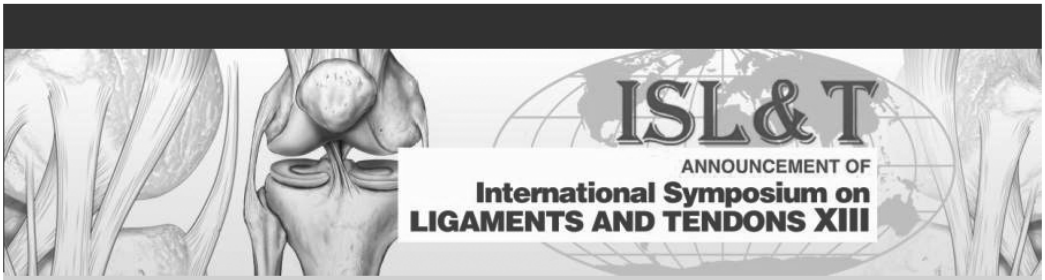

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