Scaffold-Based Cartilage Treatments: With or Without Cells? A Systematic Review of Preclinical and Clinical Evidence

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Purpose: Regenerative scaffold-based procedures are emerging as a potential therapeutic option for the treatment of chondral and osteochondral lesions. In general, we can summarize most of the recent developments to reach clinical application into 2 major trends: the use of different cell sources or the application of biomaterials as a cell-free approach. The aim of this systematic review was to analyze both preclinical and clinical studies on these new trends to understand how the available evidence supports the use of cell sources or justifies the cell-free approach for the scaffold-based treatment of cartilage lesions. Methods: The research was performed using the PubMed database by looking at studies published in the English language referring to chondral or osteochondral defect repair with scaffold-based procedures until the end of 2013. The following strings were used: (“cartilage”[MeSH] AND “tissue scaffolds”[MeSH]). Results: The search showed an increasing number of published articles each year for both scaffold-based approaches, identifying a total of 305 articles. Among clinical trials, 116 used cell-based scaffold treatments and 11 used scaffolds alone. In the preclinical setting, a scaffold/cell combination was the most used treatment approach (133 v 45 articles), with mesenchymal stem cells (MSCs) being the favorite cell type. Bone marrow was the most used cell source, but other sources are gaining interest. Among articles comparing scaffolds with or without cells, the majority reported superior results for cells (71 of 89 articles). In the clinical setting, most of the articles analyzed chondrocyte-based scaffolds, with only 7 studies using MSCs; all cells were from bone marrow. Despite the lower number of articles, cell-free scaffolds are gaining popularity, with a recent increase in published studies showing promising results. Conclusions: This systematic review underlined the difficulties in understanding the real need for cells to increase the scaffold-based cartilage healing potential because of the heterogeneity of products used as well as the design of the published studies. Scaffold and cell combinations were the most investigated option in the preclinical setting, showing generally superior results, but in the clinical setting, both strategies remain used. In particular, although chondrocytes are the most commonly used cell type, research showed increasing interest in the potential of MSCs for cartilage regeneration. However, the difficulties in managing cell cultures, together with the development of a new generation of materials able to exploit the intrinsic tissue regeneration ability, justifies the clinical use of cell-free scaffolds, with increasing literature and promising preliminary results. Level of Evidence: Level IV, systematic review of Level I to IV studies.

Chondral and osteochondral lesions are debilitating conditions, which if not properly treated lead to the development of osteoarthritis. Regenerative scaffold-based procedures are emerging as a potential therapeutic option for the treatment of these kinds of lesions, with an increasing number of publications every year in in vitro and preclinical animal studies, as well as in clinical applications. The rationale for using a scaffold is to have a temporary 3-dimensional structure of biodegradable polymers, possibly mimicking the highly organized zonal architecture of articular cartilage, to permit the growth of living cells. The use of scaffolds has been...
introduced into clinical practice to improve the results obtainable with the first-generation cell-based approach while overcoming the drawbacks and simplifying the procedure. In fact, the first regenerative approach for cartilage treatment, autologous chondrocyte implantation (ACI), proved to offer good and long-lasting results, but with evident biological and surgical limitations. Thus, 15 years ago, autologous chondrocytes were combined with scaffolds for matrix-assisted autologous chondrocyte transplantation (MACT). Cells were harvested and cultured in vitro and then on the 3-dimensional biomaterial, which favored their redifferentiation, better protection, more homogeneous distribution, and easier handling for surgical implantation.

Several scaffolds have been developed in the attempt to better fulfill the requirements of the cartilage regeneration process, with various materials proposed (natural or synthetic) in different physical forms (fibers, meshes, and gels). Polyactides, including polylactic and polyglycolic acids, are commonly used synthetic matrices. Some concerns might be raised by their degradation products and their effect on native tissue and implanted cells, but innovations in the chemical makeup of these materials have improved their biocharacteristics and biocompatibility. Natural materials—such as collagen derivatives, hyaluronic acid, alginate, agarose, fibrin glue, and chitosan—have good biocompatibility, enhance cell proliferation, and are processed in a reliable and reproducible way.

Many scaffolds have reached clinical practice, and studies are now being published with good mid- and long-term results. However, although MACT is gaining increasing space in the literature, some limits are also emerging. Besides traumatic focal lesions of the femoral condyles, which were shown to have more chance of benefit from this treatment, many other indications have more controversial results, with lower or even poor clinical outcome. Moreover, this approach also suffers from a 2-step operation, technical difficulties and regulatory restrictions for cell manipulation, and high costs.

Thus, after a decade focused on expanding and improving scaffold-based ACI, in past years both researchers and clinicians have been looking for different solutions to regenerate the articular surface. In general, we could categorize most of the recent developments reaching clinical application into 2 major trends: the use of different cell sources or the application of biomaterials as a cell-free approach. The fascinating proliferation and differentiation capacity of mesenchymal stem cells (MSCs) is gaining popularity in the field of cartilage regeneration as a new powerful tool for scaffold augmentation. Conversely, there is an increasing awareness of the role of scaffolds, which are not just carrier systems for cell delivery but may also present an intrinsic ability to promote chondral or osteochondral regeneration by exploiting the self-regenerative potential of the body.

The aim of this systematic review was to analyze both preclinical and clinical studies on these new trends to understand how the available evidence supports the use of cell sources or justifies the cell-free approach for the scaffold-based treatment of cartilage lesions.

**Methods**

The research was performed by 2 independent reviewers (A.R., G.T.) using the PubMed database searching for both preclinical and clinical studies on scaffold-based treatments for cartilage lesions. In particular, research criteria included studies published in the English language that referred to chondral or osteochondral defect repair with scaffold-based procedures until the end of 2013. The following strings were used: “cartilage”[MeSH] AND “tissue scaffolds”[MeSH]. Among clinical publications, only comparative studies or randomized controlled trials (RCTs) were analyzed in detail. After title and abstract screening, full texts were divided into 2 groups according to the use of cells: one group for scaffolds without cells and one group for scaffolds combined with cells. Articles obtained from screening reference lists were also included in our analysis (Fig 1).

**Results**

The search identified 748 articles; among these, 305 articles met the inclusion criteria and thus were included in the analysis. One hundred seventy-eight of these articles were preclinical studies and 127 were clinical studies. In the preclinical studies, 133 investigated the use of cell-based scaffolds, and 45 studied scaffolds alone. Among the clinical trials, 116 used cell-based scaffold treatments and 11 studies used scaffolds alone. The evaluation of evidence level showed only 6 randomized studies (level of evidence I/II) and 7 comparative studies, whereas the others were case series or case reports.

**Preclinical Studies**

The number of articles per year increased progressively for both scaffold-based treatments, as seen in detail in Figure 2. However, a scaffold/cell combination was the most used treatment approach for chondral and osteochondral defects, with MSCs as the favorite cell type; articles on this type of study increased each year (Fig 3). Bone marrow was the most used cell source—as bone marrow concentrate or bone marrow cultured cells (Fig 3), followed by adipose-derived MSCs (ADMSCs)—the new emerging trend for chondral and osteochondral lesions. Few studies were reported for synovium-derived MSCs
(SDMSCs), peripheral blood-derived MSCs (PBMSCs), and umbilical cord blood-derived MSCs (UCMSCs) (Table 1). Fifty-one articles used autologous chondrocytes (ACs), one study used the combination of ACs and osteoblasts, 2 articles studied autologous perichondrium cells, 2 studied autologous periosteal cells, and only one article used a combination of ACs and MSCs (Table 1).

**Fig 1.** Scheme of research methodology.

**Fig 2.** Published preclinical in vivo articles per year regarding a scaffold-based approach for chondral and osteochondral lesions. The number of articles per year increased progressively for both scaffold-based treatments.
Among articles focusing on scaffolds with cells, 89 compared the outcome using scaffolds with or without cells, reporting superior results for cells in 71 articles, doubtful results using cells in 10 articles, and the same results as seen for scaffolds alone in 8 articles (Table 2).

Clinical Studies

In the clinical setting, an increasing number of articles concerning the cell-based scaffold approach were observed each year, although a trend of decrease was seen in 2013 (Fig 4). Despite the low number of articles, cell-free scaffolds are gaining popularity; there was an increase in published studies from 2010 onward. Among cell-based scaffolds, 7 articles described the results using MSCs (concentrated only from bone marrow), whereas the others described the use of chondrocytes. We now report in detail RCTs and comparative studies.

In 2004, Visna et al. investigated the use of 3-dimensional carrier fibrin glue with autologous chondrocytes for the treatment of deep knee cartilage lesions compared with abrasive techniques. Fifty patients (25 for each group) were evaluated; a better outcome was seen in patients treated with autologous chondrograft transplantation, with better functional improvement than in those treated with abrasive techniques. One year later in a randomized trial, Bartlett et al. compared the outcomes of 91 osteochondral defects: 44 were treated with ACI-C, a first generation of ACI using a collagen membrane instead of periosteum, and 47 were treated with a MACT technique. Both treatments showed comparable results regarding clinical, arthroscopic, and histologic outcomes, with the advantages of last surgery and less extensive joint exposure for the MACT technique. In 2007, Manfredini et al. compared the results of ACI treatment and a hyaluronan-based MACT technique in 17 and 15 patients, respectively, with knee cartilage lesions. Significant clinical improvement was observed in both groups at 6 and 12 months after surgery, with well-integrated tissue similar to the surrounding healthy cartilage. Molecular analysis also highlighted that the implanted material undergoes progressive remodeling to regenerate hyaline cartilage. A similar study published 1 year later by Ferruzzi et al. showed the results after treatment of knee osteochondral lesions with an open ACI procedure (group 1) and an arthroscopic hyaluronan-based MACT technique (group 2). Ninety-eight patients were treated in this comparative study—48 in group 1 and 50 in group 2. At 5 years, similar clinical and histologic results were described, with faster recovery in the Hyalograft C (Anika Therapeutics, Bedford, MA) group. In 2009, Kon et al. compared the clinical outcomes at 5-year follow-up in patients treated with hyaluronan-based MACT and those treated with microfracture. Both groups showed statistically significant improvement in all clinical scores.

Table 1. Number of Articles Found for the Different Cell Sources Used in a Preclinical Scaffold-Based Approach

<table>
<thead>
<tr>
<th>Autologous Chondrocytes</th>
<th>Autologous Perichondrium Cells</th>
<th>Autologous Periosteal Cells</th>
<th>Autologous Chondrocytes and MSCs</th>
<th>Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC/BMSCs</td>
<td>ADMSCs</td>
<td>SDMSCs</td>
<td>PBMSCs</td>
<td>UCMS Cs</td>
</tr>
<tr>
<td>51</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
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NOTE. Bone marrow is the favorite cell source followed by ADMSCs. There were few studies for SDMSCs, PBMSCs, and UCMS Cs. Fifty-one articles used AC, 1 used AC and osteoblasts, 2 used autologous perichondrial cells, 2 used autologous periosteal cells, and 1 used ACs and MSCs. ACs, autologous chondrocytes; ADMSCs, adipose-derived mesenchymal stem cells; BMC, bone marrow concentrate; BMSC, bone marrow derived mesenchymal stem cells; MSCs, mesenchymal stem cells; PBMSCs, peripheral blood-derived mesenchymal stem cells; SDMSC, synovial derived mesenchymal stem cells; UCMS Cs, umbilical cord mesenchymal stem cells.
from preoperatively to final follow-up; however, better clinical results and sports activity resumption were seen in the MACT group at 5 years. In 2010, Basad et al.\textsuperscript{22} reported superior results with the MACT technique than with microfracture in the treatment of large articular defects: 40 patients underwent MACT, whereas 20 were treated with microfracture. A significant clinical improvement was detected in both groups, but better and more stable results over time were described for MACT. In the same year, Welsch et al.\textsuperscript{23} showed a comparable clinical outcome using 2 MACT procedures (Hyalograft C—a hyaluronan-based scaffold, or CaReS [Arthrobotics, Krems, Austria], a collagen-based scaffold), for the treatment of cartilage lesions. However, magnetic resonance imaging (MRI) analysis highlighted the superiority of cartilage repair tissue and surface for CaRes. Zeifang et al.\textsuperscript{24} aimed to test whether the MACT technique with BioSeed-C (BioTissue Technologies, Freiburg, Germany) (a fibrin, polylactic and polyglycolic acids, and polydioxanone-based scaffold) or the original ACI technique for the treatment of full-thickness cartilage defects provided a superior outcome regarding clinical efficacy and safety. Results showed no difference in the clinical efficacy at 12 and 24 months of follow-up; however, better MRI results at 6 months and postoperative improvement were detected for BioSeed-C.

In 2011, Cole et al.\textsuperscript{25} harvested healthy cartilage tissue from an unaffected area of the injured joint, mechanically fragmented it, and then embedded it into a 3-dimensional polymeric reabsorbable scaffold composed of polycaprolactone, polyglycolic acid, and polydioxanone, which was implanted into the defect. The results of this cartilage autograft implantation system were reported in a randomized study that showed better subjective results at 2 years when compared with microfracture. MRI evaluation showed no differences in defect filling, tissue integration, or subchondral cysts, although more intralesional osteophyte formation was documented in the microfracture group. In the same year, Kon et al.\textsuperscript{26} evaluated whether hyaluronan-based MACT (Hyalograft C) provided better outcomes than the microfracture approach in a challenging population of 41 professional or semi-professional male soccer players. Twenty-one patients underwent Hyalograft C MACT, and 20 patients were treated with microfracture. Despite similar success in returning to competitive sport, microfracture allowed a faster recovery but presented clinical deterioration over time, whereas arthroscopic Hyalograft C treatment delayed the return to competition but offered more durable clinical results. Macmull et al.\textsuperscript{27} performed chondrocyte implantation in 48 patients with patellar lesions: 25 received ACI-C treatment (a collagen membrane instead of periosteum was used to contain the liquid cell suspension), and 23 underwent the MACT technique. Significant clinical improvements were reported for both groups; however, better results were obtained with MACT: excellent and good results

![Fig 4. Published clinical articles per year regarding scaffold-based approach for chondral and osteochondral lesions. An increasing number of articles reporting a cell-based scaffold approach can be observed year by year, although a trend of decrease was seen in 2013.](image-url)
were achieved in 40% of patients who received ACI-C treatment and in 57% of patients who underwent MACT.

In 2012, Panagopoulos et al. described the results after ACI and MACT in 11 athletes and 8 professional soldiers, respectively, with knee cartilage lesions. Comparable improvements in functional scores between the 2 groups were detected, but with a trend toward better results for MACT. In general, only 6 of 19 patients returned to previous athletic performance. In the same year, Crawford et al. conducted an RCT to investigate if NeoCart (Histogenics, Waltham, MA), an autologous cartilage tissue implant, was superior to microfracture for treating knee articular cartilage injuries. Twenty-one patients received a NeoCart implant, whereas 9 patients were treated with microfracture. Results showed that the safety of NeoCart implantation was similar to that of microfracture surgery and was associated with greater clinical efficacy at 24 months.

Discussion

The scaffold-based approach represents a fascinating treatment option for chondral and osteochondral lesions, providing a structural basis for defect repair and stimulating the healing processes of damaged tissues. In this scenario, cells occupy a controversial role. Despite promising results reported using scaffolds and cells for clinical applications, the use of cell-free scaffolds also showed good results and avoided cell manipulation and its regulatory obstacles. Our preclinical search identified an increasing number of published articles every year for both scaffold-based approaches (Fig 2). A scaffold/cell combination was the most investigated option (Table 2). In general, superior results were reported by adding cells compared with using a scaffold alone, but the heterogeneity of these articles regarding different cell sources, dosages, scaffold materials, animal models, and defect types makes it difficult to draw a conclusion on the real need for cells in the chondral and osteochondral scaffold-based approach.

The clinical scenario was similar, showing an increasing number of articles each year both for scaffolds with cells and cell-free scaffolds, although for the cell/scaffold combination we observed a slight decrease in articles in 2013 (Fig 4). Overall good results were shown when cells were added, but there were no articles directly comparing the outcome of the same scaffold used alone or with cells. The few RCTs or comparative trials on MACT were very heterogeneous, and usually the comparison was made with standard cartilage treatments and not with the scaffold alone and thus did not clarify the real role of cells.

Because both the preclinical and clinical literature cannot clearly prove the superiority of either a cell-based scaffold or a cell-free scaffold, research is focused in 2 opposite directions to improve cartilage treatment: cell source and scaffold properties.

The analysis of the cell sources proposed for the cell-based scaffold approach showed that although MSCs were the favorite cell type in the preclinical search (Table 1), with an increase of articles through the years with respect to chondrocytes (Fig 3), chondrocytes were used for the majority of clinical studies. Recently, the use of MSCs for cartilage repair has seen a fast diffusion into the literature, with about 2,400 articles, of which more than 1,000 were from 2010. MSCs represent an appealing tool for regenerative medicine thanks to their unique characteristics and ability—their self-renewal characteristics, their maintenance of “stemness,” their potential for differentiation into cells forming multiple mesodermal tissues, and their trophic and immunomodulatory effects. Both for in vivo (Table 1) and clinical studies, bone marrow represented the favorite type of MSC as a concentrate for one-step procedures and as in vitro expanded MSCs for a 2-step approach. However, other MSC sources are emerging as valuable options: among these, ADMSCs are gaining increasing attention because they proved to have good chondrogenic potential and are easy to obtain and can be used as cell concentrate or expanded by culture. However, despite good and promising results, MSC research is still in its infancy, especially in the clinical setting, and many controversial aspects still have to be clarified, such as the best MSC source, dosage, use of concentrated or expanded MSCs, delivery, and so on.

The second direction of research development is the cell-free scaffold approach for the treatment of chondral and osteochondral lesions. Our results also highlighted an increasing number of articles for cell-free scaffolds both in preclinical and clinical articles. This is not surprising because in past years, huge steps forward were made regarding biomaterials research, with the development of a new generation of materials able to mimic the characteristics of healthy tissues and exploit the intrinsic tissue regeneration ability and avoid the risk linked to cell management (such as bacterial contamination and phenotype loss during extensive cell manipulation), reducing costs and simplifying the procedure. In this direction, biomaterials that also focused on osteochondral lesions were developed, with the challenge to guide the regeneration of 2 different tissues characterized by different healing potentials, and promising results have been shown in the clinical setting as well for the treatment of complex cases otherwise doomed to a poorer outcome with the other cartilage treatment procedures.

In the complex and rapidly evolving field of cartilage regenerative treatments, in which an increasing number of articles are documenting similar good results for a
huge number of products, several factors are driving research efforts, and not all of them are based purely on scientific evidence of superiority of one approach over the other. In fact, economic and regulatory aspects have a huge influence and may explain some strategies of treatment development. This is the case with some products previously proposed and promoted for the regenerative potential of cell-based scaffolds that are currently focused more on the advantages and benefits of the same scaffolds using cell-free scaffolds. Because there are no clinical studies directly comparing the same scaffold with or without cells, and good results have been separately reported using the same scaffolds with or without cells, the issue of the real role of cells remains. A more technical aspect could also be an important confounding factor when trying to address the problem of understanding the contribution of cells to tissue regeneration. The “quality” of the implanted cells is not reported in most of the cell-based products or in the studies documenting their results regarding clinical outcomes. It is well known that during in vitro cultivation, chondrocytes can undergo dedifferentiation, losing their phenotype and thus the ability to form stable hyaline cartilage. Even after culture in the 3-dimensional structure of the scaffolds, cells may not be completely recovered, and concerns could be raised regarding their distribution and vitality after culture as well as after the trauma of scaffold surgical manipulation and implantation. One possibility to address the problem of cell quality was shown by Vanlauwe et al., who developed a new method to score the chondrogenic potential of a cell culture through a gene marker profile to ensure implantation of a quality selected cell population (chondrocyte-characterized implantation).

A similar approach could also be used for MSCs, selecting only cell populations with the ability to differentiate into a chondrocyte phenotype and to maintain it after implantation. Other strategies are emerging to stimulate and guide the differentiation of MSCs toward a fully developed hyaline-like tissue (predifferentiation, magnetic devices, association with hyaluronic acid, and combination with growth or transcription factors).14

Limitations

Limitations of this review are the analysis of only one database and the evaluation of only articles published in the English language. However, the number of articles identified through a systematic approach and reviewed in this article clearly allowed representation of the complexity of this field as well as the research and literature development of the new emerging trends for cartilage treatments.

Further high-quality studies will have to prove the real benefit of each one of these research developments by directly comparing the newly developed treatment strategies, increasing the knowledge about scaffold-based treatment, and proving the advantages of cell-based products with respect to those focused more on scaffold intrinsic properties to favor chondral or osteochondral regeneration by exploiting the self-regenerative potential of the body.

Conclusions

This systematic review underlines the difficulties in understanding the real need for cells to increase the scaffold-based cartilage healing potential, because of the heterogeneity of products used as well as the design of the published studies. Scaffold/cell combinations were the most investigated option in the preclinical setting, in general showing superior results; in the clinical setting, both strategies were used. In particular, although chondrocytes were the most commonly used cell type, research showed increasing interest in the potential of MSCs for cartilage regeneration. However, the difficulties in managing cell cultures, together with the development of a new generation of materials able to exploit the intrinsic tissue regeneration ability, justifies the clinical use of cell-free scaffolds, with an increasing literature and promising preliminary results. Thus, the literature analysis revealed 2 opposite directions, which could be explained by a complex interaction of scientific and economic and regulatory aspects. Although we are far from understanding which could be considered the best approach, an increasing number of both preclinical and clinical articles show the huge research efforts in this field, which are likely to result in improvements regarding biomaterial properties, cell sources and cell manipulation, and application modality and, no less important, the development of a patient profile to select and treat those patients who may benefit more from this kind of treatment.

References


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