■ REVIEW ARTICLE

Articular cartilage tissue engineering

TODAY'S RESEARCH, TOMORROW'S PRACTICE?

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Articular cartilage repair remains a challenge to surgeons and basic scientists. The field of tissue engineering allows the simultaneous use of material scaffolds, cells and signalling molecules to attempt to modulate the regenerative tissue. This review summarises the research that has been undertaken to date using this approach, with a particular emphasis on those techniques that have been introduced into clinical practice, via *in vitro* and preclinical studies.

The ability to regenerate articular cartilage remains a challenging goal for researchers and surgeons. The incidence of articular cartilage pathology is increasing, and as a result more cartilage repair procedures are being performed each year.¹ As yet, no procedure has the ability to reliably reproduce the biological composition and biomechanical properties of hyaline cartilage.

Current repair strategies vary according to the nature and size of lesion and the preference of the operating surgeon. The most readily available technique is marrow stimulation, of which microfracture has gained in popularity since its inception in the late 1990s.² Following subchondral bone perforation, progenitor cells are released into the defect from the marrow cavity, producing a fibrocartilaginous repair. Satisfactory results have been reported,³⁻⁵ but some studies have shown a reduction in outcome after two years, probably as a result of the inferior mechanical characteristics of the fibrocartilaginous repair tissue.⁶

A number of studies have compared microfracture to autologous chondrocyte implantation (ACI). Knutsen et al⁷ found no difference in outcome at five years, but the failure rate of ACI was less in those whose histology was superior at a one-year biopsy. Saris et al⁸ have recently shown that characterised chondrocyte implantation (CCI, Tigenix, Leuven, Belgium) results in a superior structural repair to microfracture at one year, but with a similar clinical outcome. Three-year results have since been presented showing an improvement in clinical outcome over time with CCI.⁹

Osteochondral autograft transfer (OAT) removes plugs of hyaline cartilage with underlying

subchondral bone from an unaffected area of the knee and implants them into the chondral defect, often at the expense of donor site morbidity.¹⁰ Although hyaline cartilage is replaced, it is neither biomechanically or topographically the same as at the recipient site. The OAT procedure is associated with technical difficulty, poor tissue integration, chondrocyte death from plug impaction, and loss of normal articular topography.¹¹ As a result, it is less used than the former procedures. Good clinical results have been published, but these vary between reports.^{12,13}

Fresh osteochondral allografting can often circumvent a number of the issues that surround OAT procedures. Larger cartilage defects can be replaced with topographically and biomechanically similar tissue. Although good to excellent results in 75% to 80% of patients have been reported, tissue availability and the risks of disease transmission remain a concern.¹⁴

Tissue engineering strategies are now being employed, with the goal of improving the quality and longevity of repair tissue and ultimately patient outcome. In a previous review article, the principles of tissue engineering were described, with particular attention to the properties that materials should ideally possess for articular cartilage repair.¹⁵ The purpose of this review is to outline examples of the materials and biological additives that have been investigated in both *in vitro* and *in vivo* preclinical models and, where possible, to provide examples of their clinical use. This will attempt to demonstrate how tissue engineering may form the basis of future innovations in regenerating hyaline cartilage.

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The building blocks of tissue engineering

Hunziker¹⁶ describes tissue engineering as “the art of reconstituting mammalian tissues, both structurally and functionally”. Cells and/or bioactive molecules are delivered to a defect via a biomaterial scaffold to achieve tissue regeneration. In articular cartilage research there have been extensive studies performed both *in vitro* and *in vivo*, the results of which are now beginning to shape clinical practice. The following will detail the key concepts in each area of articular cartilage engineering.

Scaffolds. The purpose of using biomaterial scaffolds in tissue-engineered constructs is to mimic the 3D environment of the extracellular matrix, provide structural support to the regenerate and surrounding tissues, and provide an increased surface area to volume ratio for cellular migration, adhesion and differentiation.¹⁷

There are a number of essential requirements. They must be biodegradable, with non-toxic byproducts, and exhibit favourable resorption kinetics to maintain initial stability, but they should not hinder further tissue regeneration.¹⁸ They must be able to fix to the defect site, facilitate cell attachment and regulate cell expression.¹⁹ Porosity and interconnectivity are important to allow cell migration and the passage of nutrients and waste products. O'Brien et al²⁰ showed that pore size, porosity and interconnectivity affect cell adhesion and proliferation in collagen/glycosaminoglycan scaffolds. The optimum pore size to facilitate this has been shown to be between 100 µm and 500 µm.²¹

There are a number of biomaterial options for application to articular cartilage tissue engineering, which can be natural or synthetic.

Natural material scaffolds. Natural scaffolds provide a more normal environment for cell adhesion and proliferation and may be further subdivided into protein-based matrices such as collagen and fibrin, and carbohydrate-based matrices such as alginate, agarose, chitosan and hyaluronan.

Collagen is the major protein constituent of connective tissue.²² The basic molecular subunit is tropocollagen, which consists of three polypeptide chains wound together in a triple helix that forms its tertiary structure. Collagen possess ligands, which facilitate cell adhesion and can influence cell morphology, migration and differentiation.²³ The abundance of functional groups along its backbone also allow for interaction with other molecules, such as growth factors, which may be used in combination in tissue engineering applications. Although ACI was originally described using a periosteal patch over the defect,²⁴ collagen is also now used. An example is Chondro-Gide (Geistlich Biomaterials, Wolhausen, Germany), a mixture of type I and type III porcine collagen produced in a bilayered structure which allows chondrocyte adhesion while maintaining a watertight patch over the chondral defect.²⁵ This concept has evolved further to matrix-assisted chondrocyte implantation (MACI) (Genzyme, Oxford, United Kingdom), where chondrocytes are expanded on the collagen membrane *ex vivo* then reimplanted. A study by Bartlett et al²⁶ has shown

that both ACI and MACI produce similar results at two years. Dorotka et al²⁷ have also shown how a collagen type I membrane can be used to support cell migration and adhesion in a microfracture defect in a goat model. They compared this with microfracture only, as well as with microfracture in combination with a chondrocyte-seeded scaffold. Histologically, the chondrocyte-seeded scaffold was superior, but microfracture augmented with a collagen scaffold was significantly superior to microfracture alone. This has led to the clinical use of Chondro-Gide as a scaffold augmentation of microfracture, the so-called autologous matrix-induced chondrogenesis (AMIC).²⁸ Initial reports have been favourable, especially at the patellofemoral joint; however, longer term data are awaited.²⁹

Fibrin is formed from a reaction between fibrinogen and thrombin, producing a natural 3D matrix which has favourable biodegradability characteristics producing non-toxic physiological substances. Fortier et al³⁰ and Nixon et al³¹ have successfully used fibrin composites as 3D scaffolds to support chondrocytes and mesenchymal stem cells *in vitro*, and in equine cartilage defects *in vivo*. The same group further showed that chondrocytes in a fibrin composite could be upregulated by insulin-like growth factor 1 (IGF-1), producing a greater amount of tissue with increased extracellular matrix and collagen II production.³²

Alginate is an anionic polysaccharide derived from seaweed. In the presence of calcium cations, alginate chains are cross-linked through ionic bonding. When cells are dropped in calcium chloride solution in the presence of alginate, beads are formed. *In vitro*, these 3D alginate cultures are particularly useful as they aid re-differentiation of de-differentiated chondrocytes which have lost their phenotype due to monolayer expansion.³³ If calcium sulphate is used instead, a slower reaction results which provides the potential for *in vivo* injectable options.³⁴ Diduch et al³⁵ have demonstrated chondrogenesis in mesenchymal stem cells supported within alginate beads in rabbit osteochondral defects. However, there are concerns over the biocompatibility of alginate, which is not widely used in clinical practice.³⁶

Hyaluronan is a component of the extracellular matrix which stimulates chondrogenesis in mesenchymal stem cells.³⁷ It requires cross-linking by esterification or other chemical means, in order to produce a construct able to support articular cartilage repair, but this process changes its biocompatibility, which can create degradation products that can cause chondrolysis.³⁸ Hyaff-11 is an esterified hyaluronan scaffold which has been used extensively in clinical articular cartilage repair. Marketed as Hyalograft C (Fidia Farmaceutica, Abano Terme, Italy), the combination of culture-expanded chondrocytes and Hyaff have shown good results at three years comparable to those of standard ACI.³⁹ Minimal exposure is required, as Hyaff does not require further fixation to subchondral bone.⁴⁰ Results in patellofemoral lesions have also been found to be satisfactory, with International Knee Documentation Committee (IKDC)⁴¹

scores improving from 18% Class A or B to 90% at two years.⁴²

Chitosan is a bi-copolymer of glucosamine and N-acetylglucosamine. Its degradation products, which include chondroitin sulphate, dermatan sulphate, hyaluronic acid, keratin sulphate and glycosylated type II collagen, are non-toxic and are involved in the synthesis of articular cartilage.^{43,44} Its cationic nature and hence its high charge density in acid solution allow for water-insoluble cationic complexes to form with a variety of polyanionic substances.⁴⁵ This has significant application in the delivery of growth factors. An example of its clinical use is BST Cargel (Biosyntech, Quebec, Canada), which is a chitosan/glycerol copolymer hydrogel that is mixed with blood and injected into a chondral defect following microfracture. Results in sheep⁴⁶ and rabbits⁴⁷ have shown an improvement over microfracture alone. Results from an ongoing human trial are awaited.

Synthetic material scaffolds. Synthetic materials have been used extensively both *in vitro* and *in vivo*, partly because of their acceptance by the American Food and Drug Administration for their use as suture material over 20 years ago. They include polylactic acid (PLA), polyglycolic acid (PGA) and their derivatives, for example poly(lactic-co-glycolic) acid (PLGA). They have been popular because of their easy moulding characteristics, relatively easy production, and the ability to control dissolution and degradation.¹⁷ However, their major flaw is biocompatibility. They are broken down by a hydrolytic reaction, thereby high concentrations of acidic byproducts and particulates can be released, causing inflammation,⁴⁸ giant cell reaction⁴⁹ and chondrocyte death owing to a reduction in pH.⁵⁰ They also do not possess natural sites for cell adhesion, and so these often need to be added.

Williams and Gamradt⁵¹ have recently reported a series of patients who were treated with the Trufit CB osteochondral plug (Smith & Nephew, San Antonio, Texas). Trufit is a biphasic synthetic plug made of poly-L-lactide glycolide (PLG), which is supplemented with calcium sulphate. It has been designed as an 'off the shelf' osteochondral scaffold for the treatment of small, isolated full-thickness osteochondral defects. Although concerns have been raised over its biocompatibility, results at 12 months have been favourable, however, longer term data are required.

Cells. It is unclear which cell type is optimal for articular cartilage tissue engineering. The chondrocyte is the predominant cell type, but has limited potential for intrinsic repair. Adult mesenchymal stem cells, on the other hand, are readily available and possess the ability to differentiate into a number of different cell types.^{52,53} Controlling the differentiation of either of these cell types may be the key to producing quality repair tissue. The use of embryonic stem cells and induced pluripotent stem cells in articular repair is very much in its infancy and will be discussed in more detail later.

A. Chondrocytes. Brittberg et al²⁴ published the original method of using chondrocytes in suspension under a watertight periosteal patch in autologous chondrocyte implantation. In

this technique, following a cartilage biopsy cells are expanded in monolayer, which has been shown to cause de-differentiation of chondrocytes to a more fibroblastic phenotype.¹⁶ De-differentiated chondrocytes have not been shown to re-differentiate in a 2D system or in solution, as opposed to successful re-differentiation in a 3D matrix or high-density composition.^{33,54} As a result, the ability to regenerate hyaline cartilage has not been achieved in a reproducible fashion by this method, although some good results have been reported.^{11,26,55,56}

It is also unclear from which cell source the repair tissue is generated. Breinan et al⁵⁷ have shown that similar results are found when ACI is compared to implantation of a periosteal patch without chondrocytes in a canine model. It may be that the periosteal progenitor population, as well as communication with the bone marrow, is responsible for this repair tissue. However, Dorotka et al²⁷ have shown in a goat model that collagen membranes augmented with a homogeneous population of chondrocytes, added to a microfracture defect, create significantly better repair tissue than microfracture and collagen alone (AMIC), in terms of defect tissue filling and histological grade. These results suggest that adding chondrocytes to a 3D collagen matrix may be a key factor in producing quality repair tissue. The $\alpha 1\beta 1$ integrin in collagen has been shown to be essential for cell survival and can be up- or down-regulated with the addition of certain growth factors.^{58,59} In addition, Cao et al⁶⁰ have shown that deprivation of collagen-derived signals can induce chondrocyte apoptosis. Alternatively, it may be that chondrocyte and progenitor cell co-culture has a symbiotic relationship with regard to matrix production.

Another method of maintaining the chondrogenic phenotype is CCI. This involves a cell-surface marker profile predictive of the capacity to form hyaline-like cartilage *in vivo* in a constant and reproducible manner. As previously described, Saris et al⁸ have compared this technique of cell expansion and implantation to microfracture, and found superior histological scores with equivocal clinical outcome scores at one year. It remains to be seen whether this histology translates to increased longevity or superior outcome.

The major drawback of using autogenous chondrocytes is the need for a surgical procedure to procure them, and the methods used to expand them *in vitro* prior to implantation. Traditionally, bovine serum has been used in the cell expansion medium to provide essential growth factors that support cell metabolism and enhance proliferation. Concerns over infectious contamination and antigenicity have resulted in a shift towards using serum-free media containing appropriate growth factors to allow proliferation with minimal de-differentiation.⁶¹ Techniques, including using human serum,⁶² and other growth factors to maintain the expression of the transcription factor SOX9, which is vital for maintaining a chondrogenic lineage,⁶³ have been investigated and will be discussed in the following sections.

Allogeneic chondrocytes may provide a solution to the availability issue, but little research has been published on their use. Almqvist⁶⁴ presented encouraging results from a human pilot study in which allogeneic chondrocytes were used in an ACI-type application. At 12 months' follow-up satisfactory repair tissue was formed and no adverse events were reported.⁶⁴ Chondrocytes have been shown to be immunoprivileged when surrounded with extracellular matrix.⁶⁵ In ACI applications the fact that the graft is avascular and the cells are sealed off from the synovium via a collagen patch may provide a degree of immunoprotection.

B. Stem cells. Stem cells have the 'capacity for self-renewal or unlimited self-renewal under controlled conditions', and 'they retain the potential to differentiate into a variety of more specialised cell types'.⁶⁶ Therefore, these are cells with multipotent differentiation capacity.⁶⁷ There are a number of stem-cell sources, of which embryonic stem cells and induced pluripotent stem cells have recently gained most attention. However, it is the adult mesenchymal stem cell that is of most interest in articular cartilage repair. Since Friedenstein's⁶⁸ early pioneering work, where he demonstrated that bone marrow cells were capable of osteogenesis, much research has focused on the use of adult mesenchymal stem cells. They represent an autologous supply of cells which can be easily harvested from a number of different tissues, including bone marrow, adipose tissue, muscle, periosteum, and synovium.¹⁶ Many studies have compared these sources in terms of their chondrogenic ability,⁶⁹ with several focusing on comparisons between adipose tissue and bone marrow, of which bone marrow-derived cells have shown superior results.^{70,71} Bone marrow is the most readily available as it can be easily harvested in a relatively non-invasive manner. The rest of this section will therefore concentrate on bone marrow-derived mesenchymal stem cells and their application in *in vitro* and pre-clinical research.

In vitro, mesenchymal stem cells have been shown to differentiate into chondrocytes under certain culture conditions.⁷² The application of growth factors such as fibroblast growth factor 2 (FGF-2) and transforming growth factor β 1 and 3 (TGF- β 1/TGF- β 3) has been particularly useful. FGF-2 has been shown to aid the expansion of mesenchymal stem cells,^{73,74} whereas all three growth factors have demonstrated the ability to push mesenchymal stem cells down a chondrogenic lineage, maintain the chondrogenic phenotype and prevent the cells from becoming terminally differentiated.⁷⁴⁻⁷⁷ What is not evident *in vivo* is whether the mesenchymal stem cells actually differentiate into host cells⁷⁸ or whether it is their trophic affect on host cells via growth factor and cytokine release that mediates their mode of action.^{79,80}

The majority of *in vivo* research using mesenchymal stem cells has used bone marrow-derived cells in rabbits. Encouraging results have been shown with a number of studies, each using different carrier systems, with improvements in histological and biomechanical endpoints.⁸¹⁻⁸³

These groups all employed culture-expanded mesenchymal stem cells; however, Solchaga et al⁸⁴ used a fibronectin-coated hyaluronan-based sponge as a carrier for bone marrow into full-thickness defects in rabbits, without the isolation and expansion of mesenchymal stem cells. At all time points, no statistical difference in histological grade was seen between control and treatment groups. Similar findings were established in a large animal equine study by Wilke, Nydam and Nixon⁸⁵ in which bone marrow in a fibrin construct was injected into osteochondral defects under gas arthroscopy. Early healing was found to be improved as assessed arthroscopically at 30 days, but there were no differences in histology scores at eight months. This may be due to the small number of mesenchymal stem cells present in the constructs owing to lack of concentration; Pittenger et al⁷² showed that in humans bone marrow consisted of only about one progenitor cell in every 105. In order to address this, Fortier et al⁸⁶ concentrated the total nucleated cell number from bone marrow aspirate using a density centrifugation method (SmartPREP II, Harvest Technologies, Plymouth, Massachusetts). This concentrates the mesenchymal stem cells which are resident in the nucleated cell population. Using the same fibrin construct, full-thickness defects were treated in 12 horses. At second-look arthroscopy at 12 weeks, improved scores were found in treatment over control groups. At eight months, both macroscopic, histological, and MRI (T1rho and T2 mapping) scores were significantly improved in treatment over control groups. This technique is encouraging because of the ability to concentrate cell numbers in the operating theatre, thereby negating the need for cell culture facilities.

A small number of studies using mesenchymal stem cells in the human population have been published. In a pre-clinical study, Wakitani et al⁸⁷ treated 6 mm diameter full-thickness defects in rabbits with culture-expand bone marrow-derived mesenchymal stem cells embedded in collagen type I gels. At two weeks they claimed that the mesenchymal stem cells had differentiated into chondrocytes, with biomechanically superior tissue produced at 24 weeks over control defects. Using the same culture and transplant system, 24 human patients underwent high tibial osteotomy and cartilage grafting on the medial femoral condyle.⁸⁸ Of these, 12 had mesenchymal stem cell-seeded defects which, at 42 weeks, were found to have a cartilage-like appearance with hyaline-like cartilage on biopsy. However, this was not statistically different from defects without cells. In 2007, the same group published a case series using the same system, this time treating patellofemoral defects.⁸⁹ Although knee outcome scores had improved with time, biopsy revealed fibrocartilage.

Allogeneic mesenchymal stem cells represent an 'off-the shelf' option for cartilage repair. They have been shown to be immunoprivileged, therefore it is possible to deliver them *in vivo* without rejection.^{90,91} They have also been shown to produce cytokines, which may help modulate the repair process *in vivo*.⁹² Thus far, preclinical trials have

been encouraging, but it remains to be seen whether this type of cellular therapy may become commonplace in clinical practice.

Both chondrocytes and mesenchymal stem cells are troubled with fibroblastic de-differentiation and terminal differentiation to a hypertrophic phenotype *in vivo*. It is therefore likely that these cell types will require some degree of modulation to be applied successfully. This may be provided by the addition of growth factors.

Growth factors. Both chondrocytes and mesenchymal stem cells are influenced by signalling molecules within the extracellular matrix which include hormones, cytokines and growth factors. An imbalance between the anabolic and catabolic signalling factors has a significant impact on the development of osteoarthritis.⁹³ This interaction therefore also plays a significant role in the regenerative process. The ability to combine growth factors with cells and scaffolds to produce more phenotypically suitable tissue-engineered constructs is an exciting prospect. A number of different growth factors have been demonstrated to have an impact on articular cartilage repair, but it is how these are used that holds the key for tissue regeneration.

A. Transforming growth factor- β (TGF- β). This is a member of the TGF superfamily, which also includes the bone morphogenetic proteins (BMPs). It has been researched extensively in both bone and cartilage regeneration and is involved in the development and homeostasis of various tissues.⁹⁴ It is secreted in an inactive form, bound to a latency-associated peptide from which it dissociates before becoming active and binding to its target receptor.⁹⁵ Although three isoforms exist, TGF- β 1 is the most widely investigated.

In articular cartilage, *in vitro* studies have shown TGF- β 1 to induce mesenchymal cell stem differentiation to chondrocytes,⁹⁶ promote cell proliferation⁹⁷ and protein synthesis,⁹⁸ and inhibit the actions of matrix metalloproteinases.⁹⁹ However, it does have negative effects such as fibrosis and osteophytosis if given in higher doses.^{98,100}

In vivo, a number of studies using rabbits have produced good results with locally applied TGF- β 1. Fan et al¹⁰¹ applied TGF- β 1 to osteochondral defects on a gelatine-chondroitin-hyaluronate tri-copolymer scaffold with gelatine microspheres containing the TGF in the chondral layer. Significantly improved histology was found in the treatment groups over controls at each time point. Mierisch et al¹⁰² applied TGF- β 1 in calcium alginate beads into an osteochondral defect. Although not significant, improved histology was found at each time point in the treatment groups. However, when a dose of 200 ng/ml of TGF- β 1 was used, instead of 20 ng/ml, an increase in osteophytosis was noted. Similar results were found by Holland, Tabata and Mikos,¹⁰³ who incorporated TGF- β 1 into gelatine microparticles and added it to oligo(poly(ethyleneglycol)fumarate) scaffolds. In this instance no increase in osteophytosis was noted, even at a dose of 200 ng/ml. *In vitro* release studies performed using this construct had shown a reduction in the TGF burst

release profile from the scaffold. This may be partly responsible for the lack of negative effects.¹⁰³

B. Bone morphogenetic proteins. These are also members of the TGF superfamily of growth factors and are of particular interest owing to the clinical availability of both BMP-2 (Infuse, Medtronic, Minneapolis, Minnesota) and BMP-7 (Osteogenic Protein-1 (OP-1) Stryker Biotech, Hopkinton, Massachusetts). Research has shown that BMPs, particularly BMP-4, -6 and 7, have a positive effect on the chondrogenic phenotype, increasing the amount of collagen type II and proteoglycan production and reducing collagen type I.^{104,105} Kaps et al¹⁰⁶ also showed that, with BMP-7, repair tissue was protected against fibroblastic invasion. Although much work has shown the beneficial effect of BMP-2 in terms of extracellular matrix synthesis, a number of studies have also shown detrimental effects. Van Beuningen et al¹⁰⁰ demonstrated the presence of osteophytosis associated with injection into murine knee joints, as did Gelse et al¹⁰⁷ following the implantation of BMP-2 transfected mesenchymal stem cells into rat patellar grooves. Henson and Vincent¹⁰⁸ have shown BMP-2 to have an inhibitory effect on chondrocyte migration from cartilage explants on to a gelatine scaffold following a single impact injury load model. They hypothesised that BMP-2 inhibition may be partly responsible for the poor intrinsic repair response in articular cartilage.

Cook et al¹⁰⁹ incorporated BMP-7 (OP-1) on to a collagen sponge and inserted it into osteochondral defects in a canine model. At all points up to 52 weeks, a significant improvement in tissue fill and quality was noted over controls with hyaline-like tissue described in all OP-1 groups.¹⁰⁹ Similar results were found by Kuo et al,¹¹⁰ who added OP-1 to microfracture defects in rabbits, suggesting a synergistic relationship.

Kuroda et al¹¹¹ retrovirally transduced muscle-derived stem cells with BMP-4 and inserted them into defects into nude rats. Increased chondrogenesis was seen over controls at 24 weeks, with no unwanted side effects.

C. Insulin-like growth factor-1 (IGF-I). This is the main anabolic growth factor of articular cartilage.^{30,31} It has been shown repeatedly to increase proteoglycan and collagen type II synthesis as well as provide chondrocyte phenotypic stability. It is stored in the extracellular matrix, bound to proteoglycans via IGF-1-binding proteins. It is likely that the interaction between it and the binding proteins regulate its activity, as an increase in catabolic activity causes proteolysis of these proteins, thereby modulating its release.¹¹² Nixon et al³¹ showed its *in vivo* application when coupled with fibrin composites in an equine osteochondral model. Although not normal cartilage, the growth factor-treated defects showed improved histology over controls, with a significant increase in the amount of type II collagen present. Elisseeff et al¹¹³ demonstrated that by combining TGF- β 1 with IGF-1 *in vitro* during the culture of bovine chondrocytes, an increase in extracellular matrix synthesis and cell content was found. They used a novel poly(ethylene oxide) hydrogel scaffold, which contained growth factor-

encapsulated PLA spheres. A controlled release of TGF initially, followed by IGF was attained. Although other *in vitro* studies have explored the same idea with success,¹¹⁴ the perceived benefits of cell differentiation and proliferation, followed by extracellular matrix synthesis, were not confirmed in an *in vivo* study by Holland et al¹¹⁵ following a similar hypothesis. Using a poly (ethylene glycol) (PEG) hydrogel scaffold containing gelatine microparticles of TGF- β 1 and IGF-1, no improvement was seen using the combination treatment. In fact, IGF-1 alone was found to be better than TGF, either alone or in combination, in terms of tissue composition.

D. Fibroblast growth factor. A total of 22 different members of the FGF family have been identified, of which FGF-2 (basic FGF) is the most widely investigated in articular cartilage repair.¹¹⁶ It is stored bound to heparin sulphate proteoglycan in the extracellular matrix.¹¹⁷ FGF-2 is an important mitogen for cells of mesodermal origin and is a chemo-attractant for endothelial cells.¹¹⁸ Early work by Cuevas, Burgos and Baird¹¹⁹ showed that basic FGF increased DNA synthesis and cell proliferation within articular cartilage. This was later confounded by Inoue et al¹²⁰ who showed a tendency of rabbit chondrocytes to become flattened and fibroblastic in shape in presence of FGF. Kato and Iwamoto¹²¹ later showed that FGF-2 inhibits terminal differentiation of chondrocytes. This is of particular interest with regard to mesenchymal stem cells, as being able to stimulate chondrogenic differentiation and inhibit terminal differentiation is essential. This has been shown *in vitro* by Stewart et al,⁷⁴ who cultured bone marrow-derived mesenchymal stem cells in a 3D pellet in the presence of FGF-2. They found that it enhanced chondrogenic differentiation and stabilised phenotypic expression.

Weisser et al¹²² incorporated FGF-2 in agarose gel with chondrocytes and implanted the construct into rabbit osteochondral defects. Although the results were not found to be significant, they concluded that FGF seemed to stabilise the chondrocyte differentiated state. In another rabbit model, Fukuda et al¹²³ created osteochondral defects with FGF-2 incorporated into a triaxial 3D woven structure of ultra-high molecular weight polyethylene. The mechanical properties of the test group were more similar to those of native tissue, and a significant increase in defect filling was found at 26 weeks.

Although most attention has been paid to FGF-2, FGF-18 also has great potential in articular cartilage tissue engineering, with a number of recent studies showing the potential to stimulate cell proliferation and differentiation and matrix production both *in vitro* and *in vivo*.^{124,125}

E. Platelet-derived growth factor. This is a known chemo-attractant, stimulating macrophages and fibroblasts during healing.¹¹⁸ It is stored in platelets, hence it was recognised as a key growth factor in the microfracture technique attracting cells to the defect site.² Not only is it a chemo-attractant, *in vitro* studies have also showed it to have an impact on mesenchymal stem cell differentiation,⁹⁶ enhancing matrix

production and preventing the progression of chondrocytes down the endochondral maturation pathway.¹²⁶

F. Vascular endothelial growth factor. This has been shown to act synergistically along with FGF, promoting angiogenesis.¹¹⁴ Although some *in vitro* studies have shown an ability to stimulate chondrocyte proliferation, its angiogenic properties are most likely best used in the regeneration of bone¹²⁷ and meniscus.¹²⁸

G. Other signalling molecules. In addition to the growth factors, a number of other molecules have been identified which have a significant role to play in articular cartilage homeostasis. Transcription factors such as SOX9 have been shown to be essential for cell differentiation and cartilage formation.¹²⁹ Signal transduction molecules such as the SMADs have been shown to be intracellular regulators of chondrogenesis.¹³⁰ Parathyroid hormone-related peptide has been successfully incorporated in mesenchymal stem cells and chondrocyte cultures to prevent hypertrophy and ultimate endochondral ossification.^{131,132} This is important, as ossification is a particular problem associated with terminal differentiation of cultured cells. Inhibitors of interleukin-1 (IL-1) and tumour necrosis factor- α (TNF- α), which have been demonstrated to be involved in mediating cartilage matrix degradation and cell apoptosis, can also be used.¹³³ IL-1 receptor antagonist has been combined with IGF-1, with promising results.¹³⁴ Bespoke combinations of these molecules with anabolic growth factors therefore have the potential to augment scaffold-based repair.

Although good results have been found with many of these growth factors *in vitro*, translation *in vivo* and into clinical practice has not been as successful. A combination approach may be the most appropriate next step, but the timing and delivery of these factors remains a problem. A common question persists: which is the best growth factor? This is difficult because of the contradictory evidence in the literature. One could summarise that FGF-2 is good for mesenchymal stem cell expansion, TGF- β 1 and TGF- β 3 for mesenchymal stem cell chondrogenic differentiation, and IGF-1 and BMP-7 for matrix production and chondrocyte phenotypic stability. However, until all of these factors are available clinically, and safe, improved delivery mechanisms are available, it will be difficult to establish which is the best. Until then, we are left with those growth factors that have already achieved regulatory approval, such as BMP-7 (OP-1).

The extracellular matrix. In 2002, van der Kraan et al¹³⁵ stated that "functional tissue engineered cartilage can only be achieved when those matrices used for cartilage regeneration provide invading or embedded cells with the correct soluble and insoluble signals". This eloquently describes the vital importance of addressing the extracellular matrix in terms of communication between cells and matrix molecules. The importance of the α 1 β 1 integrin on collagen for chondrocyte survival has previously been described. Other integrins and binding sites, such as the hyaluronan binding site CD44, are present on chondro-

cytes and their reactions with the respective molecules are necessary for cartilage homeostasis.³⁷ Matrix proteoglycans such as decorin, biglycan and fibromodulin are intimately involved with the regulation of many soluble factors, such as TGF- β 1 and BMP-2.¹³⁵ Indeed, the cell adhesion via integrin ligation also regulates the activity of growth factor receptors. Thus, a sequence of events is initiated which allows for the production of matrix as the scaffold degrades. It is therefore vital that engineered scaffolds provide cells with the appropriate binding sites and signalling molecules similar to native cartilage, to optimise matrix production. It is there where we feel that natural material scaffolds such as collagen, hyaluronan and chitosan have an advantage over synthetics, as they are made up of constituent materials of hyaline cartilage. In addition, further molecules can be added, such as glycosaminoglycans;¹³⁶ however, a balance must be created between adding constituent materials and producing a commercially viable scaffold that can be produced on a mass scale for clinical use.

Future prospects

A. Growth factor delivery. As described above, a number of factors have been identified as having a positive effect on cartilage regeneration. Proof of the concept has most often been shown *in vitro* under controlled culture conditions. However, translation into *in vivo* models does not necessarily occur reliably, nor does interspecies translation. It is likely that the biggest controlling factor in success is the delivery of the molecules to the defect. The difficulty lies in getting high enough concentrations of the substrate to the local tissues for a prolonged period, with the appropriate factors being delivered at the correct time to optimise chondrogenesis. Most recombinant proteins have short half-lives and may therefore need repeat local administration.¹³⁷ Without these factors insufficient differentiation is seen, as is loss of transplanted cells, matrix destruction and lack of graft integration.

The concept of gene therapy was first described by Evans et al¹³⁸ in 1996. It involves the delivery of complementary DNA (cDNA) specific to a growth factor or signalling molecule, into target cells which then produce that factor *in vivo*. A vector is used to carry the cDNA. Direct application of the vector involves, for example, injecting it into the joint and transfecting synovial cells or chondrocytes *in vivo*. This is fairly easy and inexpensive, but it is more difficult to control transfection and there are potential safety issues.¹³⁷ Indirect transfer involves transfection *ex vivo*, then re-implantation of tissue-engineered grafts incorporating gene-enhanced cells.¹³⁹ A pure population of cells can be used under controlled conditions, providing localised transgene expression to the injured site. A greater safety profile can thus be maintained.

Scaffold material have the potential to deliver cDNA direct to the injured site. Capito and Spector¹⁴⁰ demonstrated that cross-linking IGF-1 plasmid DNA into a

collagen/glycosaminoglycan scaffold created controlled release dependent on the material degradation. *In vitro*, this construct was shown to increase chondrogenic differentiation as well as increasing matrix synthesis. Guo et al⁴³ performed a similar experiment with TGF- β 1 plasmid DNA in chitosan-gelatin scaffolds. Although a burst release of DNA was found in the first week, sustained release was maintained over a further two weeks. By week three, chondrocytes were noted to express TGF- β 1.

Owing to the concerns regarding control of transfection, indirect transfer seems a safer option. A number of viral vectors have been used, including adenovirus, adeno-associated virus and lentivirus.¹⁴¹ Gelse et al¹⁰⁷ injected adenoviral transfected IGF-1 and BMP-2 bone marrow derived mesenchymal stem cells into the knees of mice. Although both had a positive response in terms of cartilage regeneration, osteophytosis was noted with BMP-2. Mason et al¹⁴² retrovirally transduced rabbit periosteal mesenchymal stem cells with BMP-7, then seeded the cells on to PGA scaffolds which were implanted into osteochondral defects. At 12 weeks the histology from the BMP-7 group was found to be significantly improved over the control groups. Although benefits have been shown, further work still needs to be performed to ensure the safety of such treatments and their efficacy.

B. Platelet-rich plasma (PRP). The ability to concentrate platelets and apply them to local defects, providing a source of autologous growth factor, has become of interest in orthopaedic surgery.¹⁴³ It is a relatively inexpensive and easy way to apply PDGF, TGF- β 1, and FGF-2 directly to a defect, without the need to use recombinant proteins that need to be passed through regulatory channels before clinical use. Ishida et al¹⁴⁴ have shown the potential benefit of culturing meniscal fibrochondrocytes with PRP. Wu et al¹⁴⁵ cultured chondrocytes with PRP in a rabbit model following subcutaneous injection of the composite material, activated and gelled with bovine thrombin. This was shown to support chondrogenesis. In a recent goat model, PRP/chondrocyte composites were implanted beneath periosteal flaps, producing hyaline-like tissue.¹⁴⁶ Interestingly, those grafts that were not implanted under the periosteal flap became dislodged, indicating the essential need for mechanical stability of any type of articular cartilage graft. It remains to be seen whether these early positive findings can be translated successfully into human clinical practice.

C. Nanotechnology. This involves the production of materials on a nanoscale, thereby better simulating the dimensions of natural materials, such as collagen fibres. By reducing materials to the nanoscale, a subsequent improvement can be seen over microscale materials in terms of surface area, surface roughness and surface area to volume ratios, which can lead to superior physiochemical properties.¹⁴⁷ The application of nanotechnology seems obvious, as cartilage is made up of a dense nanostructured extracellular matrix, although little has been published on the use of nanoscale materials in articular cartilage repair.

Fig. 1

Summary of current practice of articular cartilage tissue engineering (PLLA, Poly-L-Lactic Acid; PLG, Polylactide-co-Glycolide; ACI, autologous chondrocyte implantation; MACI, matrix assisted chondrocyte implantation; CCI, characterised chondrocyte implantation; MSC, mesenchymal stem cell; ES cell, embryonic stem cell; iPS cell, induced pluripotent stem cell; TGF- β transforming growth factor beta; BMP, bone morphogenetic protein; IGF, insulin like growth factor; FGF, fibroblast growth factor; PTHrP, parathyroid hormone related peptide).

Khang, Park and Webster¹⁴⁸ have recently produced a carbon nanotube composite which can support chondrocyte proliferation and extracellular matrix synthesis. Li et al¹⁴⁹ published their *in vitro* results of mesenchymal stem cells chondrogenesis within an electrospun polycaprolactone nanofibrous scaffold. Not only did it support chondrogenesis, it also had improved technological characteristics over standard pellet cultures. It is likely that this type of material technology will form the basis of scaffold production in articular cartilage tissue engineering in the future.

D. Embryonic and induced pluripotent stem cells. Although adult mesenchymal stem cells have represented the mainstay of stem cell articular cartilage repair research, embryonic cells, and in particular the emergence of induced pluripotent stem cells, may represent the future of cell therapies. With the degree of ethical problems surrounding embryonic stem cells, in our opinion it is unlikely that they will come to represent a viable clinical option in articular cartilage repair. Induced pluripotent cells, on the other hand, represent a very exciting possibility. Having first been induced in the mouse in 2006¹⁵⁰ and then in

humans in 2007,¹⁵¹ these are somatic cells which are reprogrammed to pluripotent cells via transfection of stem cell-associated genes. The resultant cell line has the plasticity expected of an embryonic cell line, bypassing the concerns over embryo harvesting. This technology is in its infancy and is yet to be shown to be of clinical use in articular cartilage repair, but represents an exciting prospect for future musculoskeletal research and application.

Discussion

In the last 20 years we have witnessed great advances in articular cartilage tissue engineering. New materials are being developed which have greater biological affinity to host tissues and mimic the native environment more closely. The actions of signalling molecules and growth factors are more widely understood, and combination products are an exciting prospect (Fig. 1).

As articular cartilage injury is a multifactorial process, it is unreasonable to think that there can be just one type of treatment. Both biological and mechanical treatments

should be used. Mechanical alignment, instability and meniscal insufficiency should be surgically corrected, allowing biological treatments using tissue engineering in an optimised joint environment.

In our opinion, a biological polytherapy approach should be used to address the issues raised in the preceding sections. Natural material scaffolds, such as collagen, chitosan and hyaluronan, seem to have the edge over synthetics, allowing the appropriate communication between cells and signalling molecules in the host and regenerate extracellular matrix. Which growth factors are the most appropriate for articular cartilage repair remains controversial. The choice will depend on a number of factors, including cell type, *in vitro* cell expansion (or not), clinical availability and appropriate delivery mechanisms. As described, a number of growth factors, such as TGF- β 1 and FGF-2, seem to be more appropriately used in the early stages of chondrogenesis, whereas the anabolic growth factors IGF-1 and BMP-7 produce extracellular matrix in later stages. Combination therapies may be a good option, but thus far *in vitro* and *in vivo* experiments have yielded disappointing results.^{1,52} The emergence of material nanotechnology may allow improved biological and mechanical environments, including drug delivery mechanisms with more favourable release kinetics, ultimately achieving better outcomes.

From a clinical standpoint, the ability to provide 'point of service' cell-based therapy in matrices as an 'off the shelf' option, tailored to the needs of the patient and the biology of the lesion, is attractive and should be pursued. It is therefore likely that a stem cell approach will form the basis of future cell therapy, combined with signalling molecules, or indeed genetically modified cells programmed to produce appropriate growth factors. However, cartilage degeneration is not life-threatening, and so the safety profiles of these treatments must be rigorously tested before they become mainstream. A further hurdle will be the regulatory pathways by which these treatments will be assessed. Only then will they become a viable option for clinical practice.

In the meantime, numerous biological scaffolds are available in Europe for the treatment of chondral defects with or without combination cell therapy. Although the microfracture *versus* ACI debate will continue, new research is emerging showing an improvement in cell treatment.⁸ Scientists and clinicians will continue to work towards producing a simple, viable option for the treatment of articular cartilage pathology in patients, an approach that promises exciting results.

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