Clinical application of scaffolds for cartilage tissue engineering

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Abstract The purpose of this paper is to review the basic science and clinical literature on scaffolds clinically available for the treatment of articular cartilage injuries. The use of tissue-engineered grafts based on scaffolds seems to be as effective as conventional ACI clinically. However, there is limited evidence that scaffold techniques result in homogeneous distribution of cells. Similarly, few studies exist on the maintenance of the chondrocyte phenotype in scaffolds. Both of which would be potential advantages over the first generation ACI. The mean clinical score in all of the clinical literature on scaffold techniques significantly improved compared with preoperative values. More than 80% of patients had an excellent or good outcome. None of the short- or mid-term clinical and histological results of these tissue-engineering techniques with scaffolds were reported to be better than conventional ACI. However, some studies suggest that these methods may reduce surgical time, morbidity, and risks of periosteal hypertrophy and post-operative adhesions. Based on the available literature, we were not able to rank the scaffolds available for clinical use. Firm recommendations on which cartilage repair procedure is to be preferred is currently not known on the basis of these studies. Randomized clinical trials and longer follow-up periods are needed for more widespread information regarding the clinical effectiveness of scaffold-based, tissue-engineered cartilage repair.

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Introduction

The repair of articular cartilage defects in the knee of young or active individuals remains a problem in orthopedic practice. These defects have limited ability to heal and may progress to osteoarthritis. They may be symptomatic and cause pain, swelling and catching. In a study of 993 consecutive arthroscopies done in patients with knee pain [2], articular cartilage changes were noted in 66% of the knees and isolated, localized cartilage lesions in about 20% of the cases. Full thickness cartilage lesions were found in 11% of the knees and 6% were larger than 2 cm^2.

Several different surgical procedures have been applied to treat cartilage injuries, but no method has been judged superior. At present, more than 5,000 Medline citations are available on surgical cartilage treatment. Microfracture technique, as a low cost and minimally invasive procedure, is currently being used as the first choice in patients with previously untreated cartilage defects. However, histological analysis of repair tissue after these operations shows mainly fibrocartilage. Advantages of mosaicplasty or osteochondral autologous grafts are that defects can be

filled immediately with mature, hyaline articular cartilage and that both chondral and osteochondral defects can be treated in the same way. However, donor site morbidity is a concern and the long-term results of the harvesting procedure are not known. Of the numerous techniques available today, no method has yet been able to consistently reproduce normal hyaline cartilage. One such method is autologous chondrocyte implantation (ACI), described by Brittberg et al. in 1994 [11]. The introduction of ACI into clinical practice has enabled the application of tissue-engineering techniques with a cell-based therapy that allows regeneration of damaged cartilage tissue. Although the short- or middle-term clinical results of ACI were reported as good [74, 87], this procedure has potential disadvantages, such as the risk of leakage of transplanted chondrocytes from the cartilage defects, an uneven distribution of chondrocytes in the transplanted site due to gravity [101], periosteal hypertrophy [42, 104], complexity of the surgical procedure [67], and not the least the reacquisition of phenotypes of dedifferentiated chondrocytes in a monolayer culture [8, 59].

In order to overcome some of these hurdles, researchers have attempted to reconstruct cartilage in the laboratory using tissue engineering, a technique by which a living tissue can be reconstructed by associating the cells with biomaterials that provide a scaffold on which they can proliferate three-dimensionally, under physiological conditions. The scaffold technique may have the technical and theoretical advantages such as less invasive technique because of no need to harvest periosteum, as well as homogeneous distribution of chondrocytes and the maintenance of the phenotype. Carriers have been marketed and various tissue-engineering techniques, widely used nowadays, have been developed using chondrocytes seeded on biological matrices such as collagen membranes [22] or hyaluronic acid [67]. Despite the diffusion of these methods, the ideal matrix material has not been identified, and there are still some areas that would need better clarification. It has not verified that the technical and theoretical advantages of scaffold techniques have led to the better clinical and histological results compared with conventional ACI. Therefore, a review of current developments in scaffolds for cartilage tissue engineering in clinical use and future perspectives are necessary.

The aim of this review is to assess the literature of clinically available scaffolds used for the treatment of articular cartilage injuries in order to know the current status of scaffolds for cartilage tissue engineering. Although tissue engineering consists of three major parts; transplanted cells, scaffold for cell proliferation, and growth factors, we focus on the literature on scaffolds in clinical use. In addition, this article details the evidence

from studies on the clinical outcome of tissue-engineered cartilage repair. The main questions to be answered are: do advanced cartilage tissue-engineering grafts ensure even distribution of a high number of vital chondrocytes, mediate initial biomechanical stability, promote chondrocyte differentiation and the formation of cartilage matrix, thereby yielding better clinical outcomes with decreased complications, compared with conventional ACI?

Current cartilage repair strategies and tissue engineering

Historical unsatisfactory results of cartilage injury reflect the poor healing capacity of cartilage arising from its isolation from systemic regulation, and its lack of vessels and nerve supply [66]. None of the normal inflammatory and reparative processes is available for its repair. Furthermore, chondrocytes which are surrounded by an extracellular matrix cannot migrate to the site of injury from an intact healthy site, unlike most tissues [16]. Injuries which reach the subchondral bone may induce a systemic reaction and generate reparative tissue. The new tissue consists predominantly of type I collagen, resulting in the formation of fibrocartilage. Type I collagen does not have the biomechanical properties of articular cartilage. Accordingly, it cannot function as normal hyaline cartilage and eventually degenerates [32].

ACI was first described in 1994 [11]. The procedure involves the patient having to undergo harvesting through an arthroscopic procedure, followed 2–8 weeks later an arthrotomy, where the cells are injected under a cover of periosteum. The original ACI technique involved the injection of a suspension of cultured chondrocytes into a debrided chondral defect beneath a periosteal cover. Clinical results from femoral defects have ranged from 60 to 90% excellent and good between 1 to 11 years after surgery [7, 11, 14, 26, 37, 44, 46, 47, 51, 61, 62, 73, 74, 76, 77, 87–89].

As mentioned, the ACI procedure has several disadvantages. Especially, hypertrophy of tissue seemed to be the major cause for re-operations after ACI [37, 42, 78, 104]. Using collagen membranes instead of periosteum could possibly reduce the risk for re-operations. Several studies showed a lower incidence of graft hypertrophy after ACI with a type I/III collagen membrane [7, 10, 37, 42, 63, 104]. As further technological advances, researchers have initiated the use of carrier i.e., a scaffold or matrix upon which the cells are grown. The use of three-dimensional scaffolds has enabled maintenance of a chondrocyte differentiated phenotype. A further advantage of this method of cell delivery is that the scaffold may act as a barrier

to the invasion of the graft by fibroblasts, which may otherwise induce fibrous repair [29]. In addition, most of the articular cartilage engineering techniques with scaffolds can be done without a periosteal or membrane coverage and in many cases using arthroscopic techniques. Consequently, the procedures may be performed faster and with a less extensive exposure.

Scaffolds available for clinical application

The scaffold must be biocompatible, structurally and mechanically stable, must support the loading of an appropriate cell source to allow successful infiltration and attachment with appropriate bioactive molecules in order to promote cellular differentiation and maturation, and must be biodegradable, giving temporary support. Several natural biomaterials, such as collagen [20, 98, 116], hyaluronan [39], fibrin glue [48, 50], alginate [3, 41, 43, 72, 96, 106], agarose [8, 92, 105], or chitosan [99], as well as synthetic biomaterials such as polylactic acid [23] have been developed for the restoration of damaged cartilage. However, the clinical use of these materials is currently limited, mainly due to the risk of disease transmission and immunoreaction.

The inclusion criteria for studies on scaffolds available for clinical application were use of in vitro or in vivo or clinical studies on scaffolds with clinical trial. Several studies on scaffolds such as alginate, agarose and chitosan were excluded because of no clinical experience. Ultimately studies on collagen, hyaluronan-based polymer, fibrin, and synthetic copolymer scaffold were included in this review based on the inclusion criteria. Collagen and hyaluronan-based matrices are among the most popular natural scaffolds in clinical use nowadays, as they offer a substrate that would normally be found in the structure of native articular cartilage. Our literature searches are based on MEDLINE In-process and other Non-Indexed Citations, EMBASE, and CINAHL using Ovid searches of articles published between 1 January 1966, and 1 March 2008. Therefore, we acknowledge that the most important limitation of this study is the limitation of the already existing studies.

A bilayer collagen type I–III membrane

Collagen is one of matrix proteins occurring as a major component of connective tissues, giving it strength and stability. Collagen scaffolds have been used extensively for cartilage regeneration for decades [19, 20, 38, 98, 116, 117]. Collagen can be fabricated as a membrane, gel, sponge or foam and is subject to enzymatic degradation.

Matrix-induced ACI (MACI®; Verigen, Leverkusen, Germany) can be considered as an evolution of conventional ACI. The MACI technique is a tissue-engineering technique in which the principle of autologous cell culture is preserved: a bilayer collagen I–III scaffold is used to improve the structural and biological properties of the graft. MACI uses processed cells that are harvested and isolated from the patient and expanded in vitro. Once grown, the chondrocytes are seeded between layers of a bilaminate collagen in the operating room prior to implantation. The MACI membrane can be secured directly to the base of a prepared chondral defect by fibrin glue and without a cover. The surgical procedure is quite simple, requiring short operating time. The MACI technique was introduced in 1999 (Fig. 1) [22].

Other MACI membranes such as Maix® (Matricel, Hezoenrath, Germany) or Chondro-Gide® (Geistlich Biomaterials,Wolhusen, Switzerland) consist of a porcine type I/type III collagen bilayer seeded with chondrocytes. These membranes are degraded by enzymatic digestion and have two different surfaces: the external has good mechanical strength and serves as a barrier, whereas the inner surface is porous and stimulates the cells to produce cartilage-specific matrix molecules [33]. This membrane has been used extensively in dental and maxillofacial surgery since 1980 [24]. A number of in vitro and in vivo studies have demonstrated that this multilayer three-dimensional scaffold is an excellent medium for cell attachment, maintaining the phenotype of chondrocytes

Fig. 1 The matrix-induced autologous chondrocyte implantation procedure. Reprinted by permission from Cherubino et al. [22] "Autologous chondrocyte implantation using a bilayer collagen membrane: a preliminary report. J Orthop Surg (Hong Kong) 11:10–15"

overtime as well as delivering a dense superficial layer to keep the cells in the defect and limit the migration of inflammatory proteins into the repair site. [27, 29, 33, 34, 55, 79–81, 118]. Furthermore, in a recent sheep study, a multidefect cartilage repair model demonstrated that collagen matrix seeded with autologous chondrocytes produced better quantitative and qualitative results compared with microfracture [25].

Gigante et al. [35] analyzed the distribution, viability and phenotype expression of human chondrocytes seeded on a collagen membrane at the time of the implantation. The residual part of each membrane from 12 patients who underwent MACI was tested by colorimetric assay and histochemical and ultrastructural analyses were carried out. In all of the samples a large number of viable cells, homogenously distributed, were detected. The cells expressed the markers of the differentiated hyaline-producing chondrocytes [35].

Collagen type I gel

Ochi et al. [83] reported seeding chondrocytes in atelocollagen® (Koken Co Ltd, Tokyo, Japan), a type I collagen gel, and culturing the construct in a regular culture dish for 4 weeks for clinical use. They choose atelocollagen, from which telopeptides have been removed, because the antigenic determinants on the peptide chains of type I collagen reside mainly in the telopeptide regions [31, 90]. Atelocollagen has been used clinically in plastic surgery and dermatology [52]. Their in vitro and in vivo experimental [53, 57, 114] results support the hypothesis that transplanting chondrocytes cultured in atelocollagen gel are effective in repairing articular cartilage defects, not only in animals but also in humans, by maintaining the chondrocyte phenotype, reducing the risk of leakage, and distributing grafted cells evenly throughout the grafted site. The disadvantage is that the cell/gel construct needs to be implanted under a periosteal cover to prevent it from detaching.

Hyaluronan-based polymer

Hyaluronan (hyaluronic acid) is a major component of cartilage matrix. The construct, 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 [21, 68]. Hyaluronan containing scaffolds such as Hyaff-11® (Fidia Advanced Biopolymers Laboratories, Abano Terme, Italy), an esterified derivative of hyaluronan,

has a high degree of biocompatibility and can be used to culture chondrocytes in three-dimensional culture conditions that may effectively mimic an in vivo situation. It fully resorbs in 3 months with controllable degradation rates, with its main byproduct being hyaluronan, a sugar. Hyalograft® C (Fidia Advanced Biopolymers Laboratories, Abano Terme, Italy) is a tissue-engineered graft consisting of autologous chondrocytes grown on a Hyaff-11 scaffold and a network of 20-lm-thick fibers with interstices of variable sizes. Articular cartilage is harvested from the patient and grown in a two-dimensional culture in vitro. Then the chondrocytes are absorbed onto a non-woven pad made of the benzyl ester of hyaluronic acid. The cells are seeded for 2 weeks on this hyaluronic acid scaffold at a density of 1 million cells per cm^2, resulting in a total of 4 million seeded cells per cm^2 per graft. After 2 weeks, this construct is implanted into the knee. In the majority of cases, no graft fixation is required. However, depending on the size and location of the defect, fibrin glue and/or sutures may be used to keep the graft in place.

In vitro studies have shown that chondrocytes grown in a three-dimensional matrix based on Hyaff-11 return to their phenotype in a time-dependent manner, thus restoring their capacity for secreting proteins and molecules characteristic of a hyaline cartilage [1, 15, 40]. In vivo studies in animal models have also shown that hyaluronan-based scaffolds seeded with autologous chondrocytes are useful for inducing formation of native, hyaline-like cartilage tissue with integration with the adjacent articular surface [39, 102, 103]. Extensive biocompatibility studies have demonstrated the safety of biomaterials containing Hyaff-11 and their ability to be resorbed in the absence of an inflammatory response [18]. Furthermore, 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 development and remodeling of tissue-engineered cartilage [110]. Hyalograft C was introduced into clinical use in 1999 [85]. Marcacci et al. [67] have reported the use of an arthroscopic surgical technique (Fig. 2).

Fibrin

Fibrin is a protein involved in the clotting of blood. It is formed by polymerization of fibrinogen in the presence of thrombin usually at the wound site. Fibrin has been an attractive biomaterial because it is biocompatible and biodegradable. Injectable fibrin-based gels or glues have been investigated for cartilage repair [48, 50, 60]. However, in vivo animal studies have shown that cell migration

Fig. 2 Arthroscopic autologous chondrocyte implantation using Hyalograft C. Reprinted by permission from Marcacci et al. [67] "Arthroscopic autologous chondrocyte transplantation: Technical note. Knee Surg Sports Traumatol. Arthrosc 10:154–159"

and tissue repair using such material appears to be limited [12, 107]. The mechanical stability of fibrin glue is questioned as well [107]; in addition, exogenous fibrin may trigger an immune response [58]. The action of fibrin glue on transplanted chondrocytes remains controversial. Therefore, so far fibrin glue has mainly been used clinically to secure other tissue-engineered cartilage [22, 85], or perichondrial scaffold grafts [9] etc. Visna et al. [115] has reported 1-year clinical results of tissue-engineered cartilage repair using fibrin glue (Tissucol®, BAXTER, Austria) compared to abrasion technique.

Synthetic copolymer

The bioresorbable polymer scaffold in clinical use is the copolymer of polyglycolic (PGA) / polylactic (PLA) acid (polyglactin, vicryl) and polydioxanone, which is used for tissue-engineered cartilage repair as Bio-Seed®-C (Biotissue Technologies, Freiburg, Germany). The cartilage tissue-engineering graft Bio-Seed-C combines autologous chondrocytes with a fibrin gel matrix in a porous three-dimensional textile polymer scaffold. The organotypic cell culture method with the cell-polymer tissues have been shown to allow the in vitro production of bioartificial cartilage for transplantation [17, 100]. Synthetic poly-a-hydroxy ester substrates in the form of PGA and PLA-based scaffolds have also shown to enhance the promotion of proteoglycans, chondrocyte proliferation, differentiation and maturation in comparison to collagen-based scaffolds [38]. Gel-like matrices such as fibrin allow even distribution of a large number of vital chondrocytes within the graft and promote chondrocyte differentiation as well as the formation of a cartilaginous repair tissue, while the polymer scaffold mediates initial biomechanical stability and allows easy handling of the graft by the surgeon [56]. Animal studies on rabbits and horses have shown formation of a cartilaginous tissue and good integration into the

surrounding host tissue with firm bonding of the graft to the adjacent cartilage and the underlying subchondral bone [4, 86]. Such bioresorbable scaffold material has good immunogenic compatibility, and is frequently used clinically as suture material (polyglactin, vicryl). Various in vitro and animal studies have shown that the scaffold supports cartilaginous tissue development with no signs of necrosis, apoptosis, or abnormal tissue reaction [4, 56, 84, 97]. Erggelet et al. [28] has reported a technical note of using the polymer fleece 2-mm thick, loaded with 5×10^6 chondrocytes in a fibrin gel by arthroscopic technique in 2003. Further, Ossendorf et al. [84] has already shown 2-year clinical results on the use of the two component gel-polymer scaffold.

Clinical outcomes of articular cartilage repair with scaffolds

The inclusion criteria for clinical studies on scaffold techniques were prospective or retrospective studies with a clinical outcome. Fourteen studies were included in this review based on the inclusion criteria (Tables 1, 2). Of these, eight studies have been published including hyaluronan-based scaffold (Hyalograft C) [36, 65, 68, 69, 71, 82, 85, 112]. Three studies have assessed the outcome of MACI with a bilayer collagen type I–III membrane [5, 6, 22]. The remaining three studies have assessed the outcome with a collagen type I gel, fibrin glue, and synthetic copolymer [83, 84, 115]. Only very limited number of prospective, randomized or non-randomized comparative studies have been published [5, 65, 115].The majority of the published studies had no control group, represented case series or retrospective level IV studies. As mentioned, our literature searches are based on the articles published between 1 January 1966, and 1 March 2008. Accordingly, the most important limitation of this study is the limitation of the already existing studies.

Clinical and functional scores

Encouraging clinical results have been published in terms of various subjective and objective scores in all 14 available studies including arthroscopic technique [69] and patello-femoral joint injury [36] (Table 1). According to a prospective, randomized study by Visna et al. [115], the use of tissue-engineered cartilage based on fibrin glue was superior to abrasion technique as measured by the International Knee Documentation Committee (IKDC) subjective score, Lysholm score, and Tengner activity score 1 year after surgery. However, Manfredini et al. [65] showed no significant differences in International

Table 1 Clinical outcomes

Authors	Study design	Scaffold, Technique etc	N	Follow-up	IKDC score	Lysholm score	Cincinnati knee score	ICRS Functional score, evaluation form	VAS etc	Other clinical outcomes
Manfredini et al. [65]	Prospective, non-randomised	Hyaluronan (Hyalograft C) vs. ACI (Carticel)	10 vs. 17	1 year	–	–	–	77 vs. 75 NS preoperative; 53 vs. 54 NS Post-operative values in both groups significantly improved compared with preoperative values ($p = 0.005$, 0.001)	–	HSS score; Excellent or Good: 90 vs. 88% NS
Marcacci et al. [69]	Prospective, uncontrolled	Hyaluronan (Hyalograft C), Arthroscopic technique	70	Minimum of 2 years	Objective; Normal or Nearly normal: 89%. Post-operative values significantly improved compared with preoperative values ($p < 0.0005$). Subjective; Post-operative values significantly improved compared with preoperative values ($p < 0.0005$)	–	–	–	EQ-VAS; Post-operative values significantly improved compared with preoperative values ($p < 0.0005$)	–
Ossendorf et al. [84]	Prospective, uncontrolled	Polymer (Bio-Seed-C)	40	2 years	IKDC SF-36 current health assessment; Post-operative values significantly improved compared with preoperative values ($p < 0.05$)	Mean score increased from 46 to 81 in patients with posttraumatic and/or mild degenerative defects and from 47 to 79 in patients with osteoarthritic degeneration, ($p < 0.007$)	Statistically significant improvements were observed ($p < 0.05$)	–	–	KOOS; The patient's status had improved significantly ($p < 0.05$)
Trattnig et al. [112, 113]	Retrospective, cross-sectional	Hyaluronan (Hyalograft C)	15	3–42 months	–	–	–	–	–	Brittberg score; Excellent or Good: 80%

Table 1 continued

Authors	Study design	Scaffold, Technique etc	N	Follow-up	IKDC score	Lysholm score	Cincinnati knee score	ICRS Functional score, evaluation form	VAS etc	Other clinical outcomes
Behrens et al. [6]	Prospective, uncontroled	Porcine collagen I/III matrix (Chondro-Gide)	11	5 years	–	Post-operative values significantly improved compared with preoperative values ($p = 0.04$)	–	Post-operative values significantly improved compared with preoperative values ($p = 0.03$)	–	Tegner activity score; There was no significant improvement. Meyer score; Post-operative values significantly improved compared with preoperative values ($p = 0.007$)
Gobbi et al. [36]	Case series	Hyaluronan (Hyalograft C) for damaged articular surface of the patellofemoral joint	32	2 years	Subjective; 74 preoperative: 43, Objective; Normal or Nearly normal: 91% Post-operative values significantly improved compared with preoperative values ($p < 0.0001$)	–	–	–	EuroQol-EQ-5D questionnaire; 53% and 90% of patients had no pain and morbidity problems	–
Marlovits et al. [71]	Retrospective, cross-sectional	Hyaluronan (Hyalograft C)	9	2 years					2.62 ± 0.65	KOOS; pain 68 ± 24; symptoms 62 ± 15; ADL 75 ± 22; sport 53 ± 29; QOL 70 ± 22
Nehrer et al. [82]	Prospective, case series	Hyaluronan (Hyalograft C)	36	3 years	Excellent or Good: 87%, average: 60 ± 25 preoperative: 39 ± 19, Post-operative values significantly improved compared with preoperative values ($p < 0.02$)	81 ± 19 preoperative: 58 ± 14, Post-operative values significantly improved compared with preoperative values ($p < 0.05$)	7 ± 3 preoperative: 3 ± 2	–	–	–

Table 1 continued

Authors	Study design	Scaffold, Technique etc	N	Follow-up	IKDC score	Lysholm score	Cincinnati knee score	ICRS Functional score, evaluation form	VAS etc	Other clinical outcomes
Bartlett et al. [5]	Prospective, randomised	Porcine-derived collagen I/III matrix (MACI) vs. ACI with a cover manufactured from Porcine-derived collagen I/III(Matricel)	47 vs. 44	1 year	–	–	64 vs. 59 NS (modified) preoperative: 45 vs. 41 NS, Post-operative values in both groups significantly improved compared with preoperative values ($p = 0.002$, 0.01)	–	4 vs. 4 NS preoperative: 6 vs. 6 NS. Post-operative values in both groups significantly improved compared with preoperative values ($p = 0.003$, 0.001)	Stanmore functional rating; 2 vs. 2 NS preoperative: 3 vs. 3NS Post-operative values in both groups significantly improved compared with preoperative values ($p = 0.02$, 0.02)
Marcacci et al. [68]	Retrospective, case series	Hyaluronan (Hyalograft C)	141	Average: 38 months (2–5 years)	Subjective; 92% of patients improved. average: 79 ± 20 preoperative: 40 ± 14, Post-operative values significantly improved compared with preoperative values ($p < 0.0001$). Objective; Normal or Nearly normal: 96%	–	–	Functional status: 71% of the patients could do everything or nearly everything (Levels I and II)	EuroQol-EQ-5D questionnaire; 76% and 88% of patients had no pain and morbidity problems. Post-operative values (0.7) significantly improved compared with preoperative values (0.9) ($p < 0.0001$)	–
Visna et al. [115]	Prospective, randomised, controlled	Fibrin glue (Tissucol) vs. ablasive technique	25 vs. 25	1 year	Subjective; 76 ± 13 vs. 68 ± 10 Significantly better in ACI in fibrin glue ($p < 0.05$) preoperative:41 ± 12 vs. 45 ± 11	86 ± 9 vs. 74 ± 11 Significantly better in ACI in fibrin glue ($p < 0.001$) preoperative: 48 ± 11 vs. 53 ± 11	–	–	–	Tegner activity score; 5.9 ± 0.8 vs. 4.2 ± 1.1 Significantly better in ACI in fibrin glue($p < 0.01$) preoperative: 3.2 ± 0.8 vs. 2.3 ± 1.1

Table 1 continued

Authors	Study design	Scaffold, Technique etc	N	Follow-up	IKDC score	Lysholm score	Cincinnati knee score	ICRS Functional score, evaluation form	VAS etc	Other clinical outcomes
Cherubino et al. [22]	Prospective, uncontroled	Porcine collagen I/III matrix (MACI)	6	Minimum of 6 months	–	94 (range, 87–97) preoperative:46.5 (range, 18–67)	Clinical evaluation; 8.5(range, 6–10) preoperative: 4.7 (range, 2–6) Patients' evaluation; 8 (range, 6–10) preoperative: 2.6 (range, 2–4)	Evaluation form; 4 normal knees and 2 nearly normal knees	–	Tegner activity score; 6.5 (range, 5–7) preoperative: 2.6 (range, 1–4)
Pavesio et al. [85]	Retrospective, case series	Hyaluronan (Hyalograft C)	67		Mean:17.5 months	Subjective; 78 ± 18 preoperative: 37 ± 9, Post-operative values significantly improved compared with preoperative values (p < 0.01). Objective; Normal or Nearly normal: 87%	–	–	–	EQ-VAS; 88 ± 14 preoperative: 59 ± 17, Post-operative values significantly improved compared with preoperative values (p < 0.05)
Ochi et al. [83]	Prospective, case series	Collagen I gel (Atelocollagen) with periosteal cover	28	2 years	–	97 ± 5 preoperative: 71 ± 12, Post-operative values significantly improved compared with preoperative values (p < 0.001)	–	–	–	–

IKDC The International Knee Documentation Committee, ICRS The International Cartilage Repair Society, VAS visual analogue score, HSS hospital for special surgery, KOOS The Knee Injury and Osteoarthritis Outcome Score, NS not statistically significant difference, EQ-VAS EuroQol visual analogue scale, EQ-5D EuroQol 5D questionnaire

Cartilage Repair Society score (ICRS) score and the Hospital for Special Surgery (HSS) score between patients with Hyalograft C and those undergoing ACI procedure. According to Bartlett et al. [5], no differences were found in the clinical outcomes such as the Cincinnati Knee Rating System, Stanmore functional rating system, and visual analogue score between standard ACI procedure using a collagen membrane as cover and MACI. The follow-up periods in these comparative studies were short (1 year).

In a prospective study, 5 years after transplantation of cell seeded collagen grafts, 8 of 11 patients rated the knee function better than pre-operatively, and the clinical evaluation showed significant improvement in the Meyers score, the Lysholm score and ICRS score [6]. In a multicenter retrospective cohort study using Hyalograft C with a follow-up from 2 to 5 years, 91.5% of 141 patients improved according to the IKDC subjective evaluation, with patients who had traumatic injuries and osteochondritis dissecans (OCD) reported better improvement than those who had degenerative lesions [68].

The clinical outcome with MACI membrane by Bartlett et al. [5] was better in the group of patients aged under 35 years, compared with those aged over 35 years. Patients who had been treated for lesions larger than 5 cm^2 in size had poorer clinical outcomes than those with smaller lesions, although the differences were not statistically significant. Nehrer et al. [82] have also showed that patients with Hyalograft C below 30 years of age with single lesions showed significantly better improvements compared with those over 30 years with multiple defects. They suggest that implantation of Hyalograft C in older patients or those with multiple defects should only be carried out in selected patients with high compliance and lesser expectations with regard to physical activity. Regardless of the type of scaffolds, the use of tissue-engineered grafts based on these scaffolds appears to be as effective as conventional ACI, although none of these methods so far have been shown to be better.

Complications, graft failures

There are only few serious adverse events reported in the literature (Table 2). The rate of superficial infection of patients ranges from 0 to 2%, which is equivalent to that of patients using the convensional ACI technique [7, 11, 14, 26, 30, 37, 45, 47, 51, 61, 62, 64, 73–77, 87–89, 119]. There are no patients of septic arthritis, which ranged from 0–3% of patients using the conventional ACI technique [7, 11, 14, 26, 30, 37, 45, 47, 51, 61, 62, 64, 73–77, 87–89, 119]. Apart from two studies reporting the use of periosteal flaps [83, 85], the frequency of

complications such as arthrofibrosis or graft failure appear to be lower (0–18%) than those reported to occur in 3–36% of patients using the conventional ACI technique [7, 11, 14, 26, 30, 37, 45, 47, 51, 61, 62, 64, 73–77, 87–89, 119]. The frequency of reoperations related to the implantation is also lower (0–16%) than those reported to occur in 2–36% of patients using the conventional ACI technique [7, 11, 14, 26, 30, 37, 45, 47, 51, 61, 62, 64, 73–77, 87–89, 119]. Complications known to be associated with ACI include adhesions, arthrofibrosis, periosteal hypertrophy, and graft failure. Using scaffold techniques, periosteal hypertrophy does not occur due to the fact that in the majority of procedures, periosteum is not used. Periosteal hypertrophy is a common complication of ACI [11, 37, 42, 45, 61, 73, 74, 76, 78, 87, 119]. According to the only study, which compares the results of MACI (Hyalograft C) and conventional ACI (Carticel®; Genzyme, Cambridge, Massachusetts, USA) by Manfredini et al. [65], however, neither the patients with Hyalograft C nor those with conventional ACI reported any serious complications. This literature review clearly shows the need for prospective, randomized studies with sufficient number of patients. At this stage of development it appears that new techniques with scaffolds seem to reduce complications or graft failures caused by a periosteal flap.

Arthroscopic and histological findings

Nine of the fourteen articles have reported second-look arthroscopic and histological findings (Table 2) [5, 6, 36, 68, 69, 83–85, 115]. These arthroscopic assessments indicated that 66–100% of available patients had a normal or nearly normal outcome in the ICRS visual score or Brittberg scale. Histological analysis have shown that the frequency of hyaline-like repair with good integration into the surrounding tissue was ranged 0–100% (2–22 biopsies, 3–30 months after operation). The wide difference of hyaline-like repair ratio between these published studies may be due to the differences in the number of biopsies and the time of biopsies. It is well known that the validity and significance of a biopsy is limited by the number of biopsies taken and the time after surgery, and that a biopsy result presents rather a trend [6]. ACI using periosteum has consistently reported more than 34% of the biopsy specimens had at least some hyaline cartilage present, although few were composed totally of hyaline cartilage [10, 11, 37, 44–47, 51, 61, 87, 88, 95, 109]. There is not sufficient evidence to state that scaffold methods result in homogeneous distribution of cells in the scaffold, maintenance of the chondrocyte phenotype and more hyaline cartilage, all three potential advantages over ACI. Despite many positive clinical results published, the

Table 2 Arthroscopic finding etc

Authors	Arthroscopic findings, ICRS visual scoring system	Histological findings	Magnetic resonance imaging	Complications	Other findings
Manfredini et al. [65]	–	–	Most patients in both groups showed the formation of tissue characteristics similar to the surrounding healthy cartilage. Four patients in ACI group showed hypertrophic growth of the repair tissue	There were no major adverse events observed	–
Marcacci et al. [69]	A complete coverage with a hyaline cartilage-like tissue with integration with the surrounding cartilage in 12 of 15 patients	Specimens obtained from 2 patients showed hyaline-like cartilage in one patient and fibrocartilage in the other with close integration into the subchondral bone. In one patient, a tidemark was observed		There were no major adverse events observed	Results were better in young patients who practiced sport at a highly competitive level
Ossendorf et al. [84]	The implanted grafts completely filled the defects and formed a tough hyaline-like cartilage	One repair tissue appeared as a mixed tissue of hyaline-like and fibrous cartilage, whereas 3 biopsies documented the development toward a hyaline repair tissue	Analysis at 6 months and 12 months after implantation showed good defect filling	Of the 79 patients, 5 underwent reoperation comprising synovectomy, debridement, total knee arthroplasty, and removal of graft	–
Trattnig et al. [112, 113]	–	–	Repair tissue T2 line profiles normalized over time toward the control sites	–	–
Behrens et al. [6]	Four of six patients showed tissues that correspond to the genuine cartilage with good integration into the adjacent areas	Specimens obtained from 4 patients showed fibrocartilagenous tissue in 3, and fibrous connective tissue in one		There were detachment of the transplat in one knee (9%) and a softer transplant in another (9%)	–
Gobbi et al. [36]	ICRS scale: Nearly normal: 6 of 6 patients (100%)	Specimens obtained from 6 patients showed hyaline-like cartilage in 4 patients and mixed in the other 2 patients	Improvements with 71.9% of patients having > 50% or complete fill, 75% having normal or nearly normal signal, 90.6% having mild or no effusion, 84.4% having mild or absent subchondral edema. None of the defects treated resulted in graft hypertrophy or delamination	Fibrosis in 1 patient (3%) was documented	–
Marlovits et al. [71]	–	–	A complete filling in 61.5%; a complete integration in 76.9%; an intact subchondral lamina in 84.6%; intact subchondral bone in 61.5%; isointense signal intensities in 92.3%	A graft failure was found in one patient (11%) with complete dissemination of the transplant	The clinical scores were correlated with the MRI variables

Table 2 continued

Authors	Arthroscopic findings, ICRS visual scoring system	Histological findings	Magnetic resonance imaging	Complications	Other findings
Nehrer et al. [82]	–	–	–	There were no major adverse events observed	Patients under 30 years of age with single lesions showed significant improvements compared to those over 30 years with multiple defects ($p < 0.01$)
Bartlett et al. [5]	ICRS score: Excellent or Good: 66.6 vs. 79.2% NS	Hyaline-like or Hyaline-like with fibrocartilage: 36.4 vs. 43.9% NS	–	The rate of hypertrophy: 3 (6%) vs. 4 (9%)The frequency of re-operation: 9 vs. 9% Wound infection: 1 (2%)	Patients aged less than 35 years had a significantly better clinical outcome compared with those aged more than 35 years ($p = 0.03$)
Marcacci et al. [68]	ICRS scale: Normal or Nearly normal: 96.4%	Twelve of 22 patients showed hyaline-like tissue, whereas 6 were classified as mixed tissue, 4 showed a fibrocartilage appearance	–	Nine patients (4.7%) reported adverse events or complications such as arthrosynovitis. There were 10 (5.2%) graft failures	–
Visna et al. [115]	Average ICRS score in 4 patients after ACI in fibrin glue: 8.5 (6–11)	Specimens obtained from 4 patients after ACI in fibrin glue showed hyaline-like cartilage	–	Five patients had reactive synovitis and 4 had shaving of adhesion	–
Cherubino et al. [22]	–	–	MRI taken after 6 and 12 months showed the presence of hyaline-like cartilage, with restoration of the articular surface, in all the knees	No complications were observed	–
Pavesio et al. [85]	Brittberg scale:Normal or Nearly normal: 96.7%	Fourteen of 22 patients showed hyaline-like tissue, whereas 4 were classified as mixed tissue, 4 showed a fibrocartilage appearance	–	Four patients reported fibroarthrosis or periosteal hypertrophy	Longer time points from implantation may be correlated to better histological appearance. The larger the size, the greater the improvement
Ochi et al. [83]	ICRS scale: Normal or Nearly normal: 93%	Specimens obtained from 2 patients showed hyaline-like cartilage with steady integration into the subchondral bone	–	There were marked hypertrophy of the graft in 3 knees, partial detachment of the periosteum in 4 and partial ossification of the graft in one	–

ICRS The International Cartilage Repair Society, *NS* not statistically significant difference

studies have not been able to demonstrate a complete healing with normal hyaline articular cartilage.

Follow-up biopsies from conventional ACI grafts showed the four cartilage layers composed of fibrous periosteal remnant cover, transitional repair tissue, deep hyaline-like repair tissue, and calcified layer [74, 94]. On the other hand, histological evaluation of the samples of fibrin scaffold by Visna et al. [115] demonstrated only two layers of hyaline-like cartilage and calcified layer. Differing histological findings between the conventional ACI and the scaffold technique can be explained by the different surgical technique, where the first two layers in ACI technique developed as a consequence of periostal use for chondrocyte fixation [115].

Hollander et al. [49] reported a detailed study of the maturation of Hyalograft C once implanted into humans. They has shown that hyaline cartilage regeneration can be observed less than one year after implantation of Hyalograft C and that there is progressive maturation of the implants, even in joints showing signs of osteoarthritis. This outcome was observed in 10 of 23 patients, whereas in a further 10 patients, the repair tissue was fibro-cartilage, and in the remaining 3, there was a mixed type of cartilage [49].

From a cohort of 56 MACI patients, Zheng et al. [120] examined the phenotype of chondrocytes seeded on type I/III collagen scaffold, and conducted progressive histological assessment over a period of 6 months. Their data showed that chondrocytes on the collagen scaffold appeared spherical, well integrated into the matrix, and maintained the chondrocyte phenotype as evidenced by aggrecan, type II collagen, and S-100 expression. Progressive histological evaluation of the biopsies showed the formation of cartilage-like tissue as early as 21 days, and 75% hyaline-like cartilage regeneration after 6 months. While fibrin sealant appeared to act as an adhesive substance for the seeded ACI-Maix collagen scaffold, it also facilitated the maturation of the implanted chondrocytes into functional cartilage [120].

According to Pavesio et al. [85], comparison of the clinical outcomes with arthroscopic assessment and histological findings showed that hyaline-like regenerated tissue correlated with the most favorable clinical results. However, Behrens et al. [6] demonstrated that some patients can have a good clinical outcome despite generating fibro-cartilage at the repair site. The correlation between clinical outcome and histologic grading remains controversial.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is useful for the evaluation of the morphologic status of the repair tissue [91, 93]. This method can be considered as a noninvasive alternative to second-look arthroscopy. Current MRI techniques such as high-resolution, T2 mapping, and T1 mapping using delayed gadolinium-enhanced MRI (dGEMRIC) have significantly improved during recent years [111–113]. Six of the fourteen studies have published MRI findings (Table 2) [22, 36, 65, 71, 84, 112]. The MRI analysis at 6, 12, 24 months after implantation has shown good defect filling with good integration into the surrounding tissue in more than 60% of patients [22, 36, 65, 71, 84]. In addition, repair tissue has shown a gradual maturation over time [36, 111, 112]. Similar findings have been reported in studies with conventional ACI [13, 44, 46, 89, 95, 108, 109]. Marlovits et al. [70] found using high-resolution MRI that the implantation and fixation of chondrocytes seeded on type I/III collagen scaffold with fibrin glue and with no further surgical fixation leads to a high attachment rate (88%) 35 days after the implantation. According to the study that compared the results of Hyalograft C with those of Carticel by Manfredini et al. [65], MRI analysis did not reveal any significant differences, although the images of patients with Carticel showed a greater tendency towards hypertrophic growth of the repair tissue probably due to periosteal hypertrophy. Some authors [65, 71] have reported a significant correlation between the clinical outcome and the MRI analysis scores.

Conclusion and future considerations on scaffolds for cartilage repair

The tissue-engineering methods with scaffolds including the arthroscopic technique are less invasive because there is no need to harvest periosteum. These methods reduce surgical time, morbidity, and risks of periosteal hypertrophy and postsurgical adhesions substantially. The technical and theoretical advantages of scaffold techniques have led to the technique being favored by surgeons performing chondrocyte implantation. However, in this review of the short- or mid-term, clinical and histological results published by various studies, none of these methods were judged to be better than conventional ACI. In addition, there was no obvious ranking among the scaffolds available for clinical use at the present time. There is probably not an even distribution of chondrocytes. These methods seem to promote chondrocyte differentiation and formation of cartilage matrix, but so far, they have not resulted in improved clinical results.

The ultimate aim of cartilage treatment is the restoration of normal knee function by regeneration of hyaline cartilage, and to achieve a complete integration of the new cartilage to the surrounding cartilage and underlying

bone. Promising development is underway with regards to cell-based techniques in combination with scaffolds, growth factors and possibly gene therapy. Unfortunately, this effort has not been followed by appropriate or sufficient clinical studies to assess these new methods or compare them with available procedures.

The methodological level of the clinical papers is in general low. Jacobsen et al. [54] showed very low methodological quality of most studies on cartilage repair. So far, only three prospective, randomized or non-randomized comparative studies have been published. The generally low methodological quality of many studies shows that caution is required when interpreting results after surgical cartilage repair. Firm recommendations on which cartilage repair procedure is to be preferred is currently not known on the basis of these studies.

Valid clinical answers in this field will only be the results of a combination of randomized control trials (RCTs). Further, long-term follow up is needed to determine whether articular cartilage repair with scaffold is a valid alternative as first line of treatment of larger cartilage defects compared with ACI.

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