TISSUE ENGINEERING FOR ARTICULAR CARTILAGE REPAIR – THE STATE OF THE ART

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Abstract

Adult articular cartilage exhibits little capacity for intrinsic repair, and thus even minor injuries or lesions may lead to progressive damage and osteoarthritic joint degeneration, resulting in significant pain and disability. While there have been numerous attempts to develop tissue-engineered grafts or patches to repair focal chondral and osteochondral defects, there remain significant challenges in the clinical application of cell-based therapies for cartilage repair. This paper reviews the current state of cartilage tissue engineering with respect to different cell sources and their potential genetic modification, biomaterial scaffolds and growth factors, as well as preclinical testing in various animal models. This is not intended as a systematic review, rather an opinion of where the field is moving in light of current literature. While significant advances have been made in recent years, the complexity of this problem suggests that a multidisciplinary approach – combining a clinical perspective with expertise in cell biology, biomechanics, biomaterials science and high-throughput analysis will likely be necessary to address the challenge of developing functional cartilage replacements. With this approach we are more likely to realise the clinical goal of treating both focal defects and even large-scale osteoarthritic degenerative changes in the joint.

Keywords: Cartilage; repair; stem cells; scaffolds; gene therapy; tissue engineering; regenerative medicine; translational and preclinical research.

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Introduction

The consistently successful repair of articular cartilage defects is still a major clinical challenge. Although initially considered a tissue with a simple structure (Fig. 1), reproducing the finely balanced structural interactions has proven to be difficult. The field of cartilage tissue engineering has developed over the last twenty years but, despite extensive efforts to develop novel biological solutions, there is still a paucity of clinical options for treatment. Although the field has concentrated on finding therapies for focal lesions, it has now developed sufficiently to begin considering the challenge of finding novel solutions for the extensive joint damage seen in osteoarthritis.

The last two decades have borne witness to a huge expansion in biomaterial technologies, cell sources, and molecular and genetic manipulations that have had or could have positive impact on the development of a truly functional tissue engineered cartilage substitute (Chung and Burdick, 2008). In terms of cell sources, the framework provided by isolated chondrocytes allowed for a multitude of tissue engineered cartilage products to be produced *in vitro* and *in vivo* – demonstrating in practice that cartilage tissue engineering is possible (Langer and Vacanti, 1993). With the practical clinical limitations surrounding the use of autologous adult cells, the field rapidly moved towards other progenitor cell sources. Since the early work of Johnstone and Yoo as well as Pittenger and colleagues (Johnstone *et al*., 1998; Pittenger *et al*., 1999), the use of bone marrow-derived mesenchymal stem cells (MSCs) has found widespread application (Huang *et al*., 2010a). The work of Gimble, Guilak, Hedrick and colleagues has illustrated the potential of progenitor cells from adipose and other tissues (Erickson *et al*., 2002; Zuk *et al*., 2001). Even more recently, the use of embryonic stem cell (ES)-derived and induced pluripotent stem cell (iPS)-derived progenitors has expanded the cellular palette from which to construct cartilaginous tissues (Hoben *et al*., 2009; Wei *et al*., 2012; Diekman *et al*., 2012).

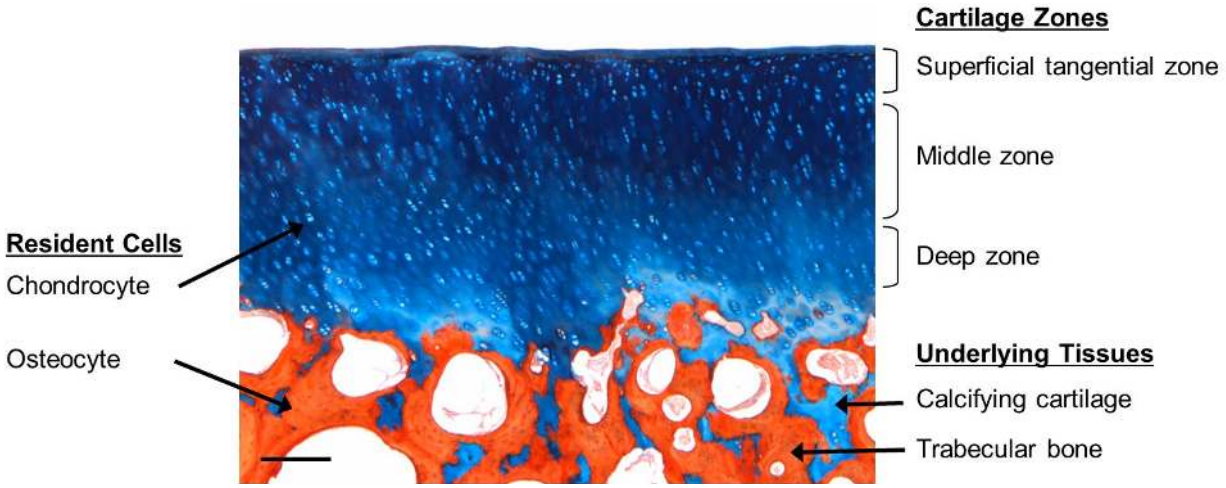

Fig. 1. Section of cartilage detailing the various zones from the upper superficial zone down to the underlying bone. Differences in cell phenotype can be detected between the superficial, middle and deep zones. These differences can still be observed during *in vitro* culture, demonstrating functional differences between the cells of each zone.

From a biomaterial standpoint, developments (initiated in the early 1990s) have spanned the use of inert and non-biodegradable hydrogel materials (agarose, alginate, and PEG networks) (Buschmann *et al*., 1992; Elisseeff *et al*., 2000; Chang *et al*., 2001) and porous formulations of biodegradable polyesters (Freed *et al*., 1998; Freed *et al*., 1993) to the present day application of advanced material chemistries based on both natural and synthetic platforms (Burdick *et al*., 2005; Lutolf and Hubbell, 2005; Lutolf *et al*., 2003). Indeed, the concept of a rapidly expanding material framework provides an almost dizzying array of choices for investigators. It is now quite realistic to engineer a material with a host of biologic inputs, while at the same time defining the time course and mechanism by which it ultimately degrades, all the while delivering agents in a controlled fashion. In addition to this cellular and material spectrum, advances in molecular therapeutics continue to provide new and interesting molecules to deliver (as soluble factors or *via* viral transduction) to cells and/or from the materials themselves. This review aims to detail the role played by the numerous factors involved in the development of tissue engineering therapies for cartilage repair in both focal lesions and osteoarthritis, and highlights the challenges that still need to be overcome in each area. The intention of this review is to illustrate and comment on these challenges; it is not intended to be a comprehensive review of each facet of the field.

Cell sources

In determining the optimal source of cells for cartilage repair, the two primary criteria that are generally considered are the performance of the cells and their ease of access. Advantages and disadvantages of various cell sources are detailed in Table 1. Regarding performance, primary or low passage articular chondrocytes provide several advantages due to their high level of matrix synthesis and lack of hypertrophy. Indeed, they are the only cell source currently approved for clinical use, albeit without clear clinical advantages over simpler methods, such as microfracture (Knutsen *et al*., 2007). However, for larger defects, which require a larger number of cells, it is generally accepted that the dedifferentiation and the concomitant advance towards replicative senescence, which occur during monolayer expansion, are significant

Table 1. Advantages and disadvantages of various cell sources.

Cell type	Advantages	Disadvantages
Autologous chondrocyte	Native phenotype Minimal risk of immunological problem	Small initial cell number De-differentiation on expansion
Allogeneic chondrocyte	Larger cell number Off-the-shelf solution	Limited donor availability Risk of disease transmission
Adult mesenchymal stem cells	Potential to produce large numbers Various harvest sites Additional paracrine signaling potential	Potential for hypertrophy Heterogeneous population of cells Stable and reproducible differentiation still problematic
Induced pluripotent stem cells (iPS)	Large source of patient specific cells Multiple cell types can be produced	Stable and reproducible differentiation still problematic Potential for teratoma
Embryonic stem cells	Off-the-shelf solution Multiple cell types can be produced	Stable and reproducible differentiation still problematic Potential for teratoma Ethical considerations

hurdles. To eliminate the need for monolayer expansion, there is growing interest in the direct reimplantation of minced cartilage; however, results are still preliminary (McCormick *et al*., 2008). Furthermore, there is concern that the iatrogenic damage caused by harvesting autologous cartilage for chondrocyte isolation may, by itself, initiate degenerative processes within the joint (Lee *et al*., 2000), although clinical evidence for this with autologous chondrocyte harvests is limited in the literature (Matricali *et al*., 2010). With the added requirement for two intra-articular procedures, one to harvest the cartilage and one to re-implant, many groups are attempting to develop other sources of cells for use in articular cartilage repair.

As cartilage is considered to be an immune privileged site, the use of allogeneic chondrocytes is being investigated. Although the results of animal studies have been debated (Moskalewski *et al*., 2002), it has long been suggested that chondrocytes are privileged by virtue of the barrier properties of their extracellular matrix. Newer data indicate that chondrocytes have immunological properties that limit host immune reaction (Adkisson *et al*., 2010b). Thus, given their ability to produce abundant matrix, allogenic juvenile chondrocytes have been studied for possible clinical use (Adkisson *et al*., 2001; Adkisson *et al*., 2010a) A clinical trial of DeNovo® ET, a *de novo* tissue derived from juvenile allogeneic chondrocytes, is currently enrolling patients for a Phase III trial. Allogeneic chondrocytes from adult donors are also under clinical investigation (Almqvist *et al*., 2009; Dhollander *et al*., 2012b). Cells from non-articular sources, such as ear, nasal and costochondral cells can also proliferate and produce *de novo* cartilaginous tissue (Tay *et al*., 2004). The robustness of the tissue produced and its ability to integrate into articular defects remains to be determined. Whether a chondrocyte derived from a different progenitor cell lineage can produce the precise extracellular matrix of articular cartilage is not established. This concern is highly relevant to the study of the many stem and progenitor cell types proposed as alternative cell sources for articular cartilage repair.

While chondrocytes are of great interest, an important advance in the field has been the identification of multiple cell sources that can readily provide large numbers of undifferentiated progenitors with osteogenic and/or chondrogenic potential. Adult bone marrow mesenchymal stem cells (MSCs) (Pittenger *et al*., 1999) are being extensively studied, as are adipose stem cells (ASCs) (Erickson *et al*., 2002; Zuk *et al*., 2001), which may provide a more abundant source of cells for musculoskeletal regeneration (Guilak *et al*., 2010). The presence of multipotent progenitor cell types in tissues such as muscle (Adachi *et al*., 2002), periosteum (Nakahara *et al*., 1991), synovium (De Bari *et al*., 2001), or infrapatellar fat pad (Wickham *et al*., 2003) provide additional cell sources that may be readily accessible during joint surgery. Although these cells have been investigated for over 40 years, the methods used for their isolation are still fairly rudimentary. For marrow, adipose and synovial cells, the most common method used is simple adherence to tissue culture plastic. There is no single marker that distinguishes adult stem cells, but the fraction may be selectively enriched using cell surface proteins (CD markers, for a review see (Harichandan and Buhring, 2011)). All the evidence would suggest that the currently used marker set is able to distinguish mesenchymal cells from haematopoietic cells, but may not be of use to select for a pure MSC population (Whitney *et al*., 2009). Moreover, it is well appreciated that there exists considerable heterogeneity in the starting MSC population, and so surface markers that identify both MSCs and their potential towards cartilage matrix formation would be especially beneficial to the field. Future progress in the control of differentiation of these cells will be hindered until more selective markers can be identified.

Once isolated, there are various protocols that are currently used to induce chondrogenic differentiation of stem cells. Typically, the protocol involves the application of a chondrogenic stimulus in the form of exogenous transforming growth factor β (TGF-β) and dexamethasone, as originally developed for postnatal bone marrow-derived MSCs (Johnstone *et al*., 1998). However, this chondrogenic differentiation includes an upregulation of genes such as collagen X, alkaline phosphatase (ALP) and MMP13 (Johnstone *et al*., 1998; Pelttari *et al*., 2006; Mwale *et al*., 2006), indicative of a hypertrophic phenotype, suggesting that the process these cells undergo is endochondral. It should be noted that the increased expression of collagen X is so rapid that doubts have been raised as to its validity as an *in vitro* hypertrophy marker (Mwale *et al*., 2006). Regardless of this fact, the molecular profile produced by stem cells differentiated *in vitro* remains quite distinct from that of articular chondrocytes treated in the same fashion (Huang *et al*., 2010b) and is seen as undesirable for the repair of a permanent articular surface. It has been proposed that one cell type that might not progress to hypertrophy would be a chondroprogenitor isolated from the cartilage tissue itself (Dowthwaite *et al*., 2004). Such cells have been isolated by their ability to attach rapidly to fibronectin-coated plastic and exhibit a high proliferative capacity (Khan *et al*., 2009). Upon *in vitro* chondrogenic induction, these cells do not express ALP or collagen X and as such may provide a useful source of material for future clinical application (Williams *et al*., 2010; McCarthy *et al*., 2012). However, there is still controversy regarding their exact identity and additional work needs to be done to characterise these cells and their function. This source suffers from the same problem as chondrocytes, in that an initial harvest of articular cartilage is required. An improved understanding of the differences between joint tissue-derived stem cells, such as from synovium or articular cartilage, and other adult stem cells such as MSCs would provide new insights into the processes that lead to the stable articular chondrocyte phenotype *in vivo*.

As noted above, ASCs are gaining increasing interest for cartilage repair due to the large numbers that can be obtained through a relatively simple liposuction procedure. In this regard, the hope is that sufficient numbers can be obtained without the need for a monolayer expansion step. As the monolayer expansion stage needs to be performed individually for each patient, and must be performed under GMP conditions, it not only increases the time before the implantation can be performed but also greatly increases the cost. While unexpanded bone marrow-derived mononuclear cells are being investigated in clinical use,

their initial yield is lower. Thus, the promise of ASCs is that they offer the largest initial cell yield, and hence the least need for expansion. How best to utilise these cells is still open to discussion. A number of groups have compared ASCs to other sources, such as bone marrow, and demonstrated that they have a lower chondrogenic potential if cultured under the same conditions as MSCs. This can be overcome in part by the addition of further factors, such as BMP-6 (Estes *et al*., 2010), but additional work needs to be done to improve their efficacy. In addition, the mixed cell population originally isolated may have a larger number of contaminating adherent cells, such as endothelial cells, that also need to be considered.

Chondrogenic differentiation using embryonic stem cells (ESCs) is much less common, though increasingly represented in the literature (Hwang *et al*., 2008; Hoben *et al*., 2009; Koay and Athanasiou, 2009). In part, this is due to these cells being highly regulated and for ethical reasons. Not only does special permission need to be obtained to allow work to be carried out with these cells, but also their clinical application for non-life threatening diseases might be limited on an ethical basis. Therefore, their study has mainly been limited to understanding more fundamental biological questions, as opposed to delivering a clinically applicable strategy. One of the main stated advantages of embryonic stem cells is their immunosuppressive properties and their protection from the host immune response, due to a lack of expression of MHC II (Drukker *et al*., 2002). This has been proposed as a rationale for using these cells in an "off-the-shelf" treatment. What is not clear is what happens to these cells once they differentiate and participate in a repair environment. If MHCII expression is induced during differentiation, these cells may then elicit an immune response. Increasingly, immunosuppressive effects are also being seen in adult MSC populations (De Miguel *et al*., 2012). Moreover, a better understanding of the population level *versus* individual cell differentiation response is required. Given the totipotent nature of these cells, even one cell that is not appropriately differentiated could lead to disastrous consequences if it initiates a different or unwanted differentiation response upon implantation. Success of this also depends on the development of additional molecular markers that can be used to identify the specific populations.

Interest in induced pluripotent stem cells (iPSCs) is increasing as they have high differentiation capacity, but also can be produced from the patient's own cells, removing the risk of rejection or disease transmission (Sun *et al*., 2010). A recent study has shown that iPSCs are capable of robust chondrogenesis using a multi-stage differentiation process involving micromass culture followed by purification of chondrogenic cells using a collagen-2 driven GFP reporter (Diekman *et al*., 2012). Although the original iPSCs were created using integrating viral vectors, the technology has developed and now episomal vectors with increased safety can be used. It is early days with this cell type and, similar to use of ESCs, issues such as efficiency, reproducibility, standardisation and control of differentiation all need to be addressed before they could be used within a clinical setting. While such cells currently have significant potential for *in vitro* studies of cartilage injury and osteoarthritis, their clinical application will require a number of years and safety trials to become a reality, so it is likely that an intermediate source would be required. Given the current clinical reality, it is likely that allogeneic chondrocytes will be the next clinical therapy attempted on a large scale, building on current autologous chondrocyte implantation (ACI) techniques. The use of autologous and allogeneic stem cells may be developed sufficiently later, with the use of either iPSCs or ESCs having even greater hurdles to overcome before widespread clinical use for articular cartilage repair would be considered.

Biomaterial platforms for cartilage tissue engineering

Articular cartilage has a highly organised hierarchical structure composed of three zones: a) a superficial zone, where chondrocytes produce and lay mainly collagen type II in parallel direction to the surface of the tissue; b) a deep zone, located at the cartilage-bone interface where the collagen fibres are aligned perpendicular to the surface; and c) a middle zone characterised by randomly oriented collagen fibres. Of the three, the superficial zone contains the highest proportion of collagen, which results in the high tensile modulus of the tissue and indicates that the main function is to resist the shear stress at the joint surface. The middle zone contains more proteoglycans, which exhibit repulsive negative charges that are neutralised by positive ions, leading to swelling pressures and its highly stable hydrated structure (Poole *et al*., 2001). These proteoglycans are responsible for the hyaline cartilage's distinctive compression-resistance properties due to the increasing drag forces between the fluid and the matrix that maintain the fluid within the tissue as the cartilage is compressed (Mow *et al*., 1992). As a consequence, hyaline cartilage of the joint becomes stiffer as the rate of loading increases (Park *et al*., 2004). This is the reason why hyaline cartilage presents a high compressive modulus. Thus, biomaterials designed with nonlinear, inhomogeneous, and viscoelastic properties that mimic the behaviour of native of hyaline cartilage are most likely to succeed in the functional repair of cartilage defects (Guilak *et al*., 2001).

Beyond the biomechanical considerations, a new generation of materials are being developed that are influenced by our knowledge of the anatomical and structural complexity of articular cartilage. The increasing capacity to design and synthesise materials with molecular resolution that scales across organisational levels is generating great excitement in the biomaterials community. The combination of technological advances and an increased knowledge in fields such as molecular and cell biology are generating a toolbox with unprecedented precision and versatility to create biomaterial scaffolds with many desired properties. In addition to being biocompatible, an ideal biomaterial scaffold for cartilage regeneration can now be bioactive, biomimetic, biodegradable and bioresponsive, providing signalling with spatio-temporal control and response that is selective to defined stimuli. Materials with such capacities that can also be implanted using minimally invasive procedures are an avenue of intense study.

Set against this idea of using complex biomaterials for cartilage repair is the concept that since chondrocytes are prolific producers of extracellular matrix, we should be able to create implants from the cells that do not require the addition of biomaterial scaffolds. Two main scaffold-less systems have been described to produce tissue-engineered structures with a cartilage-like characteristics (Kim *et al*., 2011b; Hu and Athanasiou, 2006; Ofek *et al*., 2008; Aufderheide and Athanasiou, 2007; Novotny *et al*., 2006; Kraft *et al*., 2011). These scaffold-less platforms develop a robust ECM framework of their own and permit long-time maintenance of phenotype, at least in long-term *in vitro* culture, and can improve biophysical properties by mechanical loading. Scaffold-free constructs using alginate as an intermediate step have also been produced (Masuda *et al*., 2003) and subjected to mechanical loading (Stoddart *et al*., 2006). The challenge with such scaffold-free systems is producing them in a cost-effective and timely manner for clinical use, especially with autologous cells. This is also true for scaffold-based systems, but they have the ability to direct growth, have biomechanical properties that are immediately functional *in vivo*, and can be the designed to deliver relevant bioactive factors *in vivo*.

Biomaterials currently used in the clinic

Contemporary clinical procedures for reconstructive cartilage surgery comprise marrow stimulation such as microfracture, mosaicplasty and autologous chondrocyte implantation (ACI). ACI is mainly indicated for large symptomatic defects that are surrounded by non-osteoarthritic cartilage in a stable joint (Gomoll *et al*., 2012). In fact, for the implanted cells to deposit a new tissue, the damaged site of implantation should provide a permissive environment that may not exist in the case of extensive cartilage loss. Therefore, the classical method of injecting chondrocytes under a periosteal patch (Brittberg *et al*., 1994) has been abandoned in favour of seeding the cells in biodegradable three-dimensional matrices, such as collagen patches, to physically contain the biologics within the repair site, and more importantly to provide a temporary three-dimensional cartilage-like matrix which supports and promotes healing.

In such applications, the implanted cells are attached to a three-dimensional matrix to contain physically the biologics to the repair site, and to provide a temporary matrix supporting new tissue formation. Among the clinically used scaffolds, type I/III collagen and HA-based materials (e.g. Hyaff-11, Fidia Advanced Biopolymers) are the most commonly described (Grigolo *et al*., 2001; Haddo *et al*., 2004). Other biological and synthetic materials and many combinations have been or are being considered; either for their handling properties and relevance as commercial products (e.g. injectable fibrin gels), or their ability to provide a mechanical macro-environment comparable to the one in the joint. However, the clinical data obtained, for example from applying synthetic multiphase polymer scaffolds for osteochondral repair, have been unsatisfactory so far (Dhollander *et al*., 2012a). In each of these cases, the materials have been specifically designed to be relatively simple in composition. This, combined with the early introduction into the clinic, led to a reduction in the regulatory requirements that would have to be followed by the more complex newer materials now under development.

The next generation of biomaterials

Classical carriers for articular chondrocytes consist of several matrices, including agarose (Rahfoth *et al*., 1998; Buschmann *et al*., 1992), alginate (Hauselmann *et al*., 1992), hyaluronan (Goa and Benfield, 1994), type I collagen gels and sponges (Ben-Yishay *et al*., 1995; Frenkel *et al*., 1997), type II collagen sponges (Nehrer *et al*., 1998), poly(lactic acid, PLA), and poly(glycolic acid, PGA) (Freed *et al*., 1994; Vacanti *et al*., 1991) and fibrin (Itay *et al*., 1987; Sims *et al*., 1998). The development of matrix incorporation, and change in equilibrium modulus, can vary depending on the material used (Fig. 2). Hydrogels are attractive for use as cartilage scaffolds because of their unique biocompatibility, capacity to incorporate chemical cues, and innate hydrated structure (Slaughter *et al*., 2009; Klein *et al*., 2009). Due to these benefits, ECM-like matrices made from designed molecular building-blocks, such as peptide amphiphiles (Hartgerink *et al*., 2001), elastin-like polymers (MacEwan and Chilkoti, 2010; Betre *et al*., 2006), or the commercially available Puramatrix (3DM) (Zhang, 2003), that recreate some of the structural and functional elements of the natural ECM have been developed.

Many of these materials provided good preliminary results in animal models of osteochondral defects, albeit by producing what is generally a fibrocartilaginous repair tissue. Nevertheless, an important concept obtained from these experiments is that defects treated with constructs containing matrices with cells exhibited better performance than matrices alone, supporting the idea that creating therapeutic platforms using both components for articular cartilage repair/regeneration is valid.

More recently, hydrogels based on poly(ethylene glycol) (PEG) macromers, especially diacrylated forms, have received considerable interest due to their ability to be gelled into complex defects (Elisseeff *et al*., 1999). Ehrbar *et al*. (2007) developed a PEG gel able selectively to promote cell adhesion and enzymatic degradation, demonstrating the capacity to use such PEG hydrogels as platforms to exhibit specific biomolecular signals. More recently, Nguyen *et al*. (2011) further increased the complexity of these PEG-based systems, incorporating chondroitin sulphate and matrix metalloproteinase-sensitive peptides. A large diversity of PEG hydrogels with potential applications in cartilage regeneration have since been developed (Zhu, 2010; Hwang *et al*., 2011).

Hydrogels based on hyaluronan (HA) are especially being developed for hyaline cartilage regeneration, due to the possibility of mimicking the natural tissue and the need for obtaining a highly hydrated environment. One of the major limitations, however, is the low mechanical strength of these hydrogels, which require further modification to improve their handling and mechanical properties (Facchini *et al*., 2006; Grigolo *et al*., 2001). In order to avoid toxicity complications resulting from modifications, by incorporation of chemical cross-linking agents, a number of groups are exploring innovative alternatives to improve the

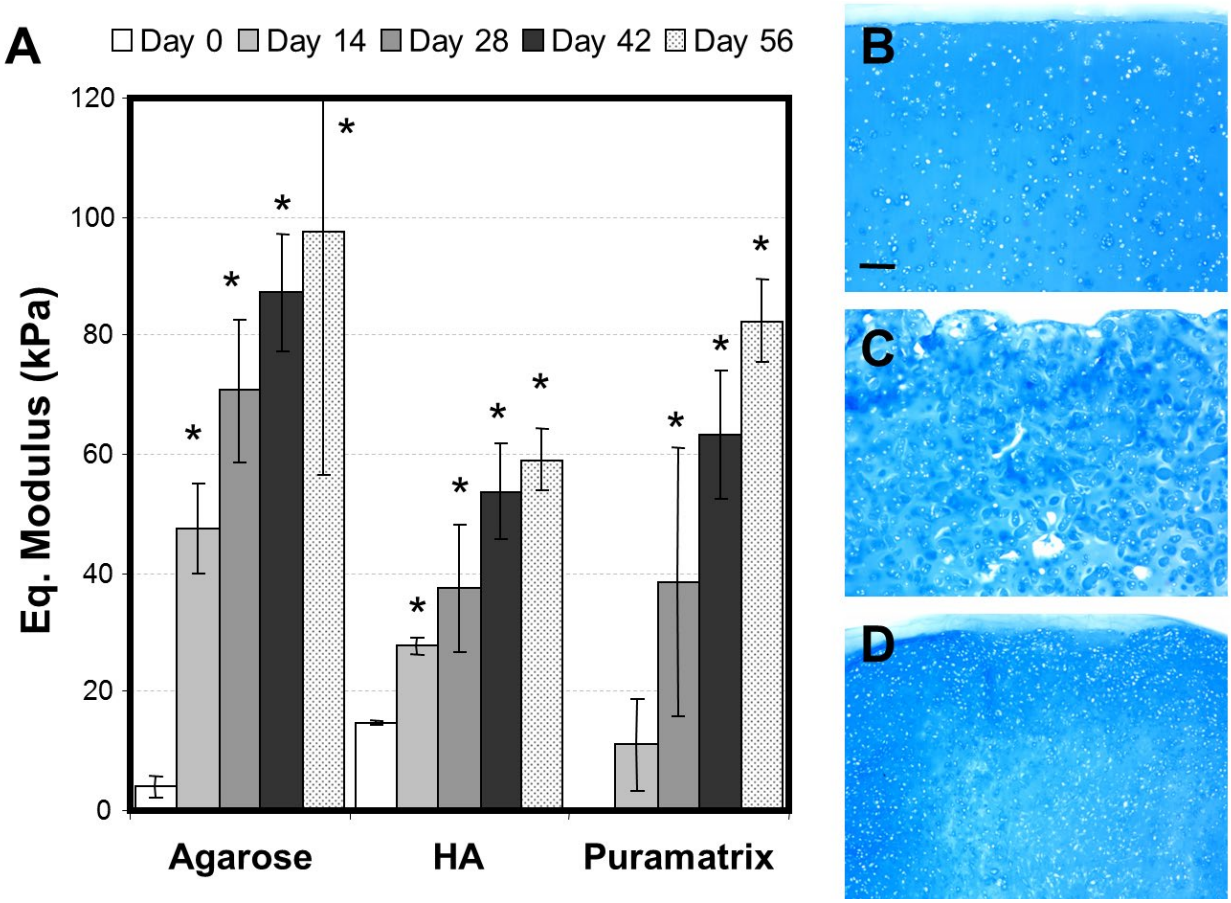

Fig. 2. The development of matrix incorporation, and change in equilibrium modulus (**A**), can vary depending on the material used. Figure adapted from (Erickson *et al*., 2009), showing maturation of MSC-seeded hydrogels based on agarose, HA, and Puramatrix. Rich alcian blue staining can be seen in (**B**) Agarose, (**C**) HA and (**D**) Puramatrix.

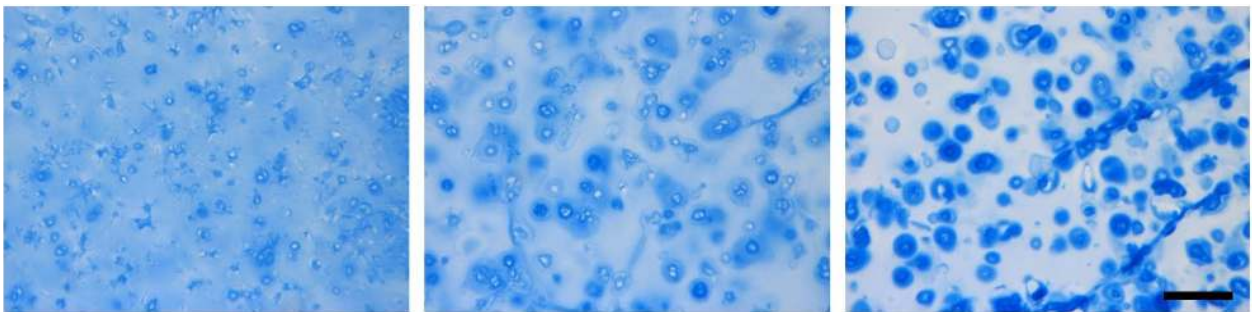

Fig. 3. Matrix distribution in photo-cross-linked MSC-seeded hyaluronic acid hydrogels after 14 d in chondrogenic culture conditions. Increasing macromer density (1 % to 5 % from left to right) increasingly restricts distribution of formed ECM, limiting overall functional properties of constructs with longer term culture. Reproduced with permission from (Erickson *et al*., 2009).

properties of HA-based hydrogels. One strategy has been the development of composite hydrogels that incorporate structural proteins within the HA material. For example, fibrin/HA hydrogels have been shown to exhibit improved mechanical properties, promote ECM production, and have potential cell delivery capabilities (Rampichova *et al*., 2010). Another example, reported by Liao *et al*. (2007), was the development of HA/collagen type I composite hydrogels, which stimulated chondrocyte growth and proteoglycan synthesis as well as overall mechanical properties. Taking advantage of the biological properties of HA molecules and diverse crosslinking mechanisms developed in the last two decades, the influence of the hydrogel mechanical properties, degradation, ability to retain and release growth factors (e.g. TGF-β1), among others have been extensively studied and demonstrate the usefulness of HA as a backbone for a cartilage biomaterial solution (Erickson *et al*., 2012; Erickson *et al*., 2009; Kim *et al*., 2011a) (Fig. 3). For example, the HA backbone has been modified with methacrylates, allowing for UV-mediated polymerisation of the hydrogel (Burdick *et al*., 2005; Smeds *et al*., 2001). These materials have been used to encapsulate both chondrocytes and MSCs with promising results (Erickson *et al*., 2012; Chung *et al*.,

2006). Another innovative approach has been recently reported by Eglin and co-workers, who developed a modified HA material that incorporates thermosensitive poly(*N*-isopropylacrylamide) (PNIPAM) segments (Mortisen *et al*., 2010). Above a specific temperature, the modified HA chains undergo conformational changes resulting in their assembly and the formation of stronger hydrogels capable of spontaneous formation and support of cell growth. This approach is highly attractive due to the possibility to develop injectable HA scaffolds that can be implanted through minimally invasive procedures and assemble at the site of injury.

A large number of self-assembling materials based on small molecular building-blocks are being developed (Capito *et al*., 2009; Wu *et al*., 2012). For example, Aggeli and colleagues have designed short β-sheet forming peptides that self-assemble into a variety of nanostructures such as tapes, ribbons and fibrils that form gels with tuneable physical and chemical properties (Aggeli *et al*., 2003). Pochan and colleagues have developed β-hairpin peptides that fold intramolecularly to acquire an amphiphilic structure to result in the formation of self-assembled hydrogel networks (Ozbas *et al*., 2007; Pochan *et al*., 2003; Schneider *et al*., 2002). Molecular self-assembling systems are also being developed to incorporate features that enhance the overall hydrogel properties and better mimic fibrocartilage. One of the most promising examples are collagen-mimetic self-assembling peptides developed by Hartgerink and colleagues (Stoop, 2008; O'Leary *et al*., 2011). These systems are highly attractive due to the possibility of creating a hydrogel that combines both the high water content and the structural integrity found in fibrocartilage.

Another promising family of self-assembling nanofibre scaffolds has been developed by Zhang and co-workers, who have developed alternating positive and negative L-amino acids that self-assemble, through β-sheet interactions, into nanofibres (Zhang, 2003; Zhao and Zhang, 2007). Liu *et al*. (2010) have used this material to promote chondrocyte growth and stimulate the production of key hyaline cartilage (aggrecan and collagen type II) markers *in vitro*. Similar results have been reported by Grodzinsky and colleagues, promoting growth and *in vitro* chondrogenesis of chondrocytes (Kisiday *et al*., 2002) and bovine bone marrow stromal cells (Kopesky *et al*., 2010). More recently, the group of Semino and co-workers reported the spontaneous formation of cartilage tissue from mouse embryonic fibroblasts cultured in self-assembling peptide scaffold RAD16-I (Puramatrix) (Quinatana *et al*., 2009). Moreover, and using another promising self-assembling platform, Stupp and colleagues have demonstrated a co-assembly system of peptide amphiphiles (PAs) able to self-assemble into nanofibres displaying a high density of binding epitopes for TGF-β-1 (Shah *et al*., 2010). This study reported the support, survival and chondrogenic differentiation of human mesenchymal stem cells *in vitro*, as well as regeneration of articular cartilage in a rabbit chondral defect.

In addition to hydrogel formulations and composites, many polymeric and natural biomaterials can be electrospun to obtain biologic and synthetic three-dimensional matrices with nano-scale fibres for chondrocyte culture and phenotype maintenance *in vitro*. Electrospinning is a relatively simple scaffold fabrication process in which a high electrical potential is applied to a polymer solution. When electrostatic repulsive forces within the solution overcome surface tension, thin strands of polymer are ejected and drawn towards the nearest grounded surface, with whipping and drawing during fibre transit resulting in the collection of stable ultra-fine polymer threads with a nano-scale diameter (Mauck *et al*., 2009). Towards cartilage tissue engineering, early work by Li and co-workers showed that 3D nanofibrous poly(ε-caprolactone) (PCL) scaffold composed of electrospun nanofibres supported the chondrocyte phenotype and matrix deposition (Li *et al*., 2003). Additional work has produced PCL and polylactic acid microfibres scaffolds with 95 and 97 % porosity (Thorvaldsson *et al*., 2008), and degradable copoly(ether) esterurethane (PDC) or poly(p-dioxanone) (PPDO) using 1,1,1,3,3,3 hexafluoro-2-propanol (HFP) as a solvent (Schneider *et al*., 2012). This type of degradable polymer is a good material platform candidate for biomaterial-based cartilage repair, in particular when the fibres are sub-micron in diameter (Li *et al*., 2006). In general, chondrocytes seeded in such nanofibrous scaffolds grow well and produce cartilage extracellular markers, such as collagen type II and IX and proteoglycans. Indeed, preliminary studies using PCL scaffolds have been performed in a mini-pig model, showing that the nanofibrous scaffold is well tolerated and can foster tissue regeneration by co-implanted chondrocytes and MSCs (Li *et al*., 2009). Recent innovations in cartilage tissue engineering with nanofibrous scaffolds have included formation of zonally distinct cartilage layers based on organised nanofibres (McCullen *et al*., 2012), inclusion of sacrificial fibres to improve composite scaffold colonisation (Baker *et al*., 2012), and use of native cartilage ECM proteins to form the nanofibres (Shields *et al*., 2004). This is an extremely active area of research, and will likely provide new and exciting materials to guide cartilage tissue regeneration.

Transition of biomaterials from the laboratory into the clinic

Articular cartilage plays an important biomechanical role in the body, serving as a load-bearing tissue with distinct mechanical and biological properties at different anatomical sites. In addition, the susceptibility to injury or degenerative disease appears to vary among different sites in the body. Moreover, clinical algorithms for articular cartilage repair are chiefly based, besides clinical symptoms and patient age, on the size and depth of the lesion, its location, and the specific environment of the joint harbouring the articular cartilage defect. The use of new tissue-engineered approaches may depend upon whether defects are osteochondral or purely chondral in nature, potentially requiring specific therapeutic platforms that depend on the type and extent of the injury. Indeed, several biomaterial platforms which overcome general aspects of cartilage disease or trauma are already in the market (with many more in the pipeline). The biological matrices used in clinics may have the ability to provide some of the biological cues that regulate the fate of the implanted

cells. Collagen and hyaluronan-based biomaterials are the most used in articular cartilage repair, but consideration of the influence of the materials on the cell fate and the type of tissue formed has only been cursorily explored. When considering the use of stem cells, which need precise spatial and temporal signals to differentiate toward a chondrocytic phenotype and avoid hypertrophy, the creation of a material that can provide biological, chemical, and physical cues leading to the optimal cell response is still an important challenge. Recently, a poly(ethylene glycol) diacrylate (PEGDA) hydrogel has been used in a pilot clinical study in combination with microfracture in focal defects of 15 patients.

Perhaps the greatest barriers to be overcome in the translation of new biomaterials into the clinic are the regulatory aspects. Many of the newer proposed biomaterial solutions would be considered as a combination product by the FDA with the scaffold being considered a device and any functionalisation a biologic. Depending on the exact composition, each component may need to be tested for safety individually as well as in combination. For a single composite scaffold with two functionalisation motifs, this could result in four sets of safety data, which would be prohibitively expensive. Each additional component adding increasing levels of testing. This aspect is critical when developing novel biomaterials and should be one of the first steps considered in the process.

Gene transfer

The concept of applying gene-transfer technologies to treat diseases that affect human joints was initially proposed by Evans *et al.* in the early 1990s (Bandara *et al*., 1992; Bandara *et al*., 1993). The strategy focused on treating patients suffering from rheumatoid arthritis (RA) with *ex vivo*-modified cells. Here, synovial cells were manipulated using a retroviral vector to carry an interleukin-1 receptor antagonist (IL-1RA) and next injected into the metacarpo-phalangeal joints of patients (Evans and Robbins, 1995). The protocol successfully led to the production of a biologically active protein, with signs of clinical improvements in the treated cases (Evans *et al*., 2005b). This approach continues to remain attractive for diseases affecting the synovium (Robbins *et al*., 2003). However, it might be less adequate to address the clinical problem of focal articular cartilage defects (Evans *et al*., 2005a; Madry *et al*., 2011).

The challenge of treating focal articular cartilage defects

The difficulty in effectively treating such chondral or osteochondral defects with gene therapy is effecting the expression of a candidate gene specifically in the site of a lesion (Cucchiarini and Madry, 2005). This goal is different from the synthesis of a therapeutic product from the synovium that mostly remains diffuse in the synovial fluid. Several groups (Che *et al*., 2010; Cucchiarini and Madry, 2005; Gelse *et al*., 2008; Gysin *et al*., 2002; Ivkovic *et al*., 2010; Kaul *et al*., 2006; Madry *et al*., 2005; Madry *et al*., 2010a; Neumann *et al*., 2013; Park *et al*., 2006; Turgeman *et al*., 2001; Vogt *et al*., 2009) are developing targeted approaches to deliver genetically modified cells or gene vectors directly into cartilage lesions, by means of an arthrotomy or *via* arthroscopy as demonstrated by Goodrich *et al*. (2007). This concept is dependent on the feasibility of adequately containing modified cells or vectors in the defect. To address this issue, cell-based strategies in conjunction with biomaterials or the careful local administration of gene vectors have been investigated as a means of providing the therapeutic formulation directly inside the lesion to allow a locally effective and durable transgene expression.

Transplantation of genetically modified cells into cartilage defects

Implantation of autologous articular chondrocytes (ACI) is a clinically accepted procedure to treat large cartilage defects. In theory, transplantation of genetically modified cells may serve the dual role of providing a cell population capable of proliferating and depositing an extracellular matrix, together with the secretion of the overexpressed protein to stimulate cartilage repair. Based on the large body of data generated from high quality clinical trials (Knutsen *et al*., 2007; Vanlauwe *et al*., 2011), articular chondrocytes are a first choice for such approaches; however, recent reports applying unmodified MSCs (Kuroda *et al*., 2007; Nejadnik *et al*., 2010) also suggest the theoretical value of genetically modified MSCs (Cucchiarini *et al*., 2012). Experimental data in small and translational animal models have convincingly shown that such overexpression enhanced the structural features of the repair tissue. Similar to the technique of matrix-assisted ACI (Safran *et al*., 2008), delivery of genetically modified cells in conjunction with biomaterials is advisable to allow their spatially controlled application to enhance local chondrogenesis. It remains to be seen whether the strategy of cultivating genetically modified cells attached to a biomaterial in a defined biomechanical environment (Grad *et al*., 2011), reflective of a clinical rehabilitation regimen (Kupcsik *et al*., 2009), enables further improvements in the performance of such genetically modified grafts (Salzmann *et al*., 2009).

Direct application of gene vectors to cartilage defects

With the development of powerful molecular biology tools, some specific gene transfer vectors have emerged for their ability to deliver therapeutic candidate sequences directly in sites of articular cartilage defects (Cucchiarini and Madry, 2005). Instead of using nonviral compounds or classical viral vectors (adeno-/retroviruses), constructs based on the small, non-pathogenic human adeno-associated virus (AAV) have evident clinical advantages, as recombinant AAV (rAAV) vectors effectively transduce most relevant tissues and cells involved in cartilage repair, including the articular cartilage and chondrocytes, the subchondral bone, and MSCs (Cucchiarini and Madry, 2005; Cucchiarini and Madry, 2010). The use of cell- and tissue-specific regulatory elements (promoter/enhancers) (Haleem-Smith *et al*., 2005; Lefebvre *et al*., 1996; Xie *et al*., 1999; Zhou *et al*., 1998) might be necessary to target a special cell type among all those permissive to rAAV

transduction, an issue particularly important for direct injection of the vector into the joint. rAAV (a mostly episomal vector that carries no viral sequences) is also more powerful (and is as much or more efficient) than nonviral vectors, and safer than highly immunogenic adenoviruses and integrative retroviral vectors that may lead to tumour gene activation. Although all viral gene vectors have safety considerations, these are being overcome, with the European Medicines Agency recently recommending an rAAV gene therapy for clinical use in the treatment of severe or multiple pancreatitis attacks due to lipoprotein lipase deficiency (Web ref. 1). rAAV has been successfully tested by direct injection in cartilage lesions using an FGF-2 gene sequence, showing promising results in terms of healing of rabbit osteochondral defects at four months *in vivo* (Cucchiarini and Madry, 2005).

Therapeutic candidates

Equally important, careful selection of therapeutic gene(s) would be essential to treat cartilage defects as there is a need here to enhance the structural quality of the repair tissue, although factors that reduce inflammatory processes (like those applied for RA) may also be valuable. In this regard, reparative and regenerative properties have been attributed to growth and transcription factors (TGF-β, BMPs, IGF-I, FGF-2, GDF-5, PTHrP; SOX, RUNX2, Cart-1 and Ets families), signalling and regulatory molecules (Wnt, hedgehog families, CD-RAP) and to components of the extracellular matrix or enzymes that produce them (type-II collagen, COMP, tenascin), several of which having been successfully reported to improve the healing of cartilage defects in various experimental models (Cucchiarini and Madry, 2005).

Much work has been already performed in various small (Che *et al*., 2010; Cucchiarini and Madry, 2005; Gelse *et al*., 2008; Gysin *et al*., 2002; Kaul *et al*., 2006; Madry *et al*., 2005; Madry *et al*., 2010a; Park *et al*., 2006; Turgeman *et al*., 2001; Vogt *et al*., 2009) and large animals (Goodrich *et al*., 2007; Heiligenstein *et al*., 2011; Hidaka *et al*., 2003). For instance, it has been shown that tissue-engineered cartilage displays better *in vitro* properties following gene-based modification with IGF-I *in vitro* (Madry *et al*., 2002). Ivkovic *et al*. (2011) used autologous *ex vivo*-transduced bone marrow *via* an adenoviral vector carrying TGF-β1. Implantation of the resulting marrow clot improved not only the histological and biochemical, but also biomechanical parameters of partial-thickness chondral defects in a sheep model after 6 months *in vivo*. Hidaka *et al*. (2003), however, failed to observe biomechanical differences of the repair tissue between groups in a horse model when implanting allogeneic chondrocytes transduced by a BMP-7 adenoviral vector. The examples listed here only serve to illustrate the important steps that have been undertaken, and critical review of the literature is recommended for a more comprehensive analysis (Cucchiarini and Madry, 2005; Evans *et al*., 2005a; Evans, 2011; Evans *et al*., 2011; Ivkovic *et al*., 2011; Madry and Cucchiarini, 2011; Madry *et al*., 2011).

Transition of gene therapies from the laboratory into the clinic

Taken together, gene transfer might be envisaged as a tool to overexpress a therapeutic factor at the site of the lesion to enhance articular cartilage repair. Thus far, no attempts have been made to replace a defective gene in such an environment. Currently, promising preclinical large animal studies have convincingly shown the potential of this approach, using both cell transplantation and direct approaches. Roadblocks that need to be addressed before this concept can be translated into the clinics include those common to gene-based approaches, such as safety given the non-lethal nature of cartilage diseases, identification of the optimal therapeutic gene(s), long-term transgene expression, ideal biomaterial scaffold and cell type for

Table 2. Advantages and disadvantages of gene transfer vectors.

Classes		Advantages	Shortcomings	Integration
Nonviral vectors		not infectious low toxicity low immunogenicity	low efficiency short-term expression	no
Viral vectors	Adenoviral vectors	highly efficient	toxicity immunogenicity short-term expression replication competence?	no
	Retroviral vectors	long-term expression	efficient, but cell selection generally needed risk of insertional mutagenesis (activation of tumor genes?) replication competence	yes
	HSV vectors	highly efficient	cytotoxicity short-term expression	no
	rAAV vectors	highly efficient low toxicity low immunogenicity long-term expression	production complex	not as a vector

HSV: herpes simplex virus; rAAV: recombinant adeno-associated virus

implantation of these genetically modified cells, together with regulatory gene sequences. The advantages and disadvantages of various viral vectors are detailed in Table 2. Possible side-effects have also to be ruled out. These include, for example, the formation of osteophytes, as observed following intra-articular injection of a BMP-2 adenoviral vector (Gelse *et al*., 2001). However, when using an *ex vivo* approach (Gelse *et al*., 2001) to directly implant modified cells in conjunction with a matrix into the defect, such effects appear unlikely, as the many animal studies that used this approach showed unchanged levels of the therapeutic protein in the synovial fluid. Moreover, it is clear that such genetic modification adds another level of costs to these procedures. That stated, the field of articular chondrocyte implantation already has all the tools necessary to move this concept into a clinical situation, including GMP level facilities for each of the steps required. The optimal cell dose would also have to be defined. In ACI, 1×10^6 cells/cm^2 of defect area are typically implanted (Niemeyer *et al*., 2013; Safran *et al*., 2008). For a human gene therapy trial, a similar dose may be selected as a starting point of evaluation.

Beyond focal cartilage defects: can we resurface the whole joint?

The great majority of approaches for cell-based or tissue-engineered repair of articular cartilage defects have focused on the treatment of focal chondral defects (i.e., less than ~4-5 cm^2) (Farr *et al*., 2011). Such lesions may be the source of significant pain and disability, and the presence of a cartilage defect may in fact initiate a degenerative condition that affects the entire joint (Lee *et al*., 2000). Joint injury is one of the primary risk factors for osteoarthritis, and cartilage defects tend to progress in people with symptomatic osteoarthritis (Davies-Tuck *et al*., 2008). Furthermore, MRI and arthroscopy studies show a high incidence of cartilage lesions are present in asymptomatic joints, particularly in athletes (Flanigan *et al*., 2010).

However, data on the overall incidence of focal chondral or osteochondral lesions are scarce. Retrospective studies suggest that nearly 60 % of knees undergoing arthroscopy possess some type of chondral lesion, although a small fraction of these are symptomatic (Curl *et al*., 1997). If other joints such as the ankle, hip and shoulder are considered, the overall incidence of focal cartilage defects is significantly increased. In contrast to such focal lesions, osteoarthritis is a painful and debilitating disease of the entire synovial joint, characterised by progressive degenerative changes in the articular cartilage, subchondral bone, menisci, synovium and most other joint tissues. The overall incidence of osteoarthritis is believed to be many times higher than that of focal cartilage defects, with estimates of over 150 million people worldwide (Web ref. 2), although the great majority of joint replacements are currently performed in the USA and Europe.

To date, there have been few approaches that have attempted tissue-engineered repair of very large cartilage defects, or to treat an osteoarthritic joint, and the primary focus of the field has been on the repair of "circumscribed" or "focal" defects. Numerous challenges exist that have hindered attempts at repair of large defects or the resurfacing of an entire osteoarthritic joint. For example, cell-based therapies for large defects may require significantly greater numbers of cells, which may not be readily available, particularly for autologous primary cells. Once implanted in an arthritic joint, cells may be exposed to biomechanical and inflammatory factors that were related to the degeneration state of the joint, and such altered biomechanics or cytokines may influence chondrogenesis or cause degradation of the implant (Majumdar *et al*., 2001; Wehling *et al*., 2009).

From a biomechanical standpoint, the larger the defect, the more difficult it will be to restore the native mechanical environment of the joint, which requires matching the mechanical properties and geometry of the implant. While a small defect may provide support around the rim of the defect to protect a graft as it matures and integrates, larger defects will be more likely to be exposed to high stresses immediately and will require more robust mechanical properties to withstand joint loading. Theoretical models of joint contact have shown that increasing defect size leads to increased stress and strain on the rim of the defect (Pena *et al*., 2007). With increasing size, a greater proportion of the joint surface is involved, requiring more complex geometries to achieve joint congruence (Latt *et al*., 2011). At an extreme, total resurfacing of the joint would require an implant that matches the geometry of the whole joint while providing functional mechanical properties upon implantation.

Despite these challenges, significant advances have been made in the past few years toward the development of technologies for resurfacing of large cartilage defects or complete joint surfaces. From a bioengineering perspective, an evolving sub-discipline of tissue engineering, termed "functional tissue engineering", has placed focus on defining the biomechanical design parameters necessary for the success of mechanically functional tissues, and the development of scaffolds and tissue constructs that have appropriate anatomical geometry and mechanical properties to promote osteochondral repair (Guilak *et al*., 2001). In this respect, a number of novel technologies have led to the development of anatomically shaped scaffolds that are designed to restore joint surface geometry and therefore congruence over large surfaces. For example, moulded agarose constructs overlying trabecular bone have been used to create a construct that matches the anatomical geometry of the patella (Hung *et al*., 2003). Rapid prototyping methods have also been used to fabricate three-dimensional femoral and tibial cartilage constructs to restore congruent articulating surfaces in small joints (Lee *et al*., 2010; Woodfield *et al*., 2009). Importantly, new classes of biomaterials and biomaterial structures have been developed that can not only provide predefined geometries, but possess functional biomechanical properties similar to those of native tissues so that they can withstand joint loading immediately upon implantation (Freed *et al*., 2009). For example, three-dimensionally woven composite materials can be designed to provide many of the complex properties of native cartilage, including anisotropy, inhomogeneity, nonlinearity, and viscoelasticity (Moutos

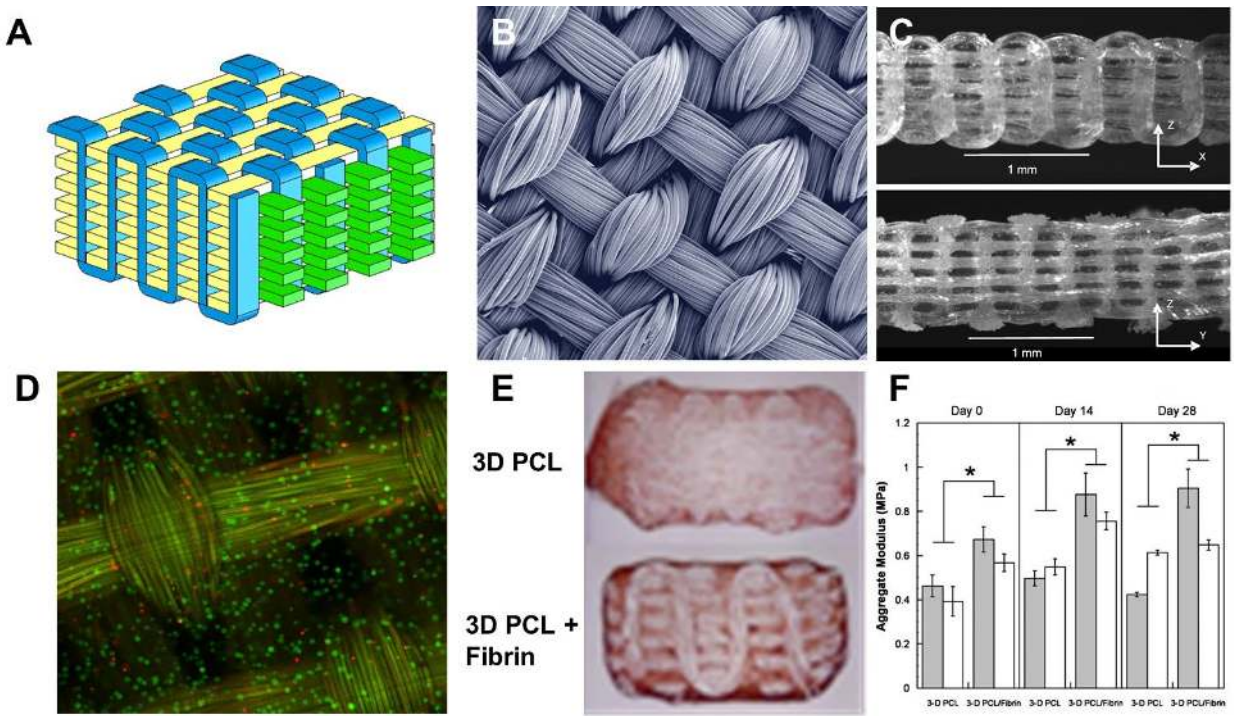

Fig. 4. Three-dimensional woven scaffolds for cartilage tissue engineering. **A.** Fibre architecture showing the orthogonal structure consisting of multiple layers of x (green), y (yellow), and z (blue) fibers. **B.** Scanning electron micrograph of the scaffold surface. **C.** Cross-sectional views of the x-z and y-z planes showing the continuity of the pore structure. **D.** Live-dead fluorescent labelling of cells seeded within the scaffold. **E.** Immunohistochemistry for type II collagen production in 3D woven PCL scaffolds seeded with adipose-derived stem cells (ASCs) with or without a fibrin gel. **F.** Unseeded and ASC-seeded scaffolds exhibit biomimetic cartilage mechanical properties over a 28 d culture period. Adapted from (Moutos *et al*., 2007; Moutos and Guilak, 2010).

et al., 2007) (Fig. 4). Similarly, other materials processing techniques, such as electrospinning, can be used to create highly anisotropic materials that can control cellular alignment and matrix deposition (Li *et al*., 2007).

Thus, while significant progress is being made in the development of cell-based and tissue-engineering strategies for the repair of small osteochondral defects, continuing advances in stem cell biology and scaffold technology may allow the extension of these methods to the treatment of large defects, or perhaps, biologically-based resurfacing of the entire joint. Nonetheless, a number of challenges still remain in this regard. In addition to the aforementioned biomechanical requirements, the development of large constructs that cover the entire joint surface will require large numbers of cells, emphasising the issues discussed regarding the ease of cell harvesting and expansion. Similarly, the exposure of an engineered tissue to the inflammatory environment of an osteoarthritic joint may inhibit stem cell differentiation, or induce degradation of the newly formed cartilage, further emphasising the need for scaffolds that can provide biomechanical function in such situations (Ousema *et al*., 2012).

In vitro to *in vivo* translation – general considerations

As work with cell sources, biomaterial frameworks and biologic intervention/gene transfer methods to further cartilage tissue engineering matures, the question that arises is how to facilitate, categorise, and benchmark such progress (all of which is occurring simultaneously in many laboratories the world over)? Further, progress towards the realisation of functional engineered cartilage constructs brings up the practical (and moral) question of when is it appropriate and/or expeditious to test these newly developed products in animal models? Secondary to this question is which animal model is best to use?

With the great spectrum of choices that one can make in cartilage tissue engineering, there now exist several critical needs for the field to tackle. First and foremost, a 'common language' must be established to allow for rapid and reliable comparisons across groups. For example, when reporting a mechanical property, it is essential that material properties be reported using the same testing configurations (that "apples" be compared to "apples") and ideally reported with respect to native cartilage tissue tested in the same fashion. Likewise, it would be a great benefit if the community could decide on (and share) a common cell source (including a common culture procedure with defined medium). While this would be difficult to implement due to the lack of consensus on which cell type is most suitable, the inclusion of an additional group which could be used as a reference between studies might be an option in the short term. Here, each new study would then be compared to identical conditions using this 'gold standard', and so relative efficacy of any new cell source or cell-material interaction could be appropriately adjudged. Finally, with the increasing number of choices, we must similarly expand

our vision in terms of outcome testing. Gone are the days when a study could be done on a sample by sample basis, while still taking advantage of the large parameter space available. We have already seen efforts in the community to develop high throughput screening (HTS) tools, much like those used in the pharmaceutical industry for drug screening, that are specific for cartilage tissue engineering biochemical outcomes (Huang *et al*., 2008). Along with these HTS assays, the idea of micro-scaling the work will enable a host of conditions to be assayed at once in an economical fashion (Khademhosseini *et al*., 2006). Still more development is required in this research space. For example, mechanical outcomes must be foremost in any cartilage tissue engineering effort (given the required load bearing characteristics of the tissue), and a focus on a reliable and reproducible mechanical high throughput screening tool for the community should be developed. These advances would allow for a mature field to make rational choices so as to better select new cells, new factors and their combination, and new material formulations for improved cartilage tissue engineering.

While much progress remains to be made *in vitro*, today there do exist cartilage constructs that approach native tissue values for certain quantitative parameters (including biochemical and mechanical properties) e.g., (Lima *et al*., 2007; Erickson *et al*., 2012; Moutos *et al*., 2007). Where then should such constructs be tested, and what outcome parameters are most important in the *in vivo* transition of such constructs?

The lapine model of cartilage repair is a good first animal screening system for any new material, provided that skeletally mature rabbits and appropriate negative controls and sample sizes are used (Orth *et al*., 2012; Laverty *et al*., 2010; Hunziker, 1999; Buckwalter and Mankin, 1998; Mankin, 1982), but the cartilage is thin, limiting most studies to the study of osteochondral (rather than chondral) healing. When transitioning to a larger animal system, however, the species of choice remains controversial, with various groups advocating mini-pig, dog, sheep, goat or horse as the ideal system (see recent reviews (Chu *et al*., 2010; Ahern *et al*., 2009)), with each model having its own benefits and drawbacks. The most appropriate clinical pathology chosen to be studied remains controversial as well; is it better to study osteochondral or chondral lesions? Clinically, the latter are seen more often, but the former may be easier to implement repair solutions for, although they are perhaps more complicated as they involve multiple tissue phases. Furthermore, not addressing a possible underlying subchondral bone pathology that either was induced in response to the cartilage loss or was the initial reason for the cartilage defect would likely diminish the clinical relevance (Gomoll *et al*., 2010; Lories and Luyten, 2011; Madry *et al*., 2010b; Madry, 2010). Similarly, there remains the open question as to whether to implant a construct early, allowing maturation to occur *in vivo*, or whether it is best to implant a fully matured construct. Again, both approaches have clinical relevance and practical issues driving selection. It is also not yet clear what rehabilitation regimens are most appropriate, and whether these can be implemented in the various animal models. In patients, it is not uncommon to institute a low load-bearing rehabilitation regimen for up to three months, but this is difficult to achieve in most animal models. Additionally, the age of the animal model will have a significant impact on outcomes; is it better to test engineered constructs in a young animal, where repair or regeneration is more likely, or in an aged animal or one with concomitant joint pathology so as to better recapitulate the clinical scenario? On one hand, demonstration of efficacy in the absence of challenge may lead to overly optimistic results, while engineering in most difficult scenario may limit progress, when every attempt is a failure. What is clear is that a realistic consensus must be achieved, and that consistent outcome measures should be applied across animal platforms. Moreover, given the distinct environments of *in vitro versus in vivo* studies, when *in vitro* constructs demonstrate a certain 'threshold' of function, they should be immediately assayed in a large animal load-bearing environment, so that realistic assessment of their potential can be evaluated. Finally, the relevant funding agencies in Europe, the US, and around the world should be educated on this process, and should support such endeavours to enable and foster progress in this area.

Concluding remarks

As can be seen from the points raised in this review, the number of potential variables in any cartilage tissue engineering strategy is vast, and those parameters that will be the most critical for functional outcomes have yet to be determined. In addition, it is clear that certain methods and models used are suboptimal for determining the effect that will be seen in a likely patient population. While they are useful for improving academic knowledge, researchers have to be more self-critical if the challenge of translation is to be overcome. Researchers must also become more aware of the regulatory requirements and associated costs that they will face in translating potential therapies into the clinical arena. It has been proposed that rather than waiting until the optimal conditions have been found, intermediate treatments should be investigated and then later improved upon (Evans, 2011). While this approach has its merits, the costs of clinical trials, and the difficulties associated with clinical acceptance and implementation, may make this approach financially nonviable. These hurdles also explain why many published clinical studies are not performed in the optimal manner. The lack of well performed, prospective clinical studies comparing and validating cartilage repair techniques makes drawing conclusions on efficacy problematic. Until a scientifically rigorous and cost effective way of testing novel therapies within a clinical setting is found, translational progress will be painfully slow.

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Discussion with Reviewers

Reviewer II: In the section on clinical translation, you mention the key challenge of getting individual investigators to work as a consortium to expand consensus and accelerate translation of cartilage tissue engineering strategies. However, this noble goal can conflict with the current pressures (and incentives) within academic research, where novelty of investigation is valued and bibliometrics such as the H-index are being used increasingly to evaluate scientific productivity. Consequently, many investigators may not reach a point of professional discretion to prioritise cost- and time-intensive translational studies until much later in their career. How might the greater community, possibly through its professional societies, better address this underlying obstacle to consortium research?

Authors: This is an excellent and challenging question. The rise in popularity of metrics that purport to measure the 'value' of an individual academic researcher would seem to be out of step with this fact, that the days of an individual researcher working in isolation are coming to an end as projects increase in complexity. There needs to be recognition of this fact from all strata of the academic research infrastructure. The metrics are not going away, so use of those appropriate for modern scientific inquiry is crucial. Many metrics fail to account for the time-intensive nature of translational studies. Perhaps this is where professional societies can play a role – advocating for more appropriate values to be used when assessing the collaborations that a young investigator has entered into. An intermediate step might be an adjustment in the value placed on journals that publish translational research. In targeting solutions to defined problems, papers can be rejected from mainstream journals for being too observational. We are already seeing major journals

producing more specialised versions with translational subjects, elevating those subjects and allowing younger investigators new avenues to higher metrics. At least funding bodies are embracing multi-centre consortium applications to a greater extent, and such a mechanism was employed by AOERB in bringing this group of researchers together, since it is unlikely that one group alone could address and solve the challenge of cartilage repair.

Reviewer II: Can you provide a specific lesson learned from your collective experience in the Acute Cartilage Injury Collaborative Research Program regarding multidisciplinary collaboration in cartilage tissue engineering? Perhaps a success story to inspire the eCM readership?
Authors: When the consortium was set up, the partners were selected from a peer-reviewed pool of individually submitted ideas. Thus, for those selected, the challenge has been to find ways to 'make the sum of the consortium greater than the total of its parts'. To this end, we began by discussing each partner's projects in detail to find all the points at which other partners could intersect with it. This openness led to agreement about which cell sources, models, tests and analyses all partners could use, exchanges of materials and techniques, and of course, the discussions of the problem that produced this review.

Reviewer III: How would you address the problem that neo-cartilage will be surrounded by normal cartilage with different mechanical properties? This could lead to accelerated destruction of the repair tissue.
Authors: This is an often-overlooked challenge, as the focus to date has been on producing large amounts of tissue. Mature cartilage replacements do not integrate well with the surrounding tissue, but we do not know what level of maturity an implant should be that would allow integration, while maintaining function and avoiding breakdown. Methods that rapidly induce maturation of neocartilage might be one direction to take (Khan et al., 2011).

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