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REVIEW ARTICLE

Cell Therapy and Tissue Engineering Approaches for Cartilage Repair and/or Regeneration

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Articular cartilage injuries caused by traumatic, mechanical and/or by progressive degeneration result in pain, swelling, subsequent loss of joint function and finally osteoarthritis. Due to the peculiar structure of the tissue (no blood supply), chondrocytes, the unique cellular phenotype in cartilage, receive their nutrition through diffusion from the synovial fluid and this limits their intrinsic capacity for healing. The first cellular avenue explored for cartilage repair involved the in situ transplantation of isolated chondrocytes. Latterly, an improved alternative for the above reparative strategy involved the infusion of mesenchymal stem cells (MSC), which in addition to a self-renewal capacity exhibit a differentiation potential to chondrocytes, as well as a capability to produce a vast array of growth factors, cytokines and extracellular matrix compounds involved in cartilage development. In addition to the above and foremost reparative options up till now in use, other therapeutic options have been developed, comprising the design of biomaterial substrates (scaffolds) capable of sustaining MSC attachment, proliferation and differentiation. The implantation of these engineered platforms, closely to the site of cartilage damage, may well facilitate the initiation of an 'in situ' cartilage reparation process. In this mini-review, we examined the timely and conceptual development of several cell-based methods, designed to repair/regenerate a damaged cartilage. In addition to the above described cartilage reparative options, other therapeutic alternatives still in progress are portrayed.

Keywords: Cartilage damage, Repair/regeneration, Cell implantation, Biological scaffolds, Micro fracture, Novel cartilage restorative approaches

Cartilage: a peculiar type of connective tissue

Chondrocytes, the unique type of cell present in hyaline cartilage develop from the highly regulated differentiation of mesenchymal stem cells (MSC), a mesodermal-derived stem cell present in several fetal and adult tissues. In the cartilage, chondrocytes are distributed either singularly or in clusters (recently divided) called isogenous groups. In these groups, chondrocytes are active in matrix production and display areas of cytoplasmic basophilia, which are indicative of protein synthesis and clear areas, which indicate their large Golgi apparatus (1-3).

The newly divided chondrocytes secrete the major macromolecules in cartilage matrix, including: a) collagen types II, IV, IX and XI, which are involved in the formation of a three-dimensional meshwork of the relatively thin and short matrix fibrils, and b) proteoglycans, which delineates the ground substance of hyaline cartilage, contains three types of glycosaminoglycan's (GAGs), including hyaluronans, chondroitin sulfate and keratin sulfate. The last two types are joined to a core protein to form a proteoglycan monomer, of which aggrecan is the most

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significant. Other cartilage proteoglycans (decorin, biglycan, and fibromodulin), also play a role in matrix stabilization (2-5).

This unique structure, including cells and a vast network of cell regulators, gives cartilage a sort of compression rigidity and facilitates the entry of water. Thus, the tissue develops a peculiar power to absorb and dissipate tension forces constantly acting on the system bone. The high degree of hydration and the movement of water in the matrix allow the cartilage matrix to respond to varying pressure loads and contribute to cartilage's weight-bearing capacity.

Throughout life, cartilage undergoes continuous internal remodeling as the cells replace matrix molecules lost through degradation. The structure of the matrix is significant, since it acts as a signal transducer for the embedded chondrocytes. Thus, pressure loads applied to the cartilage as synovial joints create mechanical, electrical, and chemical signals that help to direct the synthetic activity of the chondrocytes (2, 3, 6).

However, as the body ages the composition of the matrix changes, and chondrocytes lose their ability to respond to these stimuli. In older chondrocytes, cytoplasmic changes are visible (shrinkage) resulting from the loss of lipid droplets and glycogen stores. In addition to ageing, there are other main ways that articular cartilage can be damaged: a sudden accidental injury, osteoarthritis, osteochondritis dissecans and infection. Conventionally, the clinical management of an osteochondral injury involves the use of mechanical symptomatic measures, in most cases associated to the use of analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs) and/or chondro-protective agents (chondroitin sulfate, sulfate glucosamine, hyaluronic acid). Other protective options, involve the use of corticoids, HA, PRP, abrasion, micro fracture, radiofrequency and/or osteochondral grafts (6-8).

In most cases, the above therapeutic options are reparative but not renewable and associated to the creation of a more fibrous than hyaline tissue. However, the better understanding of the molecular, biological and structural components comprised in cartilage structure and function, has permitted the development of new prospects aimed to design biological therapies aimed to repair and/or regenerate a damaged cartilage (9, 10).

Cell-based therapies for the treatment of chondral lesions

Based on the better understanding of the cellular, molecular and micro environmental features of cartilage, it became evident that a damaged cartilage certainly could be a select target to develop tissue engineering procedures utilizing cell-based strategies. Most of these procedures have been designed to generate a neo-cartilage in an attempt to offer patients with chondral injuries, either an improvement in quality of life or a definitive cure.

Autologous chondrocytes implantation (ACI)

This early restorative procedures involves the arthroscopic procurement of a biopsy (8 mm) from the femoral groove of a healthy cartilage, area that normally is not subjected to load. Retrieved tissue is enzymatically treated to obtain a population of healthy isolated chondrocytes, which are then ex vivo expanded under conditions that preserve cell viability and function. The resulting population of chondrocytes is then injected under the periosteum, where they should grow and mature over time (11, 12).

Results from several clinical studies, including small cohorts of patients suffering diverse types of cartilage damage (early osteoarthritis, femoral condyle defects, knee joints defects, others), have shown that ACI treatment prompts pain reduction, improves quality of life and in many cases delay the need of joint replacement (13, 14). Despite these encouraging conclusions, the results of a similar but more comprehensive study revealed that effect (s) elicited by ACI are quite similar to those attained after osteochondral grafts treatment (mosaicplasty) (15).

Mesenchymal stem cells (MSC) implantation

Due to several cellular and molecular traits, mesenchymal stem cells (MSC) have been proposed to be an attractive candidate for cartilage repair. Among many others, MSC attributes comprise its abundance in various tissue sources (bone marrow, adipose tissue, umbilical cord blood, cord blood, others), self-renewal and a vast differentiation potential towards a chondrogenic. In addition, MSC produces a variety of extracellular matrix macromolecules involved in cartilage function, including collagen (s), fibronectin, glycosylaminoglycans (GAGs) and proteoglycans, as well as a vast repertoire of cytokines, growth factors, colony stimulating factors and chemokines (16, 17).

Based on the cellular and molecular features of MSC, biomedical actors developed the notion that the implantation of MSC may represent an appealing clinical alternative for regeneration of articular cartilage defects. Results of several clinical studies utilizing MSC for cartilage repair have evidenced that the procedure is feasible and safe. In addition, the intra-articular injection of MSC

proved to be effective in terms of reducing pain, improving tissue function and a robust capability to regenerate hyaline-like cartilage (18, 19).

Table 1 summarizes the outcomes of a group of comprehensive but not all-inclusive clinical studies, utilizing cell-based therapeutic approaches for the treatment of cartilage lesions. As compared to conventional procedures utilized to regenerate a damaged articular cartilage (micro-fracture, perforations, abrasion and/or mosaicplasty), it is without doubt that cell-based therapies, using either chondrocytes or MSC, represent an appealing curative alternative for cartilage regeneration.

However, there are several issues dealing with the isolation and manipulation of the 'curative' cell that require additional attentiveness. Specifically, in the case of MSC: a) selection of the most appropriate tissue source (s) (bone marrow, fat, umbilical cord blood, placenta, others), b) validation of a proper delivery route to the damaged tissue and c) a clear understanding that the curative effect of a MSC-based protocol, resides on the quality (biological attributes) and not necessarily on the quantity of the 'curative' cell (22). Accordingly, provisions should be taken during the ex-vivo processing of MSC (expansion), to protect stemness and avoid the expression of senescence-associated features (23, 24). As indicated in a recent publication, there are several challenges that must be overcome before MSC-based tissue engineering can become an effective cartilage regeneration therapy (17).

Biological scaffolds and its use in the treatment of chondral lesions

As indicated above, the limited cell proliferation and differentiation capacity of chondrocytes in conjunction with a low production of cartilage-specific extracellular matrix have seriously limited their use in regenerative strategies. Accordingly, attempts have been oriented to develop other reparative options aimed to achieve an effective regeneration of hyaline cartilage.

The enhanced understanding of the molecular structure and functional role of extracellular matrix components in cartilage dynamics (25), encouraged the construction of sophisticated platforms (scaffolds) mimicking cartilage microenvironment. As a result, these prototypes seem to represent an appealing clinical device to be used in the treatment of chondral defects. To facilitate the binding of a cartilage-repair cell prototype (chondrocytes, MSC, others) these biodegradable scaffolds have been designed to include both a proximal cell-binding surface and a distal one to facilitate their loading in the proximity of a cartilage damage site (26).

The commercial availability of a vast array of these scaffolds, have prompted the initiation of a number of clinical studies (Table 2) to explore their use in the treatment of diverse cartilage lesions. The result of several preclinical and clinical studies put forward the notion that these elaborated structures embody a safe and promising clinical option for cartilage repair.

Nonetheless, in the case that these scaffolds turn out to be loaded with MSC entails the validation of several issues including the quality of the ex vivo expanded MSC (23), the assurance that mature and not a hypertrophic chondrocytes will be generated (17), and last but not least, the absence of ancillary factors that may alter MSC's cartilage repair potential (9).

Table 1. Clinical studies assessing the capability of cell-based therapies to the repair cartilage defects: an assortment of illustrative studies

Cartilage lesion	Cell type[1]	Number of patients[2]	Most significant findings	Reference
Knee articular	ACI	431	Mild or no effects	15
Knee osteoarhritis	MSC	41	Significant improvement in knee evaluation tests and MRI scores	18
Knee articular	ACI or MSC	72	Both cell types produce no significant differences in knee evaluation tests (IKDC, Lysholm, Tegner). However, patients receiving MSC, but not ACI, improve evaluation tests and require less surgery	19
Knee osteoarthritis	MSC	18	No adverse events, improvement in knee evaluation tests, size of defect decreased, hyaline-like cartilage regeneration	20
Knee osteoarthritis	MSC	6	In 3/6 patients, cartilage thickness and knee evaluation tests improved (6 months); increase in extension of repair tissue; decrease in edematous subchondral patches	21

1: ACI: chondrocyte; MSC: mesenchymal stem cells, 2: Include both treated and control patients.

Table 2. Treatment of cartilage defects by using diverse types of biological scaffolds seeded with cartilage-repair cells

Scaffold and cell type used	Chondral lesion, number of patients and clinical outcome	Reference
Collagen I/III-based/bone marrow cells	Knee large lesions, 52/54 patients, after 1-5 year significant improvement in all knee functional scores	27
Hyaluronic acid/ chondrocytes	Knee, 141 patients, after an average follow up time (8 months) more than 70% of patients had no pain and mobility problems, histological analysis revealed hyaline-like cartilage, no side effects	28
Hyaluronic or Collagen-based/chondrocytes	In both groups (10 patients each), clinical outcome (24 months) was similar in MRI of cartilage repaired tissue, relaxation times for healthy surrounding cartilage and Zonal evaluation. However, global T2 was significantly higher in the hyaluronic group. Thus, functional outcome seems to be related to the type of scaffold used	29

The use of cell-based therapies in conjunction with biological scaffolds to repair a damaged cartilage: new challenges to overcome

It is without doubt that the development of cell-based therapies aimed to repair a damaged cartilage, using either isolated chondrocytes or MSC as such or in conjunction with biological scaffolds has been an area of intensive clinical research in the last years. The clinical results of these studies, some of them depicted in Tables 1 and 2, have revealed that these procedures are feasible, safe and in most cases beneficial in the management of patients suffering chondral defects (13-15).

Simultaneously, several attempts have been initiated to further improve chondrogenic recovery, after the utilization of several types of cell-loaded biomaterial scaffolds. Among them, there are several efforts to improve ex vivo expansion of autologous chondrocytes (15) or intended to find alternative sources of MSC aimed to obtain cell products exhibiting a solid chondrogenic differentiation program with less hypertrophic differentiation (17, 22, 23).

The above, may well permit the development of protocols easily scalable, translatable to the clinic and capable of resisting variable biomechanical loading.

Despite the remarkable development of techniques intended to be utilized in chondrogenic cellular therapies, robust data still is lacking in terms of assuring the patient a proficient therapy capable of recapitulate hyaline cartilage tissue (9, 17, 25).

Recent biomedical advances in articular cartilage repair

Cartilage structure and function can be fairly harmed by a variety of causes resulting in injury, inflammation, pain, limited movement and significant joint damage and deformity. The therapeutic modalities previously examined sustain the regeneration of a damaged cartilage only in those conditions where the extent of chondral damage is limited and/or adjacent to a tissue region that preserves its full functional structure.

Most recently, a generation of innovative biomedical procedures has been explored in an attempt to treat not only focal defects, but even large-scale osteoarthritic degenerative changes. Among them, the following can be mentioned.

The 'one step' cell free cartilage reparative method

This novel and promising therapeutic option has matured from the expertise gained after the implementation of the so called 'two-steps' procedures, as shown in Tables 1 and 2. The 'one step' cell free reparative method starts with the direct implantation of a cell-free scaffold neighboring the site of the chondral lesion. The above procedure is followed by a mechanical arthroscopic maneuver intended to create a micro fracture within the bone underlying the damaged cartilage zone. The above procedure facilitates the in situ release, migration and attachment of endogenous MSC (the 'repair' cell) to the nearby implanted cell-free scaffold. This sort of combined reparative process represents an appealing therapeutic option for the treatment of small to medium-sized cartilage defects (30, 31).

Attempts to further improve the therapeutic capacity of MSC to play a part in cartilage repair

It is well known that the binding capacity of MSC to extracellular-like molecules (collagen I/II, hyaluronic acid, fibronectin, others) is dependent, among other factors, on the cellular expression of β1-integrins, a type of transmembrane receptors (16). In addition, recent studies have shown that escalation of integrin expression facilitates the attachment of MSC to precise regions of a damaged cartilage (32). In the same vein, cellular studies have shown that the presence of L-Ascorbic acid 2-phosphate (a cul-

ture media components often utilized to grow MSC) modulates not only the differentiation of adipose-derived MSC to chondrocytes, but stimulate the production of chondrogenic growth factors (33).

Without a doubt, the translation to the clinic of specific cellular and molecular information may pave the way to the development of novel and possible more effective cell-based therapies aimed to repair osteochondral defects.

Multidisciplinary strategies associated to the development of innovative articular cartilage repair procedures

As discussed in previous sections, numerous attempts have been developed to repair focal chondral and/or osteochondral defects. However, major approaches aimed to repair large-scale osteoarthritic degenerative changes are still under development. In this respect, a number of preclinical studies have been initiated to investigate whether migratory progenitor cells and/or gene-based approaches may be valuable to repair major articular cartilage (34, 35).

Conclusions

Taken as a whole, both the current cartilage reparative options as well as several pre-clinical studies, still under development (36, 37), epitomize an evolving approach for the generation of therapies targeted to bring welfare to a large population of patients who suffer from articular cartilage damage. However, the biomedical efforts associated with the implementation of all these new medications, will bring together (as usual) new challenges linked to the safe and highly regulated translation of such procedures into the clinic (38).

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Potential conflict of interest

The authors report no financial or other conflict of interest relevant to the subject of this article.

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