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Current strategies for osteochondral regeneration: from stem cells to pre-clinical approaches

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Damaged cartilage tissue has no functional replacement alternatives and current therapies for bone injury treatment are far from being the ideal solutions emphasizing an urgent need for alternative therapeutic approaches for osteochondral (OC) regeneration.The tissue engineering field provides new possibilities for therapeutics and regeneration in rheumatology and orthopaedics, holding the potential for improving the quality of life of millions of patients by exploring new strategies towards the development of biological substitutes to maintain, repair and improve OC tissue function. Numerous studies have focused on the development of distinct tissue engineering strategies that could result in promising solutions for this delicate interface. In order to outperform currently used methods, novel tissue engineering approaches propose, for example, the design of multi-layered scaffolds, the use of stem cells, bioreactors or the combination of clinical techniques.

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Introduction

Osteochondral (OC) interfaces are part of the joint, being a specialized and integrated structure consisting of multiple connective tissue elements, including muscles, tendons, ligaments, synovium, cartilage, and bone, organized to permit stability and movement of the human skeleton.

OC injuries can lead to joint malfunction and ultimately to the development of degenerative diseases such as osteoarthritis. With an increasing aging population, OA represents a significant socio-economical burden worldwide. Although several procedures are available on the clinical market, an ideal solution has yet to be found in order to fulfil all necessary requirements for a long-term successful regenerative approach.

This paper is aimed at reviewing distinct strategies aiming at a successful OC regeneration, involving cells, scaffolds, bioreactors or a combination of these elements. The rationale for currently used techniques as well as some promising studies in animal models will also be discussed in this review in order to highlight the state-of-the-art in OC over the past few years (Figure 1).

One of the most challenging goals in bone and cartilage tissue engineering (TE) is the creation of an engineered OC interface to repair damaged areas. Similarly to the natural milieu, an engineered interface should distribute everyday mechanical stresses with low-friction load bearing, while interacting with different structural and biological needs in a stable environment. This is particularly more demanding and unique if one considers the distinctive requirements of bone and cartilage tissues as well as the several OC systems found in the human body, dependent on their location and functionality.

Several materials, shapes, stiffness and chemical compositions were described for bone [1–8] and cartilage scaffolds [8–11,12•,13–20,21•], considering the relevance of scaffold architecture to sustain the mechanical stresses of the joint as well as to guide the cells into the desired phenotype, and promoting a complete integration of the OC system in order to restore tissue functionality.

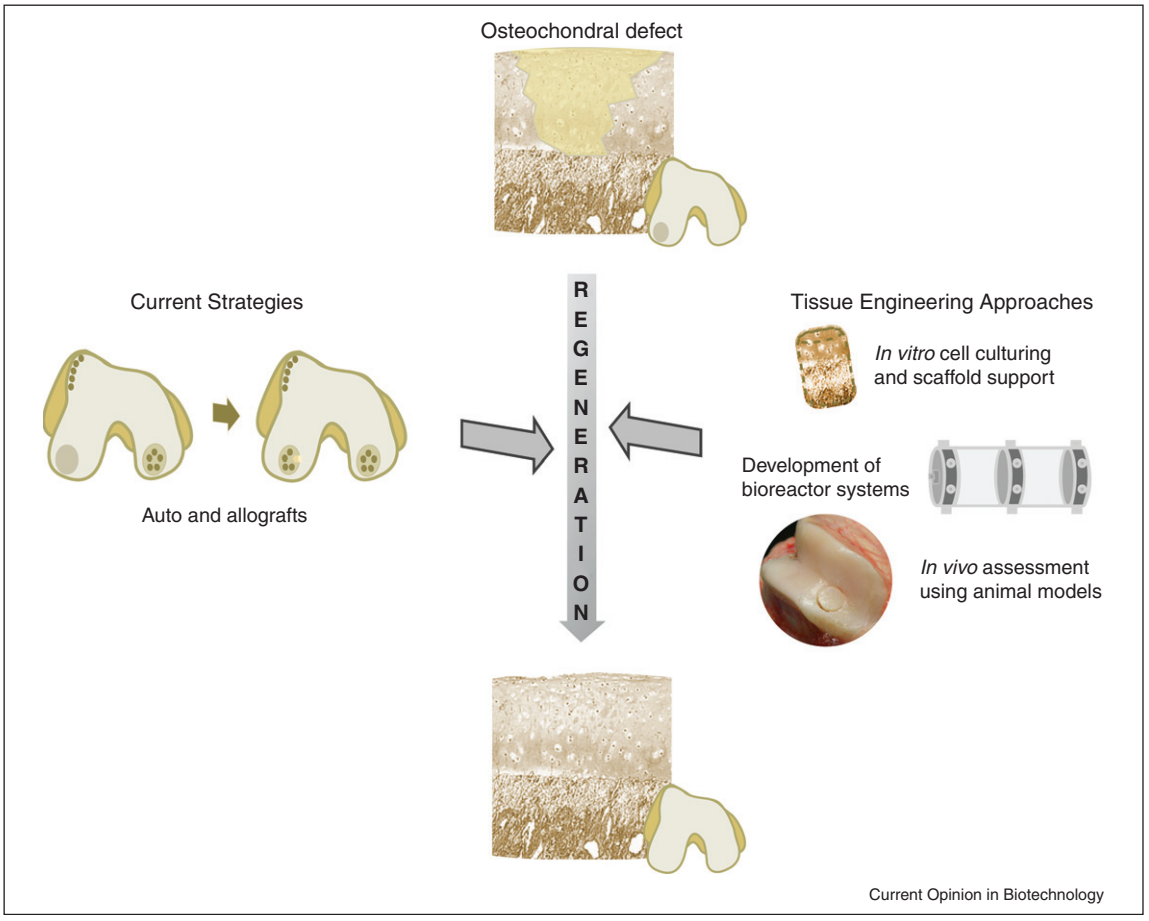
The selection of cells also plays an important element in this delicate interface headed for engineered grafts. Several potential cell sources were successfully described for bone [22–26], and cartilage [17,22–27], which are likely to be useful for OC strategies [8].

The subsequent step towards the clinical application is the up-scale and custom made production of the OC implants to fit perfectly to the injured area and to provide the biological and structural needs required to restore tissue function. In order to automate and make the system cost-effective, several bioreactor models [28,29] were designed and have been showing promising results.

Osteochondral defects (OCD)

Most OC lesions or defects (OCD) and OC injury-associated diseases lead to loss of integrity or stability

Figure 1



Clinical and tissue engineering strategies to promote the regeneration of osteochondral defects.

at the articular surface with resultant decrease range of motion of the involved joint, and, ultimately, premature osteoarthritis (OA) [30]. Although OCDs occur as a result of repetitive trauma within the joint, several factors, such as ischemia, genetics, abnormal vasculature, and metabolic disorders are associated with body processes leading to loss of cartilage [31] or to relevant changes in the architecture or composition of the bone [32]. Furthermore, joint healing is strongly dependent on age, as age is the strongest known risk factor for the development of OA [33] and depth of injury is also age associated [34]. Aged cartilage also induces changes in chondrocyte function and material properties, and responds differently to cytokines and growth factors [33].

The location of a particular defect [35] does influence repair response of the cartilage as well as the mechanical alignment of the joint [36]. OC lesions are identified most frequently in the femoral condyles [37], capitellum of the elbow [38], dome of the talus [30], and the dorsal surface of the patella [37].

Weight bearing influence in biomechanics of the joint

Homeostasis of articular cartilage depends on mechanical loads generated during daily activity. Some joint areas are particularly more affected by weight pressure than others, which may progress to a more degenerative and diffuse joint involvement that translates to the patient by causing pain, swelling, clicking, and instability. Ultimately, inappropriate joint loads are associated with focal stress and result in focal degeneration of cartilage, as that occurs in OA, and increase the stress on subchondral bone. Changes in pressure and shear stress induced by joint movement may induce changes in matrix protein expression and in the release of nitric oxide associated with joint pathogenesis [39].

The stress may also vary throughout the cartilage on a joint surface, because loading is not completely uniform, leading to gradients in stress and pressure [40]. This effect is evident in most patients, where the surface of the joints does not conform perfectly under loading and may result in an increased risk for OA progression [33].

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Current treatments in clinical field

Currently available treatments depend upon the size of the OC defect and the condition of the overlying cartilage. Using reparative surgery, cartilage treatments include arthroscopic debridement, abrasion arthroplasty, and microfracture. These procedures stimulate the body to heal the injury, mainly resulting in the formation of fibrocartilage [41]. Fibrocartilage is a scar tissue presenting diminished resilience, reduced stiffness, and poor wear characteristics when compared to hyaline cartilage. Thus, fibrocartilage is unlikely to withstand physiological loading and cannot guarantee to function successfully in long term. Nevertheless, other options are available with restorative surgery, namely, autografts recurring to mosaicoplasty procedures, allografts [42,43] and biologic replacement using cultured autologous chondrocytes [44,45]. The biggest challenge with autografts is to achieve a final round shape that mimics the surface of the articular joints. Allograft procedure is similar to autografts [46] and mostly used after other surgeries have failed. It is not recommended for patients with OA, and the limited supply of donor tissue is a major problem of this practice.

Autologous chondrocyte transplantation/implantation [44] has also been described to help restoring the structural make-up of the articular cartilage. The intermediate and long-term functional and clinical results are promising, especially regarding the durability of the repair in human patients follow-up [45].

More recently [47], tissues from the covering of bone and cartilage are implanted into the lesion through periosteal and perichondral grafting to promote the repair and functionality of cartilage.

Despite the availability of procedures, all current treatment options inflict further tissue destruction before any therapeutic effect can be achieved.

TE strategies to improve available treatments

Cells to promote healing

Despite current knowledge on OC field, the selection of a cell source to promote efficient OC regeneration is a major issue that must to be considered. Ideally, a cell source should enable insignificant donor morbidity or tissue scarcity, resurface joints with cartilage, have no limitations in the amounts available and be easy to maintain/expand *in vitro*, be readily available, have no issues of immunogenicity or disease transmission risks and be of low cost.

Tissue insufficient supply and morbidity, and host immune responses and disease transmission risks limit chondrocyte and osteoblast as ideal cells in OC strategies. Among adult stem cells, bone marrow mesenchymal stem cells (BMSCs) [15,16,19,20,22,48–51] and adipose-derived stem cells (ASCs) [9,12•,26,48,50,52] are the most investigated. Nevertheless, some studies described a higher chondrogenic [50] and osteogenic [48] potential of BMSCs when compared to ASCs. The effectiveness of autologous BMSCs transplantation for the repair of full-thickness articular cartilage defects was assessed in patellae lesions of two human patients [53]. A similar approach was also considered to repair full thickness femoral condyle defect in an athlete, who had reattained his previous activity level and experienced neither pain nor other complications [27].

Other cell sources, including synovial tissue and periosteum-derived stem cells have also showed potential for osteogenic and chondrogenic differentiation [24,54]. Cells from synovial membrane are harvested with minimal complications at the donor site due to a high self-regenerative capability [24] of synovial tissue. The periosteum is a specialized fibrous tissue composed of fibroblast, osteoblast, and progenitor cells that may also be a possible cell source for OC TE based on its accessibility, rapid proliferation and differentiation potential [54]. Furthermore, after skeletal surgery procedures, periosteum is often used as a covering layer over tissue to stimulate local regeneration. Despite the potential, periosteum-derived cells should be more investigated for cellular therapies [55].

Umbilical cord stem cells (UCSCs) together with amniotic fluid derived stem cells (AFSCs) were also introduced to cartilage and bone TE [8,23,25] presenting interesting characteristics, since they are easier to obtain and represent an almost unlimited stem cell sources. Some risks were associated with human AFSCs harvesting but, as pregnant women are older than ever before, amniocentesis is likely to become a routine procedure in future years. More recently, cells from human foetal membranes and placenta, with similar features to human UCMSCs and AFSCs, have also been successfully differentiated into osteogenic and chondrogenic lineages [56]. Although embryonic stem (ES) cells [57] hoped for a promising future in regenerative medicine, their use is still ethically controversial and have major ethical considerations associated. Notwithstanding that human ES cells express molecules which could cause immune rejection [57] and present a high genomic instability [57], ES cell transplantation in a collagen gel has shown to induce the formation of cartilage tissue [17] under mechanical condition in rats aiming at OC regeneration.

More recently, iPS technology, where iPS cells are generated by reprogramming of somatic cells through the exogenous expression of transcription factors, holds great promise for regenerative medicine in autologous cell replacement therapies and in genetic defects by restoring cellular function [58]. Nevertheless and because of iPS recent development, cell characterization and *in vivo* functionality are to be addressed in bone and cartilage fields.

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Stem cells obtained from different sources are likely to enable the most successful outcomes in OC regenerative approaches. Besides intrinsic characteristics of stem cells from a particular source, other factors should be monitored aiming at a successful strategy; such as final application, patient age, defect location and damage size. Cell culture media to induce chondrogenesis and osteogenesis of undifferentiated cells or maintain and proliferate primary chondrocytes and osteoblasts in an *ex vivo* atmosphere are commercially available. However, a common osteochondrocytic medium to co-culture or simultaneously differentiate bone and cartilage cells was not fully established yet, although some attempts have been described [8,15,59]. This approach can be advantageous to simplify cell culturing procedures and, simultaneously, reduce the time and production costs of an engineered graft towards a clinical scenario.

Biomaterials: human designs to mimic natural extracellular material

The implantation of cells in the afflicted area could be a direct approach in OC strategies, but the request for a support material to promote regeneration, especially in large sized defects, is to be critically considered. This idea is inspired in nature itself as, in the body, the majority of cells subsist in a 3D world, anchored onto a network of extracellular matrix (ECM), which scaffolding design proposes to recreate.

Scaffold characteristics will greatly influence cells and should mimic the complex and demanding environment to which cells are exposed to. Besides the tissue structural support and stimulation, either chemically or mechanically, the optimal scaffold should assist tissue functionality promoting the easy diffusion of nutrients, growth factors and cellular waste products [60]. Additionally, the ideal scaffold should be biocompatible and its biodegradability adjustable to the time required for tissue regeneration [60].

In the last few years, thousands of scaffolds have been proposed for reparative strategies made from different materials and production methodologies, with varying properties and composition. An OC scaffold should combine the better of the two worlds in a functional and integrated system. Lots of effort has been undertaken in order to achieve this goal and the most common approach is an independent cartilage or bone strategy, likely because chondrocytes and bone cells present different function-related characteristics including metabolic and structural features, yet communicating and interacting, in a unique culturing system.

Natural based polymers such as agarose [15,61], starch [9], chitosan [9,13,14,62], silk [14], gellan gum [12•], hyaluronic acid [16], collagen [17,63] or blends of these materials [9,14,18,21•], and synthetic materials such as polylactic acid (PLA) [8], polycaprolactone (PCL) [20] and oligo-polyethylene-glycol fumarate [49] have been proposed for cartilage applications. Most of these materials are processed into hydrogel and gel based matrices, which hold particular relevance for cartilage strategies because of their high water content, tissue-like elastic properties and the ability to encapsulate cells [64]. Also, gel structures partially tolerate shock absorption and deformation mimicking articular cartilage characteristics.

However, cartilage repair in OC interfaces should be accompanied by an adequate restoration of the underlying subchondral bone, enhancing the *in situ* integration of the OC system.

The minerals and the collagen fibres in the matrix are responsible for bone hardness and resistance. Nevertheless, the constant remodelling makes bone very plastic and capable of internal structural changes according to the stresses it is subjected to. Thus, bone regeneration requires scaffolds with high mechanical and osteoconductive properties, and structurally strong enough to sustain weight bearing loads and avoid cartilage calcification, which leads to tissue malfunction and death. Scaffolds should also be biodegradable to keep up with the natural bone remodelling process. Despite the brittle behaviour and low tensile strength, inappropriate for significant torsion areas such as long bones, hydroxyapatite (HA) and tricalcium phosphate (TCP) are the most studied ceramics because of their osteoconductive and high mechanical properties, and are already used in some clinical applications [4,6]. Other materials, including silk [2,7], PCL and PCL blends [1,3,5], and PLA [8], have also been effectively tested as delivery systems [2] or artificial ECM [8], mimicking and recreating in some extent the structural organization of bone [1,3,7].

Some OC approaches successfully evaluated the *in vivo* application of scaffolds made of collagen fibrils with HA nanoparticles without implanted cells [65•], which can be of particular importance if one considers the practical and commercial standpoint, as the engineered product could be a ready-to-use graft for surgery procedures. Furthermore, this approach would avoid tissue morbidity and scarcity of autologous cell sources or even immune reactions from allogenic sources and problems related to cell culturing methodologies (e.g. animal origin supplements).

Other strategies focus on the cellular interactions of implanted cells in the tissue surroundings, considering the reduced metabolism of cartilage. Chondrocytes in adult individuals do not divide or establish cell-to-cell contacts but are responsible to produce cartilage dense ECM [34], thus maintaining cartilage integrity.

Especially in elder patients, implanted cells could meliorate the native ECM properties, and improve the functionality of damaged tissue by stimulating fresh ECM

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Table 1

Overview of the scaffold-cells constructs that have been studied for osteochondral tissue applications in pre-clinical models over the last few years

Scaffolds	Cells	Ref.
OPF with gelatin microparticle hydrogel	Cell-free/marrow mesenchymal stem cells	[49]
PCL/TCP-PCL scaffold	Cell-free/marrow mesenchymal stromal cells	[20]
Hyaluronic acid gel sponges	Autologous mesenchymal stromal cells	[16]
Hyaluronate-type I collagen-fibrin scaffold	Cell-free/autologous chondrocytes	[18]
Hyaluronic acid-atelocollagen/β-TCP bilayered scaffold	Cell free/chondrocytes	[21•]
Collagen/HA gradient scaffold	Cell free/autologous chondrocytes	[65•,66•]
Poly(lactide-co-glycolide)/nano-HA scaffold	Cell free/marrow mesenchymal stem cells	[51]
Polylactic acid (PLA)-coated polyglycolic acid (PGA) scaffold	Cell free/autologous marrow mesenchymal stem cells	[19]
Collagen/β-TCP bilayered scaffold	Cell free	[63]

production. In bony defects, the integration of cells in the implant may stimulate bone marrow cells and establish a metabolic balance favouring the neobone formation. Furthermore, in critical sized defects, cells are likely to participate in a molecular communication level bridging the native tissues to the implant towards a successful OC regeneration.

Different approaches to design an OC scaffold including hydrogels [49], combination of two distinct layers [21•,29,62,63] or a gradient scaffold [65•], usually an association of a gel or a foam and a ceramic, have been developed as alternatives to this problem (Table 1).

These complex scaffolds favour the integration into the native tissue after implantation and guide the cells, into the desired phenotype, according to the prearranged environment created from scaffold physical and chemical properties.

More recently, emerging approaches include the incorporation of bone and/or cartilage growth factors in scaffolds [49,63] to stimulate native tissue formation and differentiation *in vivo*. The inclusion of growth factors can ultimately recruit host cells into the damaged site, initiating a healing pathway, which could be promising for the treatment of OCDs.

Assisted devices: bioreactor systems

The limited diffusion in static culture environments may constrain tissue ingrowth in engineered scaffolds. Bioreactors are usually designed to control the transport of nutrients and oxygen to cells in constructs promoting cellular expansion, and in some cases, enabling mechanical stimulation of cultured cells, thus enhancing cell differentiation and ECM formation.

The challenge is, once again, finding a compromise considering the different intrinsic properties of cartilage and bone tissues. In a bioreactor system, dynamic compression should be applied for cartilage ECM stimulation while, for bone, medium perfusion is required to control mass transport and provide shear-stress to stimulate neobone formation. To overcome this issue, studies have focused on the development of double chamber bioreactors with physical separation; described to fulfil the needs of tissue-specific mechanical forces for OC stimulation [28,29].

The next step, barely explored, would be the automation of bioreactors controlled by computer software. The customization of engineered grafts through the development of anatomically moulded surfaces [61] has showed potential results headed to translational OC interfaces. As follows bioreactors would be a reliable system of automation and standardization of cell and scaffold methodologies reducing the time and production costs of functional custom-designed grafts.

In vivo models for osteochondral tissue engineering

Animal studies still represent an essential tool to understand the biologic behaviour of healing and tissue regeneration *in vivo*, though differences in the anatomy and metabolism of animal models must be considered in an experimental setup with human correlations.

Different animal models have been used in OC studies [16,18–20,21•,49,51,63,65•]. Rats present distinctive characteristics, such as athymic nude or transgenic animals, not easily available in larger animal models. This model has been used to test the efficacy of a poly(lactide-co-glycolide)/nano-HA scaffold seeded with undifferentiated mesenchymal stem cells in OC defects [51]. After 12 weeks, defects treated with these constructs showed smooth and hyaline cartilage with abundant glycosaminoglycan and collagen type II deposition.

Rabbit also demonstrated to be a successful model for OC [16,20,21,49], especially in femoral regions with the successful application of hyaluronate-atelocollagen/beta-TCP-hydroxyapatite scaffolds in the patellar grove [21•], which promoted, in some extent, OC regeneration without the formation of fibrocartilage.

Sheep is also a popular animal model because of their weight-bearing limbs and with metabolic and bone remo-

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delling rates similar to that of humans as well as the sequence of events in bone graft incorporation and healing capacities. An OC interface was evaluated in sheep by the implantation of a composite scaffold of collagen and HA with or without autologous chondrocytes into a condyle critical defect [65•]. Both conditions showed to support neobone formation and hyaline-like cartilage regeneration. With a similar implant, collagen/TCP, OC regeneration was evaluated in the trochlear groove of minipigs [63]. Although cells were absent in this strategy, the *in situ* incorporation of growth factors in the construct leads to fibrocartilage formation and partial reconstruction of the subchondral bone integrity in a short-term follow-up.

A pilot clinical trial with 13 patients using the collagen/HA cell free tri-layer scaffold [66•] mentioned above indicated promising results with tissue recovery in some extent after a six-month follow-up.

Conclusions

The currently available treatments based on 'damage to heal approaches', have a limited success. With an increasing aging population, tissue engineering strategies provide important cues and hope for the treatment of OC degeneration. Ultimately, the tissue engineered implant should be able to stimulate and replace old tissue and native lethargic cells in order to accomplish both regeneration and restoring functions for a successful clinical achievement.

The challenge stands for the replication of the natural functional architecture and the translation of promising strategies towards patient needs. Success lies on the delicate balance of cartilage and bone characteristics combined in an engineered graft, and its integration *in vivo*. The implant must participate in the regenerative process, considering the specific properties of each OC interface, which can only be achieved through the design of scaffold materials accommodating the specific characteristics of bone and cartilage tissues, and providing stem cells with the necessary cues to satisfy both tissue cellular needs. The application of cells in critical defects or elder patient injuries is likely to be beneficial in stimulating native cells into the regenerative process. The use of bioreactors can improve the functionality of such constructs, accelerate the production, create custom-made systems, and reduce time costs for obtaining implants for OC applications.

Conflict of interest

Authors declare no conflict of interest.

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