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Third generation of cellular therapies for chondral defects: a bibliographic research

Etudiant
Laure-Méline Piotet

Tuteur
Prof. Lee Ann Laurent-Applegate
Unité de thérapie régénérative

Co-tuteur
Dr. Nathalie Hirt-Burri
Unité de thérapie régénérative
Dr. Virginie Philippe
Service d'Orthopédie et Traumatologie

Expert
Dr. Robin Martin
Service d'Orthopédie et Traumatologie

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UNIL | Université de Lausanne
Faculté de biologie et de médecine

CHUV

Table of contents

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Introduction

The knee is one of the most complex organs of the body. It consists of two joints: the tibio-femoral joint, which binds the femur (thigh bone) and the tibia (shin bone) (Fig. 1), and the patella-femoral joint, which links the kneecap (patella) to the femur. The fibula is a smaller bone that runs alongside tibia, and it also participates in overall joint stability.

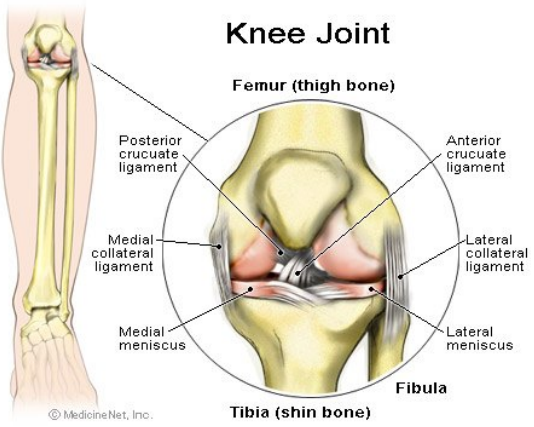

Fig. 1 *Tibio-femoral joints and the main ligaments (Image courtesy of ©burleighphysio.com)*

Different ligaments (Fig. 2) join the knee bones and provide stability to the knee: they can either be intra articular (anterior cruciate ligament, posterior cruciate ligament, the transverse ligament and the menisco-femoral ligaments) or extra articular.

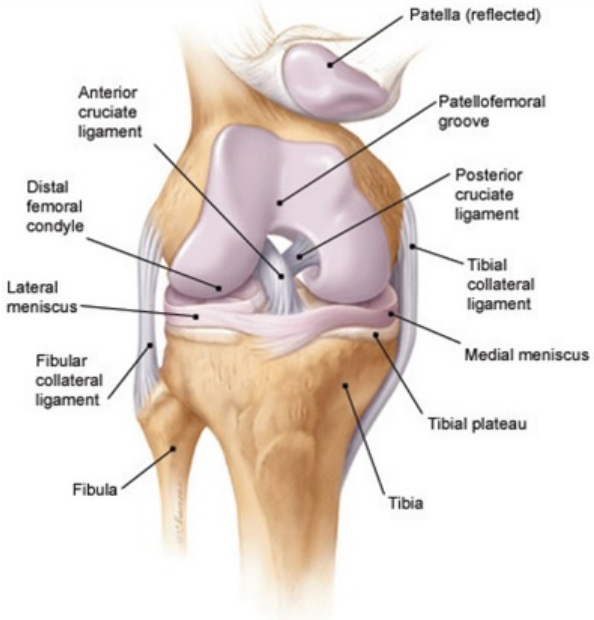

Fig. 2 *Anatomy of the knee (Image courtesy of ©www.slideshare.net)*

The articular capsule is an elastic and fibrous membrane, which surrounds and delimits the joint. The articular capsule contributes to maintain the contact of the joint structures and to ensure stability.

The two articular discs of the knee are called menisci: they are composed of fibrous cartilage, and serve to protect the ends of the femur from being damaged, while they also permit load bearing, stability and joint lubrification. The rest of the articular surface is covered with hyaline cartilage which can wear over the years.
The knee articular surface is submitted throughout life to strong mechanical loading. Over years of use (degenerative lesions) or after a traumatic event, cartilage can wear out and become damaged. Most of the lesions are developed while practicing sports: football, rugby, skiing and snowboarding have the highest damage potential. The damage can occur to the cartilage on its own as an isolated condition, or simultaneously with other injuries (the most frequent accompanying lesions concern the anterior cruciate ligament, or the meniscus [89]). Injuries to the cartilage of the knee lead to local inflammation that can frequently result in osteoarthritis. The symptoms are knee swelling, reduced range of motion, grinding sensation when moving, stiffness, and sometimes pain when straightening the knee. In severe stages, the pain can even perturb sleep at night.

Even if most of the chondral lesions remain asymptomatic, injuries of the articular cartilage of the knee are disabling, principally due to the biological nature of cartilage. Indeed, cartilage is both a resistant and flexible tissue composed of chondrocytes distributed in an extracellular matrix (ECM), a gel-like substance that gives cartilage its form and function. Three types of cartilage exist: elastic, fibrous and hyaline which is also called articular cartilage (Fig. 3). The most wide-spread within the body is hyaline cartilage, which is found in joints, trachea, ribs, larynx and in the nasal septum. In the joints, articular cartilage covers the bone ends and allows them to roll against another. Therefore, hyaline cartilage has low friction properties and the capacity to resist shear and compression [2].

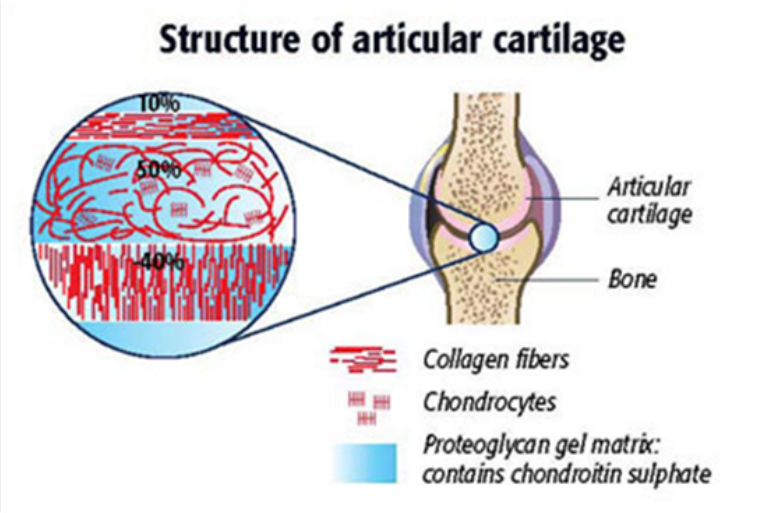

Fig. 3 *Structure of articular cartilage*
(Image courtesy of ©http://acner.org/img/care_and_prevention/)

Chondrocytes, which are derived from mesenchymal stem cells, correspond to only 2% of the total articular cartilage volume. Chondrocytes are separated from each other and immobilized in a rigid microenvironment where they self-develop and maintain: there is no direct contact between the cells, as each cell lies in a small cavity of cartilage which is called lacuna. The chondrocytes interact with the environment through complicated stimuli such as growth factors, mechanical loading, piezoelectric forces or hydrostatic pressure. Therefore, cellular survival depends on their mechanical and chemical environment.

The extracellular matrix (ECM), which surrounds the chondrocytes and keeps them in position, is mainly composed of water, which contributes up to 80% of cartilage weight and allows nutriment diffusion to the chondrocytes. Collagen fibrils represent 60% of cartilage dry weight. Type II collagen represents up to 95% of collagen in the ECM (collagen I, IV, V, VI, IX and XI are also present but in minor proportions) and is mingled with proteoglycans [1], which are proteins that are heavily glycosylated. A common proteoglycan consists of a big “core protein” with one or more linear glycosaminoglycan chains covalently attached (Fig. 4). Aggrecan, also called cartilage-specific proteoglycan core protein, is the largest and the most abundant proteoglycan in cartilage, representing up to 10% of its weight. It provides cartilage the possibility to resist compressive loads. This function of the articular tissue depends on a high Aggrecan/GAGs concentration being present in the MEC. The highest aggrecan concentration in cartilage is reached in young adulthood followed by a slow degradation over time, while its degradation products slowly accumulate. The proteolyse of terminal globular domain of Aggrecan has been associated with the development of cartilage deterioration and/or arthritis.

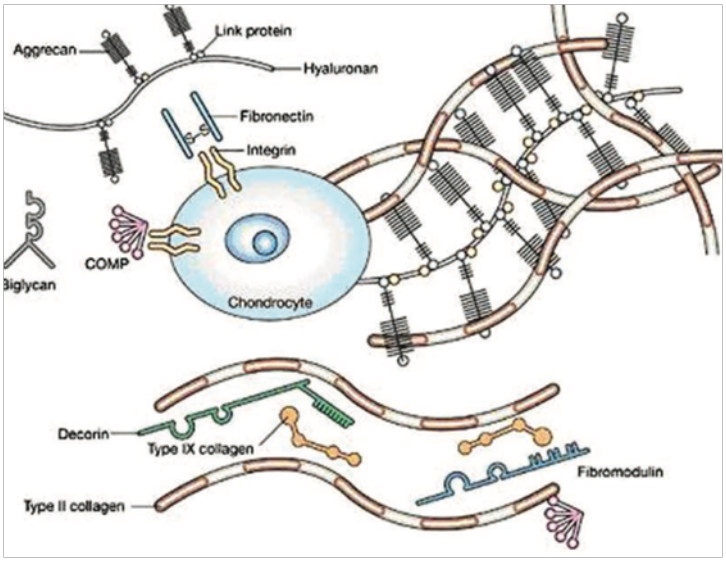

Fig. 4 *Extracellular matrix of cartilage. Two major load-bearing molecules are composed in this ECM: collagen fibrils (mostly type II collagen) and proteoglycans (principally Aggrecan) (reprinted from Chen et al, 2006 [76])*

If biological characteristics allow cartilage to withstand forces and constraints, this also implicates that cartilage has weak self-repair capacity. The inability of the chondrocytes to migrate to an injured part of the tissue combined with the avascular and aneural nature of the cartilage prevents spontaneous healing. Inferior repair commonly occurs, but stable regeneration of hyaline cartilage has never been documented [90].

Therefore, traumatic injuries and degenerative diseases of a joint frequently lead to a chondral defect, which is defined as a loss of material in the articular cartilage at the end of the bone [2]. As mentioned above, chondral defect can lead to disabling symptoms, mostly because mesenchymal stem cells and/or fibroblasts promote repair with fibrocartilage, which is biomechanically inferior to hyaline cartilage and can lead to osteoarthritis. In conclusion, treatment for symptomatic articular cartilage damages focus on regenerating hyaline cartilage to prevent osteoarthritis [3]. The International Cartilage Repair Society (ICRS) has defined a five grades classification of chondral diseases that are shown in Figure 5.

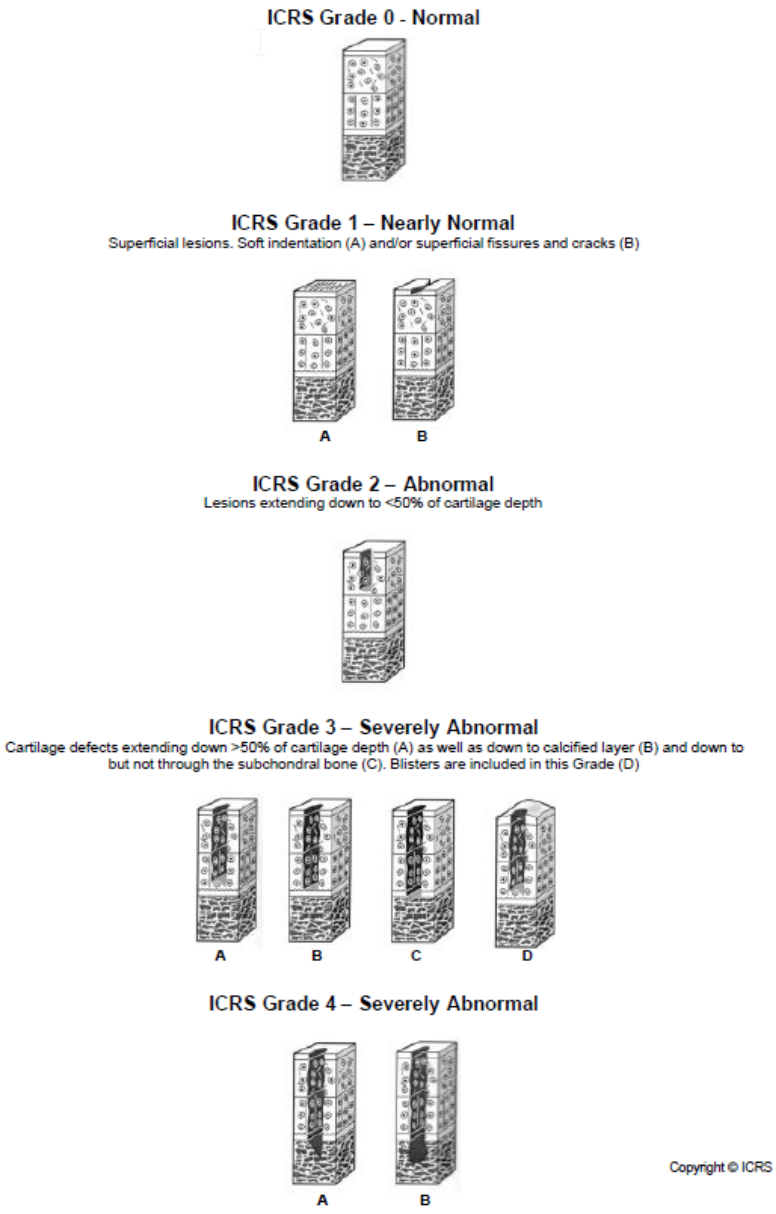

Fig. 5 *ICRS Chondral Injury Classification,*
The International Cartilage Repair Society (ICRS) uses a 5 grades scale to classify chondral injuries, with Grade 0 normal; Grade 1 nearly normal (soft indentation and/or superficial fissures and cracks); Grade 2 abnormal (lesions extending down to < 50% of cartilage depth); Grade 3 severely abnormal (cartilage defects > 50% of the cartilage depth) and Grade 4 severely abnormal (through the subchondral bone). (Image courtesy of ©www.maitrise-orthopedique.com)

Chondral lesions are common: a recent study found chondral injuries in 66% of a series of 993 consecutive knee arthroscopies [79]. Most of the lesions were isolated large lesions of the femoral condyles. The frequency of chondral lesions have been found to be increased in collegial, professional and world-class athletes [80]. Sports activities with a higher risk to induce an acute chondral injury are soccer, basketball and football [81][82]. That kind of injury can limit

the athletic activity while the patient is pre-disposed to early joint degeneration [83]. Moreover, chondral lesions are the most common cause of disability in athletes [84]. Due to their poor healing properties, articular cartilage injuries are a therapeutic challenge in active and young patients, and their early management has long-term implications. While improving the function and the mobility of the joint, the ideal therapy may allow the patient to return to sports (pivoting sports included), or at least it must reduce the symptoms of the patient and allow him to perform normal daily activities.

Common and traditional therapies for chondral defects imply conservative mesures (eg. oral analgesia or weight loss), palliative treatment (eg. arthroscopic chondroplasty) or replacement therapies (eg. joint replacement); they address pain but fail to address the lesion itself. In the past years, various therapies have been developed in the attempt to solve this issue, with different benefits and limitations noted (Table 1).

Table 1

Current clinical options to treat chondral injuries [3] [4] [5] [6] [31].

Treatment	Description	Benefits	Limitations
Non-surgical	Analgesics, weight loss, physical re-education, physiotherapy	No surgery Not expensive price	-Chronic analgesic drugs uses -Palliative
Arthroscopic chondroplasty	Minimally invasive arthroscopic resection of detached cartilage fragments to prevent further joint's irritation	More simple procedure Immediate weight-bearing Brief pain relief	-Palliative - Its benefits are not scientifically established
Microfracture	Arthroscopic procedure consisting in creating tiny breaches in the underlying bone to release osteoprogenitor cells	No graft needed For defects smaller than $2cm^2$ Fast recovery	-Produces mostly fibrocartilage which is biomechanically inferior to hyaline cartilage -Variable regain of function with rapid deterioration
Joint arthroplasty	Consists in replacing the arthritic joint with an implant	Pain relief Variable recovery of function	-Variable return to function Risk of infection of the implant -Implants wear out overtime (possible re-operation)
ACI (Autologous chondrocytes implantation)	Harvested autologous chondrocytes are cultured and expanded before being re-implanted in the lesions	May generate hyaline cartilage	-Graft delamination -Periosteal hypertrophy -The generated repair tissue may not be hyaline cartilage -Expensive
MACT (Matrix-assisted autologous chondrytes transplantation)	Harvested autologous chondrocytes are implanted and cultured in a 3-D scaffold, and then implanted into the defect	May generate hyaline cartilage May conduct to less periosteal hypertrophy compared to ACI	- Graft delamination - The generated repair tissue may not be hyaline cartilage - Expensive

In addition to the therapeutic mesures mentioned in Table 1, Osteochondral allograft transfer (which uses allogenic grafts to fill in the defect) and mosaicplasty (which consist in doing multiple autografts) also exist but their use is indicated in the case of osteochondral lesions.
The search of an ideal treatment that would restore the low friction properties of the cartilage while resisting wear over years implies generating a hyaline-like matrix fully integrated with the surrounding host tissue. The emergence of tissue engineering, which consists in generating replacement tissue by combining knowledge in material engineering and in cell therapy, has permitted a promising therapeutic technique called autologous chondrocyte implantation (ACI), which was reported for the first time in 1994 by Brittberg et al. as a treatment for isolated

condyle lesions [7] (Fig. 6). In this first generation of ACI, the surgeon implanted cultured autologous chondrocytes into the defect under a periosteal patch.

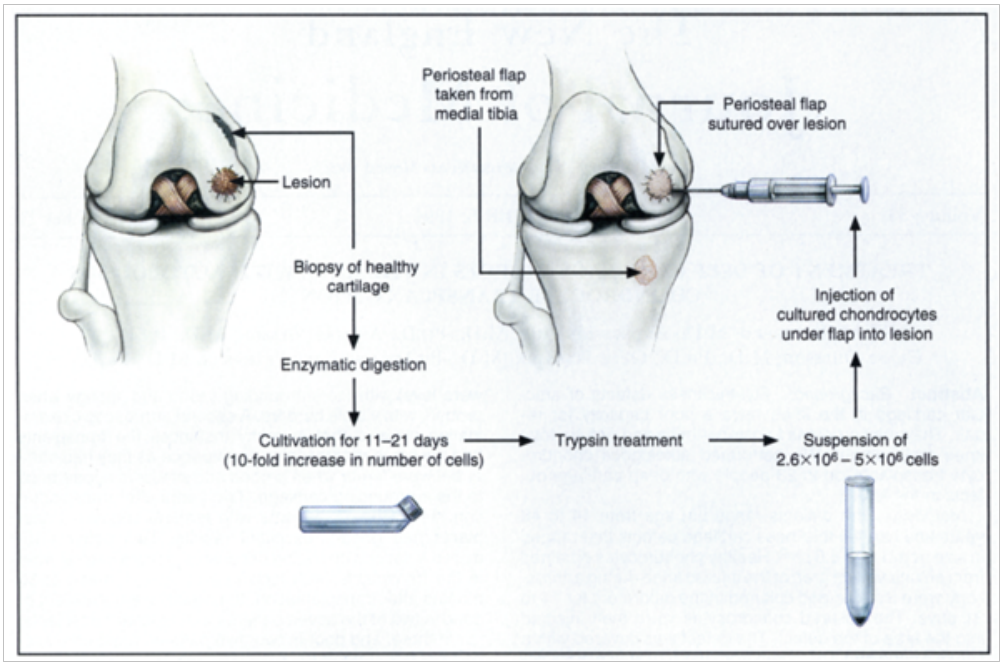

Fig. 6 *Brittberg diagram for autologous chondrocytes implantation to treat isolated condyle lesions (reprinted from Brittberg et al. 1994 [7]).*

Since 1994, ACI has been the subject of numerous studies which could show better clinical results than conservative treatment. Disadvantages of ACI compared to microfracture or osteochondral autograft transfer (OAT) (two procedures that also have obtained better clinical outcomes than conservative treatments) are the need of a 2-stage procedure. The procedure is rarer compared to other therapies, such as prothetic joint replacement, due to the strong cultural habits of the medical population. Because of its rarity and of its complexity, the procedure implies higher cost [31]. All those reasons imply that the beneficiaries of ACI procedures are typically young patients with symptomatic ICRS grade III to IV lesions who have lesions with a diameter that would be ideally larger than 2mm [33] [5].

If mid-term and long-term results of several clinical trials have proven ACI procedures as safe and with satisfactory outcomes. Llimitations also have emerged, such as chirurgical complexities, graft-site morbidity (eg. graft detachment or graft hypertrophy) or the difficulty of maintaining the chondrocytes alive and with good phenotypic features into the lesion.

This last concern led to the development of second-generation ACI [39][48][68], in which the periosteal flap has been replaced with a bilayer collagen membrane which covers and protects the graft with the purpose to reduce the development of graft hypertrophy.
The second-generation was then overtaken by the third-generation of ACI procedures, also called matrix-assisted autologous chondrocytes transplantation (MACT), which uses cell-seeded scaffolds to repair articular lesions. They indeed use a temporary 3-dimensional structure of biodegradable material that mimics some characteristics of articular cartilage, which allows the growth and repartition of the chondrocytes as close as natural cartilage as

possible [34]. The objectives of the third-generation ACI's are to be less invasive by allowing arthroscopic procedure [34] and a better maintenance of the cells.
In all the previously mentioned ACI generations, the implanted cells have always been autologous chondrocytes. The procedure is fully described in Figure 7.

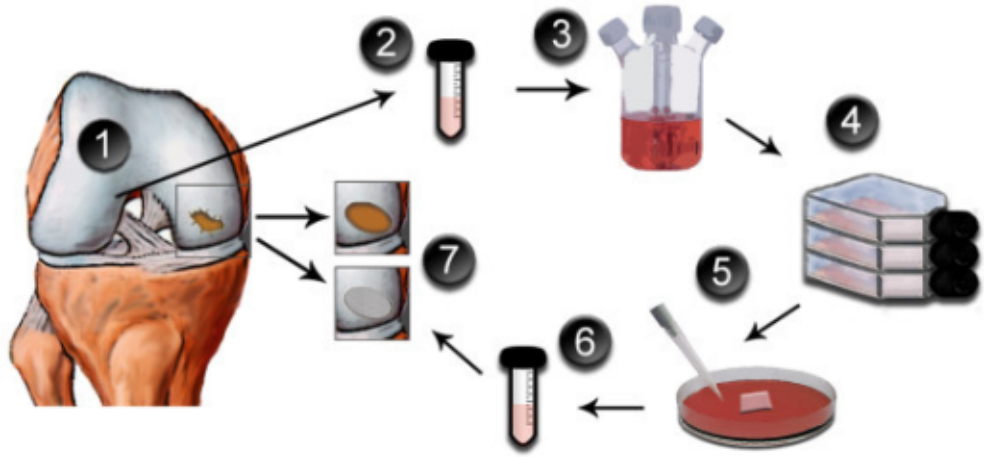

Fig. 7 *Steps of a MACT procedure: 1. Biopsy is taken through an arthroscopy procedure, 2. Biopsy is sent in a container to the GMP production center, 3. Chondrocytes are enzymatically extracted and cultured, 4. Chondrocytes are expanded, 5. The cells are seeded onto the scaffold, 6. Newly-formed tissue is sent back to the surgeon, 7. The cell-seeded scaffold is implanted into the defect*
(Image courtesy of ©https://bmcsportsscimedrehabil.biomedcentral.com)

Concerning the scaffolds, several absorbable matrices have been developed in the past years with various materials (natural or synthetic) in different physical forms (fibers, meshes, gels) [8][9][30]. Natural materials such ags type II collagen, chitosan, hyaluronic acid, alginate, agarose or fibrin glue have good biocompatibility, in addition to their capacity to enhance cell proliferation. Polylactides (eg. polylactic and polyglycolic acids) are the most frequent synthetic scaffolds (Table 2). Natural materials are more frequent, because they are derived from the the collagen membrane that was used as a chondrocytes cover in the second generation of ACI. Synthethic scaffolds are less frequent, even though innovation in their chemical components provide them with good biocompatibility [32] because their development started later.

Table 2
List of the main commercial scaffolds, which means that those scaffolds are being sold as compatible devices for MACT in human beings

Brand's name	Scaffold's description	Firm, Country
ACI-MAIX	Porcine I-III collagen membrane	Matricel, Germany
Chondro-Gide	Porcine I-III collagen membrane	Geistlich Pharma, Switzerland
Hyalograft C	Hyaluronan	Anika Pharmaceutics, USA
Bio-seed C	Resorbable polymers on polyglycolic acid	Biotissue, Germany
Novocart 3D	3D collagen biphasic structure	TETEC, Germany
CaReS	3D collagen type 1 structure	Arthro-Kinetics, Austria

Neocart	3D collagen type 1 structure	Histogenics, USA
Chondron	Fibrin gel	Regrow®, India
MACI®	Porcine I-III collagen membrane	Vericel, USA
Chondrosphere	Spheroids in suspension from autologous chondrocytes	Co.don AG, Germany

As already mentioned, MACT procedures mainly concern the knee. Several clinical scores exist to assess the post-operative knee function. The three main scores are presented below: all of them are subjective surveys that focus on functional abilities and symptom occurrences.

The first one is the IKDC score (International Knee Documentation Committee, Annexe 2), a survey that focuses on function and activity of daily living. It tries to correlate the occurrence of pain with physical activities and movements, while assessing the remaining function of the knee. It looks at three categories: symptoms, sports activity and knee function. The final score is obtained by the addition of all individual items, and then transforming the total to a scale number that range from 0 to 100, with 100 representing a perfectly functional knee. Standards are adapted with age and sex:

	18-24 y.o	25-34 y.o	35-50 y.o	51-55 y.o
MEN	89 +/- 18	89 +/- 16	85 +/- 19	77 +/- 23
WOMEN	86 +/- 19	86 +/- 20	80 +/- 23	71 +/- 26

With the population average age of 35 years old, an acceptable IKDC score would be 85 +/- 25.

The Tegner-Lysholm (Annexe 3) is in fact two scores put together. The Lysholm is a short questionnaire which asks eight questions to determine the degree of infringement of the knee and is symptom-centered. Each of the eight questions has different formatted answers and every answer corresponds to a number of points. The Tegner activity scale evaluates the knee function, especially the ability of practicing sportive activities. The grading of this score is the following: < 65 points corresponds to a poor result, between 65 and 83 points is described as fair, 84 to 90 as good, and scores greater than 90 as excellent. These two scores are sometimes used separately (with a different grading system).

The KOOS (Knee Injury and Osteoarthritis Outcome Score, Annexe 4) is the longest questionnaire; it has five subscales: pain, other symptoms, function of daily living, function in sports and recreation, knee related quality of life. The previous week is the time period considered when answering the questions. Standardized answers are given for each subscales and each question is assigned to a score from 0 to 4. Every subscale must be analyzed separately from the others, which differs from the IKDC scoring method. The KOOS is very precise and its items evaluate the function of the knee in various situations compared to the two other scores. However, its complexity limits its use because of difficulties in comparing outcomes from one patient to another.

If Magnetic resonance imagery (MRI) is frequently used as a diagnostic tool, it can also be used to evaluate the success rate of ACI procedures. For that purpose, the MOCART (Magnetic resonance Observation of Cartilage Repair Tissue) score [43] [48] has been developed in the last years [78]; as listed in Table 8.

Variable	Description	Cases
1 Degree of defect repair and filling of the defect	Complete	3
	Hypertrophy	3
	Incomplete >50% of the adjacent cartilage	4
	Incomplete <50% of the adjacent cartilage	4
	Subchondral bone exposed	2
2 Integration to border zone	Complete	8
	Demarcating border visible (split-like)	4
	Defect visible <50% of the length of the repair tissue	3
	Defect visible >50% of the length of the repair tissue	1
3 Surface of repair tissue	Surface intact	2
	Surface damaged <50% of repair tissue depth	8
	Surface damaged >50% of repair tissue depth	6
4 Structure of repair tissue	Homogenous	0
	Inhomogenous or cleft formation	16
5a Signal intensity of repair tissue Dual T2-FSE	Isointense	1
	Moderately hyperintense	0
	Markedly hyperintense	15
5b 3D-GE-FS	Isointense	0
	Moderately hyperintense	0
	Markedly hyperintense	16
6 Subchondral Lamina	Intact	3
	Not intact	13
7 Subchondral bone	Intact	4
	Non-intact	12
8 Adhesions	No	15
	Yes	1
9 Effusion	No	6

Fig. 8 *MOCART evaluation system*

In the context of the emergence of third-generation ACI procedures (MACT), the aim of the present work is to list the existing types of scaffolds that are being developed in animal models and/or used in clinical trials. Although it was initially decided to not only focus our research on MACT procedures in the knee and to include articles concerning other joints, our results will be focused on the procedures that concern the knee, as they represent the large majority of the MACT procedures. This should also have the purpose of obtaining more homogenous results.

Methods

The research was performed by using the PubMed and the Cochrane database for both animal studies and clinical studies concerning MACI treatment for chondral defects. The following strings were used: (("chrondral defect"[Mesh] OR “articular defect”[Title/Abstract] OR “cartilage repair”[Title/Abstract] OR “osteochondral defect”[Title/Abstract] OR “cartilage tissue engineering”[Title/Abstract] OR “autologous cartilage implantation”[Title/Abstract] OR “allogeneic chondrocytes implantation”[Title/Abstract]) AND ("scaffold"[Mesh] OR "matrix"[Mesh] OR "Guided Tissue Regeneration"[Mesh] OR "Tissue Engineering"[Mesh]) AND (chondrocytes[Mesh] OR biomaterial*[Title/Abstract]) OR tissue engineering[Title/Abstract] OR implant*[Title/Abstract] OR transplant*[Title/Abstract] OR cell

therapy*[Title/Abstract] OR cell*[Title/Abstract] OR autologous cell*[Title/Abstract] OR growth factor*[Title/Abstract])). Articles found by reading references lists were also included in the analysis.

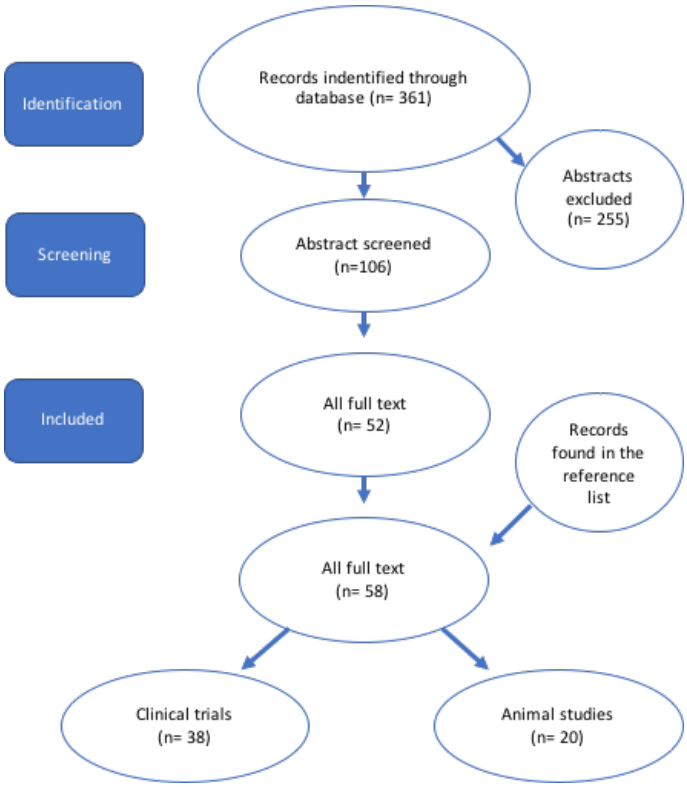

Fig. 9 *Scheme of search methodology*

Research criteria included studies that concerned third-generation ACI procedures, which enrolled matrix-assisted autologous and/or allogeneic chondrocytes implantation procedures from 1998 to 2016, either in an animal model or in human beings. All localization were included in the purpose to show the different possible utilization with MACT. All type of studies were included in this work, from case reports to level I studies.
Studies that did not fulfill those criteria were excluded. The selection process is described above in Figure 9. As we can see, the previously mentioned keywords were not precise enough to ensure close-up results.

General characteristics of the included studies

We can observe a clear augmentation in the number of published papers among the years (Fig. 10), showing an increase of the interest in the field from 2009 on.

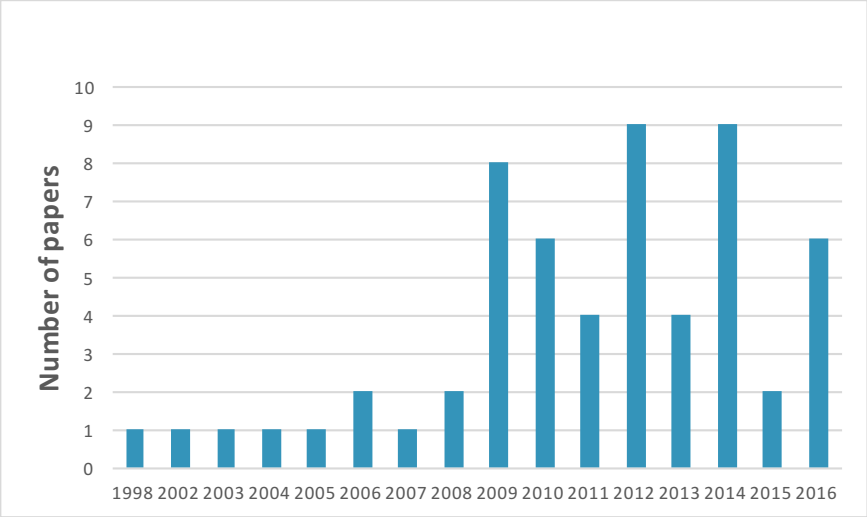

Fig. 10 *Included studies distributed by year of publication*

Numerous countries show interest in the field (Fig. 11). Interestingly, Germany is the most represented country while it also the country of origin of various commercial scaffolds, as mentioned in Table 2.

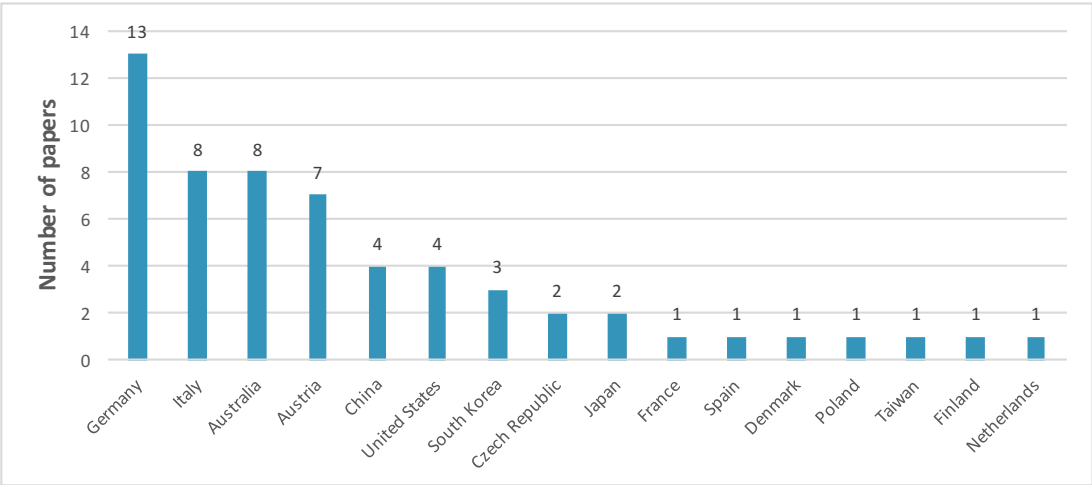

Fig. 11 *Included studies distributed by country of origin*

Of the 58 retained papers 20 concern animal studies and 38 concern human clinical studies. This work has been separated into two categories, the first treating the animal studies and the second category for the clinical trials

Animal studies

Most of the selected studies were conducted with rabbits [11][12][13][16][17] [21] [22] [23] [24 [26 [29] or in miniature pigs [14] [20] [27] [28]. The use of big animal models such as sheep [15] [18], dogs [10], horses [25] or goats [19] is less frequent, perhaps because of its cost and the need of a devoted infrastructure. Regarding the localization of the default, 4 studies had defects in the trochlear groove, 4 processed with condylar defects, 11 studies had unspecified trochlear defects and 1 study dealt with ear defects. The size of the chondral defects depended of the animal model (Fig. 12).

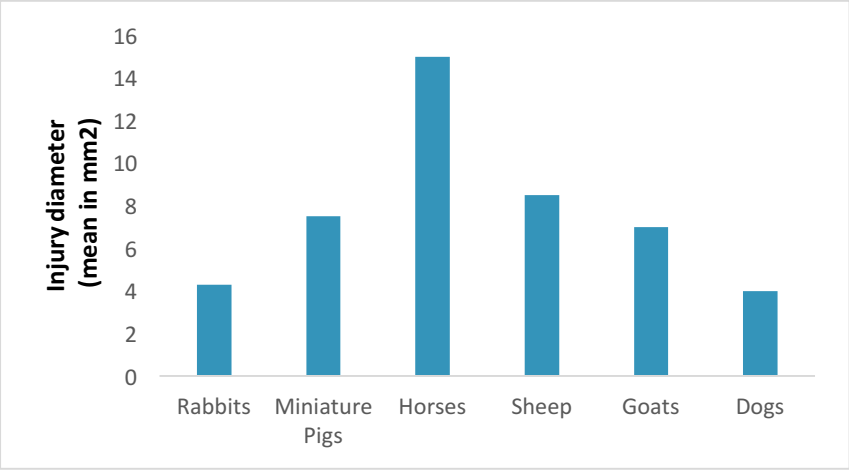

Fig. 12 *Mean diameter size in mm of the lesion, distributed by animal type*

The average study duration was found to be 6.6 months whereas the longest follow-ups lasted 12 months [19][25] and the shortest only 4 weeks [16][11]. The follow-up duration must also be correlated with the chosen animal model: shorter follow-up duration was mainly indicated for MACT procedure in rabbits [16][11], while bigger animals imply longer follow-ups. The cells that were used in the procedures are indicated in Table 3.

14 studies used a scaffold based-on natural materials (Table 3), and 6 of them on synthetic materials which were mostly polymers (Table 4). There was no different distribution in the use of the different matrix types according to time. One tested matrix is one of the commercial product mentioned in Table 2, Chondro-Gide [20]. In other studies, some components of the scaffold are the same as in the commercial ones. For example, Hyaff-11 [19] is a hyaluronic acid-based substance which is also the main component of Hyalograft C. Nevertheless, most of the scaffolds did not really correspond to the scaffolds present nowadays in the market, which differentiates the animal studies from the clinical trials, which mostly test commercial products.
As described in Table 3 and Table 4, 10 studies used allogenic chondrocytes, 9 studies used autologous chondrocytes, and one study used xenogeneic cells (the cells were of human origin) [29]. 18 studies used articular chondrocytes, while 1 study used nasal chondrocytes and 1 study used autologous auricular chondrocytes.

Table 3
Animal studies with a natural composite-based scaffold

References	Country	Year of publication	Composition of the scaffold	Animal Type	Cells Type
[10]	Austria	1998	I or II collagen	Dog	Allogenic Chondrocytes
[11]	Japan	2005	Atellocolagen sponge + PLLA mesh	Rabbit	Allogenic Chondrocytes
[12]	Czech Republic	2008	Hyaluronan + fibrin + type I collagen	Rabbit	Allogenic Chondrocytes
[13]	South Korea	2009	Biphasic atelocollagen & hyaluronate & hydroxyapatite	Rabbit	Autologous Chondrocytes
[14]	Czech Republic	2010	Hyaluronic acid hydrogel	Miniature pig	Autologous Chondrocytes
[15]	Italy	2010	3D magnesium, hydroxyapatite & type I collagen	Sheep	Autologous Chondrocytes
[16]	Taiwan	2012	Hyaluronan & collagen II microspheres	Rabbit	Allogenic Chondrocytes
[17]	Finland	2012	collagen II gel	Rabbit	Autologous Chondrocytes
[18]	Germany	2013	Allogenous sterilized bone with collagen surface	Sheep	Allogenic Chondrocytes
[19]	Italy	2013	Hyaluronic acid membrane (Hyaff 11) with autologous platelet- rich fibrin	Goat	Autologous Chondrocytes
[20]	Denmark	2015	Chondro-Gide	Miniature pig	Autologous Chondrocytes
[21]	Spain	2016	Plasma-derived albumin hydrogel	Rabbit	Allogenic Chondrocytes
[22]	China	2016	Chitosan hydrogel -demineralized bone matrix	Rabbit	Allogenic Chondrocytes
[27]	South Korea	2010	Biphasic Hyaluronate–Atelocollagen/ Tricalcium Phosphate Hydroxyapatite membrane	Miniature pig	Autologous Chondrocytes

Table 4
Animal studies with synthetic scaffold

References (n°)	Country	Year of publication	Composition of the scaffold	Animal Type	Cells type
[23]	United States	2003	Polyglycolic and polylactic acid polymer with calcium alginate	Rabbit	Allogenic Chondrocytes
[24]	Poland	2006	Polysulphonic membrane	Rabbit	Allogenic Chondrocytes
[25]	United States	2009	Polydioxanone	Horse	Autologous chondrocytes
[26]	France	2009	Silanized hydroxypropyl methylcellulose (Si-HPMC) hydrogel	Rabbit	Autologous nasal chondrocytes
[28]	Germany	2013	3D polyglycolic acid	Miniature pig	Autologous auricular Chondrocytes
[29]	China	2016	Gelatin polylactic acid nanofibers 3D +/- hyaluronic acid	Rabbit	Xenogeneic Chondrocytes

The tools used to evaluate MACT procedures in animal models are numerous. All studies accomplished a macroscopic examination of the repaired area: they observed the quality of the adhesion of the graft, and noted its color and apparent composition. Five of them used the ICRS macroscopic evaluation of cartilage repair [13] [18] [19] [20] [27], one also used the ICRS visual histological assessment score [27]: if those scores may not be the better ones, they are at least popular and their systematic use would at least permit easier comparison between the studies. Other studies applied different scores such as the O'Driscoll [13] [19] or gave a subjective description of the repaired area, which again limits the possibility of comparing the studies with one another. Safranin-O staining, glycoaminoglycans (GAGs) and type II collagen presence are the most common procedure to define the histological phenotype of the tissue.

Most of the studies obtained histological results that were compatible with the production of hyaline-like tissue in part of the defect [10]. In some articles, it was specified how much cartilage-like tissue was obtained in percentage: Nehrer et al. [10] obtained an average 2% of hyaline cartilage in the repaired area in a dog model with a type I and type II membrane after 18 months, while Ochi et al. [11] found 80% of hyaline cartilage with a scaffold mixing collagen and polymers in rabbits 4 weeks after the implantation, bearing in mind that the success of the experiment is higly variable from one species to another. This showed the difficulty of interpreting results, as the results are drastically different from one study to another. If some studies obtained a large majority of hyaline cartilage through MACT [15][24][25], the procedure led frequently to the development of a mix of cartilage-like tissue and fibrocartilage and/or fibrous tissue [10][12][16][20][21]. Final outcomes were described as better with MACT compared with left-empty defects. Studies in which the analyses were repeated multiple times described a little improvement of the outcome over time [18] [19] [29] [25] [26], mostly between 6 months and 1 year. No duration was correlated with the development of a precise or satisfying amount of cartilage-like tissue. In a rabbit model, obtaining a tissue that is partly cartilage-like could be feasible in 4 weeks [16] [11].

Some studies reported biomechanical analyses [12] [13] [14] [17][19] [28] with decent outcomes. For instance, Filova et al. [12] compared the loading diagrams of native cartilage and four different composite matrices (that mix atelocollagen and hyaluronan) and found similar nonlinear biomechanical characteristics in both groups. Other interesting findings concern the study of Rampichova et al. [14]: they measured Young's modulus of their scaffold (hyaluronan hydrogel) while it was cell-free, then again after the chondrocytes implantation, and repeated the calculation on the regenerated cartilage (6 months after implantation). They found that regenerated cartilage, when compared to native cartilage, was more rigid. Biomechanical analyses could not properly be compared between the studies, as all of them employed different scaffolds.

Radiological analyses were led in several studies with micro-computed tomography [13] [17] [20] or MRI [20] [22] or radiography [25]. A pain evaluation score was included in only one study [25] conducted in horses. They use lameness and flexion as markers of pain while the horse was trotting, and synovial effusion in the femoropatellar joint. The three items were evaluated with three scores: their results peak ten weeks after the operation, but corresponded with slight or mild pain.

Compared with MACT procedure, scaffold-alone implantation was sometimes described as prone to fibrocartilage development [12][19]. One study done in sixteen pigs treated with a biphasic atelocollagen scaffolds (that contained also hyaluronate and hydroxyapatite) showed that scaffold-alone implantation lead to superior outcomes in terms of cell viability and cell distribution than the cell-seeded scaffold. Otherwise, Mariotti et al. [19] found that subjects who underwent a MACT procedure ended up with a harder tissue and biomechanical outcomes more similar to cartilage than scaffold-alone and untreated groups.

Concerning the cells that can be used in MACT, all studies used articular chondrocytes that were either autologous or allogeneic, except two [26][28] that employed auricular and nasal chondrocytes. Auricular chondrocytes [28] implantation seemed to lead to an inferior quality of the repaired tissue concerning macroscopic appearance and biomechanical stiffness, but histological measures were slightly superior compared to articular chondrocytes implantation. As the use of nasal chondrocytes could be considered as safe for MACT [85], Vinatier et al. [26] used nasal chondrocytes to repair articular defects, with satisfying outcomes. Further studies implying nasal or auricular chondrocytes could be interesting, as they are easier to extract and can lead to the development of one-step procedures [26].

As seen in the previous paragraphs, various limitations exist in analyzing the outcomes of MACT procedures in animal models due to the multiplicity of scores and evaluation systems. Moreover, it is more difficult to harvest pertinent outcomes while working with animal samples because it is impossible to evaluate the post-operative care. Another important limiting factor in analyzing the studies outcomes is the large variety of scaffolds. Nevertheless, MACT has reported better results than other procedures (microfracture or scaffold-alone implantation) or than the left-untreated group [10][11][12][13][15][19][25][26] [27][28][29].

Human studies

38 studies were identified. While the longest and the shortest length of follow-up are respectively 180 and 12 months, the medium follow-up length is 41.2 months. Two studies [60][61] concerned the same pool of patients.
The 38 retained studies represented a pool of 1614 patients that underwent a MACT procedure to treat 1755 lesions, as few patients had multiple lesions but the reports did not indicate if the lesions were located in the ipsilateral or contralateral joint [60][61]. 1665 lesions concerned the knee (Fig. 12). The patients medium age was 35 years old (13-66 years old) and the medium BMI was 24. In the studies in which the patient gender was mentioned (n=31), only 540 patients of 1504 were women.

I. Localization of the lesions

Most of the treated lesions were situated in the knee (93%), while the remaining 7% concerned lesions in the hip (n=50) or in the talus (n=40) including a total of 7 studies [46][66][70][48][38][52][51].
If we focus our analysis on the knee (Fig 13), we see that 53% of the lesions treated with MACT were located in the median femoral condyle (MFC) and 14% in the lateral condyles (LCF). In 14 studies, some patients suffered from multiple lesions, mostly without indicating if they were located on the ipsilateral or contralateral joint.

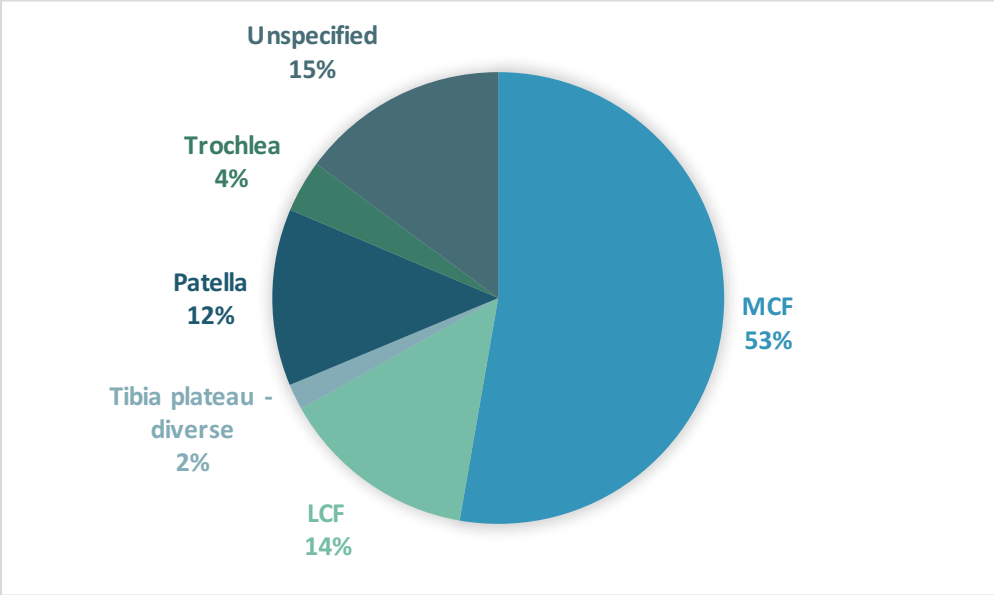

Fig. 13 *Lesions of the knee distributed by frequency*

Table 5
The sum of all the treated lesions, distributed by location – unspecified concern lesions with unclear localization, which mostly concern undescribed femoral condyles

References	Type of Lesions	Number of lesions
[46] [64] [62] [69] [43] [65] [42] [45] [47] [50] [57] [63] [74] [44] [60] [61] [49] [59] [71] [40] [56] [35] [72] [36] [67] [73]	MFC	878
[46] [64] [62] [69] [43] [65] [42] [45] [47] [50] [74] [44] [61] [49] [71] [40] [56] [35] [72] [36] [67] [73]	LFC	235

[46] [69] [43] [65] [42] [63] [39] [44] [60] [61] [41] [58] [71] [40] [56] [35] [54] [67]	Patella	211
[46] [64] [43] [65] [42] [39] [41] [58] [40] [56] [67]	Trochlea	62
[62] [37] [41] [71] [35]	Tibial plate	31
[46] [62] [47] [39] [60] [61] [41] [58] [35] [75] [54] [67] [27]	Unspecified	248

Concerning the size of the defects, the medium was about 4.756 cm^2, with outliers from 0.7 cm^2 [69] to 20 cm^2[46].

II. Clinical evaluation methods

Different evaluation tools could be used to estimate the success of MACT in the considered papers. These procedures were clinical scores, MRI and 2-look biopsy (detailed in Fig. 14) but rarely functional tests [43][74]. Clinical scores that were used are summarized in Figure 15, they are mostly subjective scores based on surveys answered by the patients: they question the pain and its conditions of appearance and of duration.

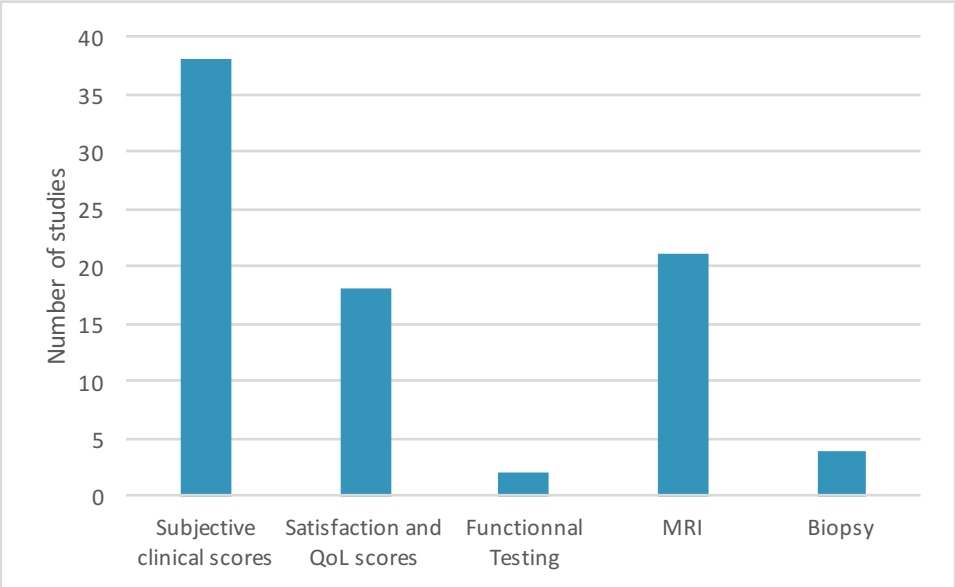

Fig. 14 *Evaluation tools distributed by frequency: in most of the studies, more than one test is used to assess the results of MACT procedures*

As mentioned before, most of the selected studies focused on the knee repair, therefore it was decided that the analysis would mostly be centered on MACT procedures in the knee and the discussion of the studies will also concern the knee. The most widely used clinical scores to assess the knee's function were IKDC (Annexe 2), Tegner-Lysholm (Annexe 3), KOOS (Annexe 4). Besides those three scores, visual analogue scale (VAS) for pain was also commonly used: quick and easy, it can allow pain evaluation in less than a minute.
All the previously mentioned subjective scores are to be repeated several times before and after the operation, according to the chosen length of follow-up. In most of the studies, those scores increased post-operatively when the procedure did not fail, which means that MACT was rather effective from a clinical point of view. Their elevation followed an interesting curve: in most of the studies the scores increased up to two years, then they remained stable or they decreased a little (mainly after five years) [43] [71]. This is illustrated in Table 6 in which are indexed all the studies that use IKDC score as a clinical evaluation.

Table 6

IKDC scoring system distributed by length of follow-up in collagen or hyaluronan-based MACT. The medium post-operative IKDC score reaches the lower limit of the standard range, which is 60. The values written in bold are considered as satisfactory clinical results, M stands for Months, Y stands for years and W for weeks. As for the results, +/- represent the standard deviation.

Cat. 1 – Follow-up: up to 1 year

Reference n°	Number of patients	Lesions	Medium IKDC pre-op	Medium IKDC post-op
[37]	1	Lateral tibial plate	27.5	(12M) **88.5**
[64]	7	4 MFC 2 LCF 1 trochlea		(12M) Improvement described as significant p (<0.05)

Cat. 2 – Follow-up: up to 2 years

Reference n°	Number of patients	Lesions	Medium IKDC pre-op	Medium IKDC post-op
[36]	8	6 MFC 2 LFC	57 +/- 25	(24M): **76** +/- 17
[67]	23	7 MFC 4 LFC 2 trochlea 8 patella 2 MFC + patella	36 +/-15	(12M) : **69.3** +/- 15.3 (24M) : stable
[75]	21	22 distal femoral lesions	44 +/- 14	(6M) 43 % of the patients had > **64** (12 M) 76% > **64** (24M) 79% > **64**
[49]	31	22 MFC 10 LFC		(3.4M): **71.2**

Cat. 3 – Follow-up: up to 5 years

Reference n°	Number of patients	Lesions	Medium IKDC pre-op	Medium IKDC post-op
[44]	21	13 MFC 4 LFC 7 patella	30	(5y): **74.3** +/- 20
[72]	21	17 MFC 4 LFC	37.9	(12M) : **66.2** (36M): **70.29**

Cat. 4 – Follow-up: > 5 years

Reference n°	Number of patients	Lesions	Medium IKDC pre-op	Medium IKDC post-op
[43]	16	11 MFC 2 LFC 4 trochlea 6 patella	44 +/- 26	(60M): **74** +/- 11.9 (120M): 59 +/- 27
[58]	32	20 patella 8 trochlea 3 patella + trochlea 1 FC + patella	46 +/- 19	(2y) **77** +/- 17 (10y) **78.6** +/- 16.4
[71]	53	44 MFC 7 LFC 2 patella 2 tibial plate	41.4	(2y): 55 (4y)**: 72** (7y): 69

22 studies used MRI to assess the success or the failure of the MACT procedure: most of them did this examination between four weeks [49] and one year [37] [47] [44] [64] after the MACT operation. Long-term follow-up (more than 2 years) with MRI is rare [42] [43] [50] [57] and MRI-analysis are mostly qualitative: it mostly serves to attest the graft's integration into the surrounding host tissue, to determine if the defect is correctly filled with the graft, and to make sure that there is no graft-hypertrophy. Sometimes MRI-examination is also used to assess if the newly formed tissue expresses signals that look like the hyaline cartilage[59]. In a few studies, a MRI composite score was used to obtain quantitative results [50] [57] [74].
Some studies used the MOCART score (Magnetic resonance Observation of Cartilage Repair Tissue) [43] [49] [54] [70], which also improves with time. As the MOCART allows comparison between the studies, it would be favorable to apply it systematically in the future to homogenize the MRI analysis and to reduce its limitations.
Ebert J. et al [50] centered their study on the hypothesis that there is a correlation between MRI composite score findings and two subjective scores: KOOS and a random satisfaction scale. No correlation emerges from their research between MRI findings and those two scores: an increase in the KOOS score is not linked with MRI satisfactory result, and vice-versa. Another study [57] obtained similar results when they tried to correlate the patient subjective feeling (represented by the KOOS score) and MRI findings. Moreover, they described their MRI findings as unsatisfactory at 60 months. The results of the KOOS were also disappointing. These scaffolds and their clinical outcomes will be discussed in detail later in this work.

III. Scaffolds

In 34 studies, the scaffolds corresponded to a marketed product (Table 6). 4 studies did not mention any commercial brand, but the composition of their scaffolds was similar to the marketed product (Table 7). 2 studies used two different scaffolds, without comparing one to another. 11 different scaffolds brands were identified: most of them used collagen as the main component. Two major trends in scaffolds engineering emerged from Table 6: porcine type I-III collagen membrane (ACI-MAIX, Chondro-Gide, MACI®) and hyaluronan (Hyalograft C). In the remainder of this section, these scaffolds and their clinical outcomes will be further analyzed.

Table 7

Commercial scaffolds brand encountered in the studies

References	Brand's name	Scaffold's description	Firm, Country
[65], [42], [45], [47], [50], [57], [63], [74]	ACI-MAIX	Porcine I-III collagen membrane	Matricel, Germany
[37], [44], [61], [68]	Chondro-Gide	Porcine I-III collagen membrane	Geistlich Pharma, Switzerland
[43], [41], [49], [58], [59], [71]	Hyalograft C	Hyaluronan	Anika Pharmaceutics, USA
[38], [40], [52], [56],	Bio-seed C	Resorbable polymers on polyglycolic acid	Biotissue, Germany
[54], [67], [46]	Novocart 3D	3D collagen biphasic structure	TETEC, Germany
[35], [72]	CaReS	3D collagen type 1 structure	Arthro-Kinetics, Austria
[36], [75]	Neocart	3D collagen type 1 structure	Histogenics, USA
[51], [73], [73]	Chondron	Fibrin gel	Regrow®, India
[43], [70], [65]	MACI®	Porcine I-III collagen membrane	Vericel, USA
[51]	Chondrosphere	Spheroids in suspension from autologous chondrocytes	Co.don AG, Germany

Table 8
Scaffolds that are not associated to a brand

References	Scaffold's description
[62], [66]	I-III collagen membrane
[64]	3D I-III collagen structure
[69]	Atecollagen gel

i. Porcine I-III collagen membrane in the knee

Porcine I-III collagen membrane choncerned 15 studies (Table 7) and was, by far, the most popular scaffold in the selected studies. 14 of them concerned MACT procedure in the knee, and were therefore eligible for further analysis. The two others concerned MACT procedure in the hip and the talus.

a. Failures and adverse events

These clinical studies included a panel of 540 patients. 27 MACT procedures (5%) were considered as failures (by failure the authors mean that the patient needed a re-operation) were reported. The causes of the failures were, when mentioned, graft hypertrophy (n=10) [47] [57] [68] [74], graft detachment or poor integration (n=10) [57] [61] [68] early post-operative failure (n=2) [57] and persistent pain (n=2) [44]. When mentioned, post-operative adverse events included the following: 8 post-operative tendinitis[56], 2 deep veins thrombosis[42], 2 transitory graft hypertrophy[44], 1 subchondral cyst recurrence[44], 1 patella baja[42], and 1 acute synovitis[56]. Most of those adverse events were in fact surgical complications and are not provoked by the scaffold itself. One study explained that no adverse events nor failures occurred [74]. With regards to the number of patients who underwent a MACT, only few side effects were described and few patients needed a re-operation: the procedure seems to be almost harmless.

The study of Angele et al. [46] focuses only on adverse events and side effects in a cohort of 423 patients who underwent a MACT procedure (with a 3D biphasic collagen structure, Novocart 3D). They found a higher proportion of failure with a follow-up of one year length. Indeed, they reported that 44 patients underwent a second procedure because of the following reasons: graft failure (n=13), delamination (n=6), arthrofibrosis (n=7), synovitis (n=7), adhesions (n=5), and pain (n=6). They also reported a larger variety of adverse events with 32 effusion phenomenon, 10 arthrofibroses, 7 adhesions, 7 delaminations, 3 graft-hypertrophy, 3 deep joint infections, 2 chondromalacia, 14 synovitis, 7 hematoma, 1 locking syndrome, 1 superficial infection and 29 patients experienced persistent pain. As a matter of fact, when comparing those results with those mentioned above, it seems very likely that some of the studies did not report all of their side effects and/or failures, which is unfortunate because this information is necessary and pertinent.

Angels et al. found that adverse events and failures were not correlated with patient cartilage defect size. On the contrary, younger patients had shorter recovery potential. Degenerative lesions and osteochondritis dissecans are prone to adverse events and failures. In a smaller group of 38 patients, Behrens et al.[61] have had similar results for the defect size, but find no difference correlated with patients age. Ebert et al.[50] also observed that shorter and more acute injuries were correlated with better prognosis.

As mentioned above, most of the adverse events were surgical complications. To limit their venue, the development of 1-step procedure may be beneficial, even if it implies the use of allogenic chondrocytes or mesenchymal stem cells. Nasal or auricular chondrocytes might be easier to extract.

The lack of comparison between the localization was explained by the cohorts small size, and the rarity of certain defects localization, such as trochlea, patella or tibial plates. Studies with larger cohorts would be necessary. Another study with another scaffold found no difference of outcomes compared to location [56].
Nevertheless, none of the adverse events or the failures seem to have been specifically correlated with the scaffold type. Indeed, porcine type I-III collagen scaffold seemed safe and was correlated with few failures (5% of the patients).

b. Clinical results

The heterogeneity of the results makes the comparison between the studies hazardous. Nevertheless, the studies global tendency was the improvement in all clinical scores [37][42][44][50][74][65]. In the study of Aldrian et al. [43], the medium IKDC score improved from 44 +/- 26 to 59 +/- 27 at 120 months post-surgery.
On the opposite side, Bauer et al.[57] showed that the KOOS score improved from baseline until 3 years. At 3 years, a threshold was reached and the results remained stable at 6 years. At this last follow-up, a small decrease was observed without being significant. This kinetics of evolution is common [56][58][72], and some studies found that most of the scores that increased occurred before 12 months [56][72].
No study showed that defects location impacted on the clinical scoring. No difference in the scores improvement was found between women and men [61], and age does not seem to be a limiting factor. The size of the injuries was not discussed. Few results reported worst clinical outcomes when the injury was degenerative or considered as advanced with ICRS chondral injury classification [42][61][74]. Anders et al. obtained similar results in a study concerning chondral injuries of the talus [70].

c. MRI analysis

As seen below in Table 9, 9 studies contained MRI-analysis. The results were heterogeneous due to the difficulty of describing an MRI objectively. Indeed, the comparison between some characteristics, such as cartilage appearance was difficult. The easiest factor that can be examined was the degree of infill of the defect. It is also the most reported characteristic, mostly because it determines the success of the graft implantation after the surgery. The MRI-analysis showed that the graft fills the defect in a satisfactory way in more than 60% of the patients in all studies and that it integrates well the surrounding tissue. Ebert J. et al. found that the presence of pre-operative subchondral bone edema, a frequent comorbidity of chondral injuries, was not correlated with the clinical evolution of the patient [47].
MOCART analysis occurred in only one study [43].

Table 9
MRI-examination findings of MACT procedure with porcine type I-III collagen, with a focus on the degree of infill.

Reference n°	Total of patients	Localisation of the lesions	MRI results
[43]	16	11 MFC 2 LFC 4 trochlea 6 patella	(10y): Medium MOCART was 70.4. 73.9% of the patient had a complete defect filling.
[65]	15	13 MFC 4 LFC 15 patella 5 trochlea	(24M): 90% of the MACI graft completely fill in the defects had complete defect, and 88% had good integration. Subchondral bone was intact in 60% of the graft.
[42]	41	22 MFC 11 LFC	(5 y): 67% complete graft infill Integration was good to excellent in 83%. Signal intensity is described as good to

		11 patella 9 trochlea	excellent in 96% of the graft. No effusion or subchondral bone edema were described.
[47]	56	33 MFC 16 LFC 7 trochlear groove or patella	(12M): 85% had good to excellent infill
[50]	104	73 MFC 27 LFC 1 medial tibial 3 lateral tibial	(5-y): MRI composite score was 3.0 +/- 0.7 points. Patients with higher scores are described as young, with a short traumatic injury.
[57]	18	18 MFC	(12M): MRI composite was 2.37 (24M): MRI composite was 2.45 (60M): Unsatisfactory with 33% of poor radiological result (5 patients obtain poor results).
[74]	35	27 MFC 10 LFC	(24M): MRI composite score was classified as good to excellent in 100% of patients
[37]	1	lateral tibial plateau	(6 & 12M): good infill, hyaline-like signals
[44]	21	13 MFC 4 LFC 7 patella	(3M & 12M): 82% of complete infill (2y): complete bone integration in 95% of the graft sites

d. Histological examination

Zheng et al.[63] accomplished histological examination of grafts that failed at 48 hours, 21 days, 6 months, 8 months and 1 year. Histological evaluation of the biopsies demonstrated the formation of cartilage-like tissue as early as 21 days. After 6 months, 75% of hyaline-like cartilage was obtained. Behrens et al. [61] obtained four biopsies at least 12 months and later after the surgery. In three of them, the regenerated tissue in the matrix corresponded only to fibrocartilage, while the last one consisted of fibrous connective tissue.
The fact that the biopsies were taken after the graft-failure implied that their histology may not correspond to the composition of successful regenerated tissue.

ii. Hyaluronan-based scaffolds

Six of the selected studies concerned MACT procedure in the knee with hyaluronan-based scaffolds. They imply a total of 188 patients.

a. Failures and adverse events

41 cases of failures were reported in five studies [41][49][58][59][71]: they included 25 patients that experienced persistent pain, 3 graft failure and 1 surgical failure. 12 other failures were mentioned without a precise cause [71]. Two of the 5 studies reported no failure [49][59]. No adverse events were reported with the use of this type of scaffold in the present articles.
Nehrer et al. created a classification in which the occurrence of failure is compared with the preoperative risk [71], which depended of the type of injuries. Procedures were then stratified in simple, complex or salvage, depending on the mentioned above type and degree of the injury: complex cases mean defects larger than 4 cm^2 on the femoral condyles, defects on the trochlea, tibia, and patella or multifocal defects, while kissing lesions, as well as early osteoarthritic changes, were defined as salvage cases. Simple procedure had a corresponding rate of failures of 1 of 22 patients (4.4%), complex procedures 4 of 22 (18.2%), and salvage 7 of 8 (87.5%), meaning that the more complicated and advanced the injury was, the more the procedure failed and the lower alevel forre the clinical outcomes.

b. Clinical results

Clinical results were described as good for most of the patients. Clinical improvement was described as increasing up to five years in one of the study by Clar et al. [59], with a stable Tegner and Lysholm score.
The localization of the lesions had a direct impact on clinical results for Kon E. et al [58]. They did indeed describe that patients with trochlear defects had higher results in IKDC than patients with patellar or multiple defects, but that differences were not significant statistically. The same study also found that women tended to have less recovery of function compared to men, whereas other factors such as age, lesion size or BMI did not influence the result. There was no difference between accelerated weight-bearing and delayed weight-bearing after the surgery [49].

c. MRI analysis

MRI analysis were done in 3 studies, their findings are reported in Table 10. The same limitations as mentioned above apply, such as the lack of an objective score to assess the quality of the regenerated tissue or the quality of its integration into their surrounding tissues. Moreover, the small amount of studies also limits the analysis possibilities.

Table 10
MRI-findings after MACT with Hyalograft C

Reference n°	Total of patients	Localisation of the lesions	MRI results
[43]	16	11 MFC 2 LFC 4 trochlea 6 patella	(10y): Medium MOCART was 70.4, 73.9% of the patient had a complete defect filling
[49]	31	22 MFC 10 LFC	(4W): MOCART: 59.7 points of 100
[59]	1	1 MFC	MRI: good hyaline-like signals

d. Histological analysis

The study of Brun P. et al [41] concerned a cohort of 63 patients who underwent a 2nd-look arthroscopy with biopsies after a MACT procedure. This second intervention occurred between 5 and 33 months after the first surgery. Patients underwent this 2nd operation either because they still experienced symptoms (n=22), or without any medical reason (asymptomatic group, n=41). The biopsies, when grouped together, had the following outcomes: 27.2% of them were composed of hyaline cartilage with cell distribution in cluster and columns, 51.4% had random cell distribution as in fibrocartilage, and 21.4% obtained a mixed-type tissue containing both hyaline cartilage and fibrocartilage. Histochemical analysis showed that collagen type II was mainly expressed in hyaline cartilage, whereas high concentration of type I collagen may be principally expressed in fibrocartilage. Brun et al.[41] found that the percentage of hyaline regenerated tissue was significantly greater after 18 months: indeed, the biopsies taken after a longer follow-up period had 45.4% hyaline cartilage, versus 23.7% in the biopsies taken within the first 18 months. Fibrocartilage was present in 55.9% of biopsies taken within 18 months after implantation and in 27.3% of those taken after that period. Mixed tissue was present in 20.3% of biopsies taken within 18 months after implantation and in 27.3% of those taken after that period. They also discovered the repaired tissue in 22 symptomatic patients was mainly composed of fibrocartilage, while the development of fibrocartilage was rare in the asymptomatic group.

IV. Global histological analysis

4 studies included 2nd-look arthroscopies with biopsies. As mentioned before, Brun et al. [61] used hyalo-graft C. They took biopsies in patients that still experienced symptoms and who had to undergo a second arthroscopy, and in some patients (n=3) who volunteered to have a second operation and who were asymptomatic. The percentage of hyaline-tissue was higher after 18 months compared with more early results. Biopsies of symptomatic patients (n=60) corresponded mainly to fibrocartilage or mixed fibrocartilage and hyaline tissue, when asymptomatic patients had an higher percentage of hyaline cartilage.
Enea et al. [62] obtained similar results with an I-III collagen membrane. They examinated their samples with ICRS scoring system, obtaining the following outcomes: 10 biopsies (30%) were normal, 17 (51%) nearly normal, 4 (12%) abnormal and 2 (6%) severely abnormal. They did not find any correlation between clinical outcomes and histological results, but they also reported that results improved with time. The histological outcome was not significantly related either to the macroscopic appearance of the lesion or to the functional status of the patient at the time of biopsy.
Behrens et al [61] had histological results from 20 patients that needed to undergo a 2nd operation: 8 were hyaline, 5 hyaline-like, 4 fibrocartilage and 3 mixed-tissue. They showed no correlation between the symptoms and the histological results, as Enea et al. and Brun et al.
Zheng et al. [63] took biopsies of patients (n= 11) after a MACI® implantation, at different times. They reported that hyaline-like cartilage appeared as early as 6 months post-operatively. At 8 months, 75% percent of the samples contained hyaline-like repaired tissue.

Conclusion

Comparing MACT procedures with one another is a difficult task, due to the large variety of existing scaffolds and the multiple tools used to evaluate them. The included studies are very heterogeneous and sometimes undetailed, and they do not correspond to an exhaustive list of all the existing articles.

Analyzing the animal studies has met the same limitations as mentioned above: the heterogeneity of the procedures and the short follow-up times made precise comparisons impossible. Nevertheless, the procedure seems globally safe for the animal: none of them died and no handicaping restricted range of motion occurred, but the impossibility of evaluating pain of the animal could question this latter point. The composition of the scaffold which were being tested in animals varies much more. Moreover, synthetic scaffolds were more common in animal studies, probably because most of the products are still not eligible for the treatment of human beings as they are more recent. As a matter of fact, collagen is the most commonly used material not only because it is correlated with better results, but also because this component has been used since the first generation of MACT.
The other promising finding of the animal studies was the possibility of using chondrocytes that are not articular, such as auricular and nasal chondrocytes, which would be easier to extract but would still imply a 2-steps procedure.

Concerning the clinical trials, MACT procedures seem safe for clinical use. Indeed, the patients satisfaction mainly increased post-operatively, clinical scores described a recovery of function, and there was only a little proportion of adverse events and/or failures. Besides that, a few items remain unclear, such as the impact of different factors such as gender and age. Localization might be the limiting factor, as different physical forces are encountered in different portions of a joint. The small cohort sizes restricted comparison between the different localization as some of them were more rare, such as trochlea and tibial plate lesions. However, some studies describe different outcomes depending of the lesions localization. As

an example, Peterson et al. in 2012 [88] showed that patellar lesions obtained only 65% of good to excellent grade of outcomes after a autologous chondrocytes transplantation, which corresponded to the results of Kon et al. [58], who also described that patellar lesions could be correlated with lower outcomes.
All in all, the defect size does not seem to be a major limiting factor in the success of MACT procedure.

As for the indications, it is reported in several studies that degenerative and old lesions are prone to fail. By failure, it means that the patient stays symptomatic or experiences little improvement. As MACT procedures are still very expensive [31], Samuelson et al. mentioned $70,000 per procedure (including the surgical treatment and post-operative care) in 2012, the nature of the injury must be taken in consideration and the chances of success should be carefully evaluated. As the procedure is more successful with recent and traumatic lesions, it is understandable that doctors reserve the technique for young (and athletic) patients to avoid a joint replacement. Despite these indications, the age range found in this research was from 13 years old to 66 years old, a larger panel than expected. Nevertheless, if few studies showed that age had no impact on the outcome [50][58], the age must be considered from a financial point of view, and the cost of MACT procedures must be compared to the price of a total joint replacement.

Histological findings are primordial: every patient that undergoes another operation must be biopsied, and obtaining biopsy specimens of successful procedures would be very convenient. Indeed, if the procedure seems to obtain satisfying outcomes, it is still difficult to assess if it does really produce hyaline (or hyaline-like) cartilage or if it only protects the injured area from shocks and wear. As seen in the results of the animal studies, a lot of MACT procedures do not produce full hyaline-like cartilage: they produce instead a mix of hyaline and fibrocartilage, if not only fibrocartilage. As biopsy extractions in healthy patient are impossible to justify from an ethical point of view, histological results may remain unclear for a few more years, unless improvements in MRI analysis occur and make histological analysis possible.

Concerning the scaffolds, even if the scaffolds that are authorized for clinical use are less variable as the scaffolds that are being developed in the laboratories, they are still very different from one to another. Synthetic materials are more rare (Bioseed-C concern only 4 studies), but the development of these types of scaffolds began later than the collagen-based one or the hyaluronan-based ones.
This study points out the fact that there is a necessity to set up studies that will compare the scaffolds between themselves. As a matter of fact, if all the scaffolds are proven to be safe in several studies, no study tries to determine if a scaffold is better for a special situation and/or localization, probably because no research group has the finances and/or the legal authorization to test several scaffolds at the same time. It does limit the appreciation of the material and it also contributes to the scattering of the clinical data.

Comparing scaffolds with one another would also be useful for the 4th generation of MACT, new procedures that are nowadays being developed. It consists in replacing the chondrocytes by mesenchymal stem cells (MSC) that are conditioned in becoming chondrocytes, as they have impressive proliferation and differentiation capacities [9]. Those procedures imply a compatible biomaterial in which adequate cells (eg. MSC) are implanted, after specific bioactive substances enhanced an appropriate cell differentiation and specific tissue formation such as bone, tendon and cartilage [86]. A study examined MACT with MSC in sheep and compared it to the same procedure with chondrocytes: the MSC-implanted scaffolds lead to superior histological results [87]. The fourth generation also implies the emergence of one-step procedures, which will be more convenient for the patient.

If I were to conduct a clinical trial concerning MACT procedures, I would try to standardize the procedure as much as possible. The follow-up would last 5 years, as few studies already show that outcomes remain globally stable after that point. As clinical scores, I would use the VAS and the IKDC for the purpose of obtaining outcomes that could be easily understood and further use. The patients cohort must be large enough to analyze the impact of the following: the defect's size and its localization, its ICRS chondral injury classification, the elapsed time since the beginning of the symptoms and the gender of the patients. The study would compare a collagen-based scaffold with a synthetic one, or at least compare 3D collagen-based structure with collagen membrane.

To conclude, I believe that if Matrix-induced chondrocyte transplantation is a promising technique, it still needs to be further studied, which is restricted by its complexity and its cost. On one hand, MACT procedures are indeed expensive and requires the investment of the medical team and of the patient. On the other hand, it lowers long-term health cost by improving the quality of life. However, if MACT is proved to function and to provide better outcomes compared to other techniques, it is not nowadays always considered as the first-line treatment. The democratization of MACT will occur when the clinical results will be more precise and when its cost will allow its use easily. Nevertheless, the fact that more and more countries authorize this procedure proves, such as Switzerland with the Neocart scaffold, and its recent development into 1-step procedures with stem cells makes me optimist for its future.