Abstract

Introduction
Articular cartilage can often get damaged due to degenerative joint disease and congenital anomalies. Due to the limited self-healing ability of articular cartilage, current joint repair focuses on surgical techniques, which are limited in their capacity to restore previous function and structure. Tissue engineering techniques, however, may hold promise to return the cartilage tissue to its native normal state. The purpose of this review is to describe developments in articular tissue engineering and determine their potential use in clinical application. As such, this review will discuss how limitations in early cellular therapy (autologous chondrocyte implantation) have lead to investigations into adult mesenchymal stem cells and embryonic stem cells for articular cartilage restoration. This report also discusses extracellular investigations including how natural and synthetic scaffolds can be applied as a method to simulate cartilage regrowth. Success in using mechanical loading to enhance chondrocyte function and induce stem cell chondrogenesis through the use of bioreactors will also be discussed.

Conclusion
The authors conclude by predicting how future research into seeding cell growth factors and scaffolds may push forward tissue engineered articular cartilage for clinical application.

Discussion

Autologous chondrocyte implantation
Chondrocytes are responsible for the production and maintenance of the extracellular matrix components (collagen Type II and proteoglycans), hence considered a good cell choice for cartilage restoration. The first human tissue engineering application to restore cartilage defects has utilised chondrocytes. Originally described by Brittberg et al. in 1994 and Grande et al. in 1995 autologous chondrocyte implantation (ACI) involves harvesting autologous chondrocytes, expanding them in mono-culture and reimplanting the chondrocyte suspension into the patient’s chondral defects 6 to 8 weeks later, sutured under a watertight periosteal patch. Despite ACI being widely used among orthopaedic surgeons and clinical studies showing favourable results, for young patients and has considerable functional limitations even for an inactive individual.

For a more effective, long-term solution, techniques replacing chondral lesions with cells which are able to produce cartilage that become fully integrated with host cartilage are being actively investigated. Over the last two decades, tissue-engineering strategies have been called upon to achieve this by combining cells, growth factors and scaffolds with appropriate mechanical stimulation. The aim of this review is to summarise the advances in engineering cartilage and to identify the limitations and challenges that remain to be addressed for successful repair and regeneration of articular cartilage for clinical application.
periosteal hypertrophy, arthrofibrosis and delamination of the graft have all been reported. A recent Cochrane review has highlighted that there is insufficient evidence to draw conclusions on the use of ACI for treating full thickness articular cartilage defects in the knee and that further good quality randomised controlled trials with long-term functional outcomes are required. Furthermore, there are many problems with the ACI technique such as obtaining enough autologous chondrocytes during harvesting, loss of cellular differentiation potential when cultured in vitro, and decreased capacity to produce hyaline extracellular matrix (ECM). Utilising tissue-engineering and biomaterials principles may refine ACI and allow greater success. Specifically, considering different cell sources, modifying the transport medium of the cells (the scaffold material), providing optimal signalling molecules to produce a stable cell phenotype and appropriate culturing environment though mechanical stimulation ACI are increasingly investigated (summarised in Figure 1).

**Stem cells**
Mesenchymal stem cells (MSCs) are being considered for many tissue-engineering applications due to their capacity for self-renewal and multidifferentiation potential. Due to ease of harvesting and the ability to undergo proliferation without losing their capacity to differentiate into articular cartilage, MSCs have been considered ideal for repairing cartilage defects. MSCs obtained from the bone marrow have already been clinically utilised to restore auricular defects. Warkitani et al. obtained 10 ml of bone marrow from the iliac crest and expanded the bone marrow mesenchymal stem cells (BMMSCs) in vitro without any chondrogenic inducing media. After 20 days, the cells were attached to a collagen gel, supplemented with antibiotics and autologous serum, and implanted into the medial femoral condyle defect of 12 patients covered by autologous periosteum. Forty-two weeks after the transplantation, the defects were covered with soft white tissue and hyaline cartilage was partially observed. The arthroscopic and histological grading was better despite no difference in clinical score with the cell-transplanted group compared to the cell free group. The same group utilised BMMSCs for a further 18 patients and demonstrated success for articular defects. The authors have recently confirmed that after 11 years and 5 months, no tumours or infections have been reported in the 41 patients followed, highlighting the long-term safety of the procedure. In addition, Nejadnik et al. has recently compared ACI against BM MSCS and found no difference in clinical outcomes at 24 months in 72 matched (lesion site and age) patients. Other sources of stem cells are being considered including adipose stem cells (ADSCs) and embryonic stem cells (hESCs). ADSCs have been shown to differentiate into chondrocytes and produce cartilage specific matrix components. With greater abundance, easier isolation method and reduced donor site morbidity than BMMSCS, several studies are highlighting the potential for these cells in cartilage engineering. However, the reliability of chondrogenic differentiation of ADSCs and the optimal method for inducing differentiation is still to be identified before utilisation of such cells in clinical studies. Furthermore, studies have shown decreased chondrogenic potential of ADSCS compared with BM MSCS. The use of hESCs is considered to be a favourable option as the cells could be used as an “off the shelf” product, omitting the need for the harvesting procedure and decreasing cost and donor site morbidity. However, current culturing techniques of hESCs have shown limited capability of providing large quantities of chondrocytes, with

**Figure 1:** Left: Process of autologous cartilage implantation (ACI). Autologous chondrocytes are harvested from the patient and then expanded in vitro for 3 to 4 weeks. The chondrocyte suspension is then implanted via a periosteal patch at the site of the chondral defect 6 to 8 weeks later at the second operation. Right: The tissue engineering improvements that are being considered for ACI including the use of stem cells, addition of growth factors, providing mechanical stimuli during implantation or modifying the scaffold material.

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**FOR CITATION PURPOSES:** Griffin M, Butler PE, Seifalian AM, Szarko M. Update into articular cartilage tissue engineering. OA Musculoskeletal Medicine 2013 Dec 01;1(3):28.

Competing interests: none declared. Conflict of interests: none declared. All authors contributed to the concept on, design, and preparation of the manuscript, as well as read and approved the final manuscript. All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.
difficulty in determining their end phenotype\(^\text{15}\).

**Scaffolds**

There are a multitude of natural and synthetic polymers being considered for cartilage tissue engineering. Natural polymers including collagen, silk, chitosan, cellulose, hyaluronic acid, fibrin glue have been utilised for cartilage engineering. Synthetic polymers including polyactic acid (PLA), polyglycolic acid (PGA) and polyethylene glycol (PEG) have been used to form cartilage scaffolds. Biomaterial scaffolds must be able to provide a structure to support cell attachment, migration, proliferation, differentiation and production of a cartilage matrix until new cartilage is formed\(^\text{16}\). Biodegradable polymers are considered to be a favourable option as the polymer does not need to be removed through a surgical procedure at a latter date\(^\text{17}\). Porosity, pore size and interconnectivity of scaffold materials are important parameters to consider when choosing the material as they determine chondrocyte migration and diffusion of nutrients and oxygen which support cell function\(^\text{18}\). They determine chondrocyte migration, proliferation, differentiation and production of a cartilage matrix until new cartilage is formed\(^\text{16}\). The efficacy of such scaffolds for articular cartilage repair in the midterm\(^\text{22}\). Fillardo et al. in 2013 reported the combined treatment of Chondro-Gide\(^\text{®}\) with microfracture to be a valid and safe cartilage repair option for small- to medium-sized cartilage defects of the knee\(^\text{20}\). However, further studies with long-term follow-up are needed to determine whether the grafted area will maintain structural and functional integrity over time\(^\text{21}\). Hyaluronan-based scaffolds have also been investigated for cartilage engineering due to its high concentration in the native ECM of articular cartilage. Hyalograft\(^\text{®}\) C, a commercially available hyaluronan based scaffold, has shown to support cartilage repair in the midterm\(^\text{22}\). Filardo et al. illustrated good clinical outcomes at 7 years in 62 patients with chondral lesions of the femoral condyles with an average size of 2.5 ± 1.0 cm\(^2\) treated with arthroscopic implantation of the bioengineered tissue\(^\text{22}\). A better outcome was found in young active men without prior surgery\(^\text{22}\). Further randomised clinical trials with long-term follow-up are still required to confirm the efficacy of such scaffolds for articular reconstruction.

**Signalling molecules**

Several chemical stimuli including cytokines, growth factors and hormones have shown important in vivo for regulating chondrocyte behaviour. There, a number of growth factors including transforming growth factor (TGF-β), insulin-like growth factor (IGF-1), bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs), which have been considered to promote the chondrocyte phenotype\(^\text{21,23}\). Growth factors from the TGF-β family have shown to play an important role in chondrocyte proliferation, apoptosis and differentiation. TGF-β1 has produced a hyaline cartilage by enhancing collagen Type II and glycosaminoglycan expression whilst reducing collagen Type I expression from stem cells\(^\text{23,24}\). BMPs have shown to be important in both chondrogenesis and osteogenesis\(^\text{23,24}\). BMP-4 transduced MSCs have acquired a chondrocytic phenotype in vitro and significantly improved articular cartilage repair in rats\(^\text{25}\). BMP-7 transfected BM-MSCs have shown the capability of expressing glycosaminoglycan and collagen Type II\(^\text{26}\). IGF-1 is important for proteoglycan synthesis and breakdown of chondrocytes and cell homeostasis\(^\text{27}\). The combined treatment of BMP-2 and IGF-1 has enhanced chondrogenesis of ADSCs, which showed mature chondrocyte-like cells and formed cartilage nodules\(^\text{27}\). These cells also produced Type II collagen with a reduced production of matrix metalloproteinase-3\(^\text{27}\).

Despite the amount of research into these signalling molecules, several questions remain regarding their potential use in cartilage regeneration. For instance, the optimal choice of specific growth factor, importance of co-delivery of growth factors, the dosage required and long-term efficacy of these stimulating factors remain unknown. Ha et al. performed a Phase I clinical trial for localized delivery of allogeneic chondrocytes expressing TGF-β1 directly to the damaged knee joint, TissueGene-C (TG-C)\(^\text{28}\). Untransduced human chondrocytes (hChon) cells were also incorporated into the TG-C product at a 3:1 ratio to help fill in the defect and as target cells for the actions of the expressed TGF-β1\(^\text{28}\). Knee evaluation scores seemed to indicate a dose-dependent trend toward efficacy; however, patient numbers were not sufficient to determine statistical significance\(^\text{28}\). Swelling, effusion...
and minor localised reactions such as warming sensation or itching was also observed in a dose-dependent manner at the injection site. It is clear that further understanding is required before growth factors can be clinically used to maintain a chondrocyte phenotype in human studies.

**Mechanical stimulation**

The optimal goal of tissue engineering cartilage is to produce cartilage, which mimics the original tissue function and structure. Recently, mechanical stimulation has shown to be important in the development of functional cartilage tissue. Articular cartilage in vivo is affected by several mechanical forces including direct compression, tensile and shear forces, and hydrostatic pressure. These mechanical forces are required to maintain cartilage structure and function. How chondrocytes sense these biomechanical forces and convert them into intracellular signals is currently under extensive investigation in hope to fully appreciate the mechanisms behind load-induced changes in cells.

Cells sense mechanical forces from the ECM by converting them into biological signals by restructuring their cytoskeleton. Mechanical stimuli are detected by transmembrane receptors (integrins) acting as a bridge between the cytoskeleton and the ECM. Once these mechanotransduction pathways are triggered their regulate gene expression and protein production, consequentially controlling cell apoptosis, migration, proliferation, differentiation and the release of various autocrine/paracrine molecules. Mechanical stimuli can also be transduced to the cell by the activation of various cell membrane ion channels and transporters. For example, an influx of Ca \(^+\) has been reported following cell deformation from shear stress and hydrostatic pressurisation, which is known to act as a second messenger in numerous signalling pathways.

Most investigations into the effect of mechanical stimulation on chondrogenesis apply either static or dynamic uniaxial compression to mimic the direct compression of two joint surfaces in vivo. Dynamic compression has increased the glycosaminoglycan and hydroxyproline content of chondrocytes grown on agarose gels, which results in a tissue with a greater elastic modulus. Dynamic compression has also induced MSC chondrogenic differentiation and increased collagen II and aggrecan expression. As a joint is loaded, hydrostatic pressure (HP) is exerted from the interstitial fluid in the joint. Cyclic and static HP has shown to increase chondrocyte production of matrix components. HP combined in the presence of TGF-β with has also been applied to MSCs and been reported to effectively initiate differentiation towards a chondrocytic phenotype.

To mimic the natural environment, bioreactors are now being explored to induce combined complex types of mechanical stimuli. Bioreactors systems replicate the in vivo environment providing precise control on nutrient transport growth factors, oxygen tension and mechanical stimulation. Traditionally bioreactors were thought to precondition implants to improve their quality before implantation. However, bioreactors have provided the opportunity to study the cellular response to mechanical stimulation under precise conditions. For instance, bioreactors that induce shear stress and compression have shown to enhance the production of the ECM components by human chondrocytes including collagen Type II, sulphated glycosaminoglycan and hydroxyproline content. The present understanding of how mechanical stimuli affects cartilage regeneration is still limited predominantly by (1) the type of load applied in studies being varied which makes comparisons difficult, (2) the use of custom-built machines that are not validated making comparisons problematic and (3) the limited knowledge of how in vivo cells respond to multiple mechanical forces.

**Conclusion**

Tissue engineering is beginning to provide alternative solutions for healing damaged articular cartilage. However, there are still many issues that need to be addressed before engineered cartilage is a clinical therapy. The optimal cell source needs to be determined for in vivo cartilage engineering. Although stem cells are favourable over chondrocytes due their abundance and ease of isolation, the capacity of long-term cartilage formation with original function and structure must be determined. Second, the optimal scaffold to support in vivo chondrogenesis remains to be defined with several natural and synthetic scaffolds materials currently emerging and being investigated. Though a few natural scaffolds have been tested clinically, lack of in depth clinical evidence highlights that current biomaterials are not fulfilling all the requirements for effective cartilage engineering. The use of signalling molecules such as growth factors is still in its infancy with multiple questions remaining about correct choice, need for multiple simultaneous growth factors, and optimal dosages.

The role of mechanical stimuli in cartilage formation has become an important and extensively researched area of work with evidence demonstrating that mechanical stimuli aids in the production of cartilage with appropriate mechanical properties and ECM components. However, further understanding into how cells translate biomechanical stimuli will allow researchers to provide the correct mechanical stimuli for optimal cartilage engineering. With these ongoing developments, cartilage tissue engineering will provide the future techniques to repair articular cartilages for the clinical setting.

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