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Bioengineering of articular cartilage: past, present and future

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Abstract

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Keywords

present, cartilage, past, articular, future, bioengineering

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Summary

The treatment of cartilage defects poses a clinical challenge due to the lack of intrinsic regenerative capacity of cartilage. The use of tissue engineering techniques to bioengineer articular cartilage is promising and may hold the key to the successful regeneration of cartilage tissue. Natural and synthetic biomaterials have been used to recreate the microarchitecture of articular cartilage through multilayered biomimetic scaffolds. Acellular scaffolds preserve the microarchitecture of articular cartilage through a process of decellularisation of biological tissue. Although promising, this technique often results in poor biomechanical strength of the graft. Biomechanical strength could be improved however, if biomaterials could be incorporated back into the decellularised tissue to overcome this limitation.

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Background

Cartilage damage can result in pain and loss of function for many patients, and the management of moderate to severe defects has been difficult due to the lack of intrinsic capacity for cartilage to regenerate [1,2]. The fibrocartilage formed differs substantially from hyaline cartilage; therefore the goal is to form regenerative tissue with compressive and hydrodynamic qualities similar to hyaline cartilage. Many reports relate compromised function associated with repaired cartilage and loss of function of the articular surface [3].

Traditional methods of repair of osteochondral defects include debridement, marrow stimulation, osteochondral grafting and autologous chondrocyte implantation (ACI) [4-9]. Arthroscopic debridement and lavage provides symptomatic relief but does not change the natural course of the disease and has similar outcomes to placebo surgery [10,11]. Marrow stimulation, usually in the form of microfracture, relies on local recruitment of marrow based stem cells and

growth factors to the site of articular repair [12]. The resulting fibrocartilaginous repair does not resemble surrounding hyaline cartilage, consisting of less collagen type II [13]. A prospective study on microfracture showed good to excellent in 67% of patients following a mean postoperative follow-up period of 3.6 years [14]. However, results of microfracture deteriorate over time due to the formation of fibrocartilage in the repair tissue [15,16].

Osteochondral grafting and ACI techniques however aim to regenerate hyaline cartilage. Recent 10 year follow-up study showed superior clinical results of osteochondral grafting compared with microfracture in young athletes with focal osteochondral defects [17]. An earlier clinical study of ACI showed an improvement in symptoms in 14 out of 16 patients at 2 years who had femoral condylar lesions [18]. Peterson et al showed good to excellent clinical results following ACI in a 2- to 9-year followup period, in particular in patients with isolated femoral condyle lesions and osteochondritis dissecans of the knee [19]. At 10 to 20 years post ACI implantation, 74% of 224 patients reported improvements in symptoms [20]. Similarly, Vijayan et al recently reported 12 out of 14 patients with good to excellent clinical outcomes at 2 to 8 year followup (average 5.2 years) post matrix-induced autologous chondrocyte implantation (MACI) [21]. However in a 5 year long term randomised controlled trial, ACI results have been comparable to microfracture, although subgroup analysis of that trial showed patients with onset of symptoms less than 3 years had better outcome with chondrocyte implantation than microfracture [22].

Disadvantages of osteochondral grafting include limitations on donor site availability and morbidity [23]. The space between cylindrical grafts may impair the quality of the repair as Lane et al. found poor integration of full thickness gaps in experiments in goats [24]. ACI is technically challenging with high reoperation rates of 9-20% and associated higher costs [14]. In one study, 36% of periosteal patches required debridement of the graft due to periosteal hypertrophy [25]. It also requires *ex vivo* expansion of chondrocytes which necessitates two operations typically at an interval of 2 weeks.

New products have since been introduced for clinical trials and clinical use. These products are based on traditional methods of repair, enhanced with tissue engineering techniques, and are summarised in table 1. Although a few have shown some promise, the majority lack long term studies and complications have been reported [26,27].

Although techniques such as osteochondral grafting and ACI have shown improvements over microfracture in certain cases, further improvements can be made to increase the longevity and consistency of clinical results achieved through current standards of care. As a result substantial research continues to focus on advancements in tissue engineering of cartilage to overcome the limitation of current repair methods and to develop a bioengineered cartilage regeneration therapy. Biomimetic scaffolds using natural and synthetic biomaterials have attempted to reverse engineer the complex microarchitecture of hyaline cartilage. Recent developments in acellular biological scaffolds, which aim to preserve the native microarchitecture of cartilage to aid in regeneration of cartilage defects, may hold the key and the future of articular cartilage regeneration.

Tissue Engineering

Tissue engineering has the potential to overcome the limitations of current treatment options for osteochondral defects. Tissue engineering combines the use of cells, biomaterials and stimulatory factors to regenerate and reconstruct the osteochondral unit. 3D tissue grafts can be shaped, engineered and tailored to specific needs to improve structural, biological and biomechanical properties of current repair processes [28].

Cell Source

Chondrocytes, fibroblasts, stem cells and genetically modified cells have been explored as sources for cartilage regeneration, the goal of which is to identify a source that can be reliably used to regenerate good quality articular cartilage [2].

Chondrocytes

Chondrocytes are responsible for the secretion and maintenance of extracellular matrix and appear to be the logical cell of choice. Mature chondrocytes secrete type 2 collagen and sulphated glycosaminoglycans (GAGs) as extracellular matrix to maintain and remodel the cartilage matrix [29]. However the use of chondrocytes is limited by two major concerns. Chondrocytes are limited in number comprising only 2-5% of cartilage tissue and thus require expansion prior to use [29-31]. Furthermore, the process of expansion and cell culture causes dedifferentiation of mature chondrocytes so synthesis of proteoglycans and collagen Type II is decreased and collagen expression converts to collagen Type I [32-34]. A variety of methods have been used to prevent or limit the degree of dedifferentiation such as three dimensional culture and scaffolds, bioreactors, reduced oxygen tension and addition of growth factors such as transforming growth factor β (TGF-β), FGF and insulin like growth factor (IGF) [35-40]. These methods have produced hyaline cartilage, with varied success, in in-vitro studies.

Stem Cells

To avoid the limitations of chondrocytes, mesenchymal stem cells (MSCs) have been used for chondrogenesis and osteogenesis [41]. MSCs are found in a variety of human tissue including bone marrow, periosteum, synovial membrane, skeletal muscle, dermis, blood and adipose tissue [40,42-44]. Bone marrow-derived stem cells (BMSCs) have been most extensively studied. However, BMSCs have been shown to express markers showing hypertrophic chondrogenesis (type X collagen and MMP-13) that mineralize when exposed to osteogenic stimuli [45-47]. Adipose-derived stem cells (ADSCs) are commonly used for the generation of chondrocytes due to their ease of harvest and the availability of larger numbers of stem cells [48]. Together with various growth factors such as TGF-β and scaffold or culture media, such as alginate or agarose gel, these cells have been shown to undergo chondrogenesis with enhanced production of collagen Type II and aggrecan [49-54]. However, MSCs tend to produce inferior matrix in terms of mechanical integrity compared with chondrocytes [55].

Human embryonic stem cells (hESCs) represent an alternative cell source for chondrogenesis due to their vast differentiation capacity into various somatic cell lineages and proliferative capabilities. A recent study demonstrates the ability for hESCs to undergo efficient chondrogenic differentiation using a hyaluronic acid hydrogel method of delivery in a rat model. They also showed complete integration of the hESCs engineered cartilage with surrounding cartilage in two-thirds of animals without the development of tumours at 12 weeks [56]. Hwang et al showed that mesenchymal stem cells derived from hESCs, are capable of multilineage differentiation into fat, cartilage and bone in vitro, and achieving normal cartilage architecture in rat osteochondral defect repair [57].

Recently, induced pluripotent stem cells (iPSCs) have been used to different both osteogenic and chondrogenic cell types [58,59]. Like hESCs, iPSCs has the potential to provide great scope for cellular expansion and differentiation compared to mesenchymal stem cells, without the same ethical problems [60]. There is always a risk of tumourigenicity associated with the use of stem cells and in particular the use of viral vectors. Newer methods that generate iPSCs without viral vectors have been developed to reduce the risk of tumourigenicity [61-64]. Overall chondrogenic differentiation of iPSCs is still in its formative stages of development and further work is required to evaluate its full potential in the field of osteochondral regeneration.

Scaffolds

Scaffolds provide the environment into which cells can grow and produce cartilage tissue and extracellular matrix. As related above, chondrocytes require 3D culture to avoid dedifferentiation of their phenotype [65]. Furthermore, the process of dedifferentiation can be reversed when chondrocytes are relocated into a three-dimensional (3D) environment [66-68]. Scaffolds can be made from a diverse range of materials including natural or synthetic materials or a hybrid of both. They can also be designed in forms of hydrogels, sponges, or fibrous mesh. Hydrogels support the transportation of cells and bioactive agents and can suspend cells in a three dimensional

environment. They can also be injected to fill defects of any size and shape. However they have inferior mechanical properties compared with other forms of scaffolds [69]. Sponges are porous scaffolds that facilitate cell adhesion. Pore size variation affects cell adhesion, migration and deposition [70]. Meshes can also be made to variable porosities governed by fibre diameter and direction. They exhibit greater mechanical strength but irregular filling into the mesh itself may compromise the quality of the graft and affect tissue integration. 3D constructs of woven fibres and electrospinning have been used to mimic the native cartilage material and 3D environment [71,72].

Natural materials

Natural materials used in cartilage engineering include collagen, hyaluronic acid (HA), chitosan, alginate, fibrin, silk, gelatin, bacterial cellulose, and cartilage derived matrix. Examples of these materials are summarised in table 2 along with their respective advantages and disadvantages. Collagen and hyaluronic acid are two of the most common materials used in cartilage engineering and clinically most relevant, with many products already in clinical use and trial which are based on tissue engineered collagen or hyaluronic acid materials. Thus, these materials will be discussed further.

Collagen has the advantage of being biodegradable, biocompatible and ability to be crosslinked [73]. Therefore it is a versatile materials used in tissue engineering. Collagen can be formed into different types of scaffolds including sponges, membranes, films, gels and fibres using a variety of fabrication methods [74]. Each fabrication method produces a different set of mechanical and biochemical properties. Methods that induce pore formation such as freeze-drying process result in greater porosity which allows greater cellular and soluble factor infiltration into the materials whilst decreases the inherent biomechanical strength of the material [75]. Collagen hydrogels are easy to make and forms a gel that can absorb large amounts of fluid which aids in cellular infiltration. However in the gel form collagen fibres are not aligned and therefore do not aid in manipulation of the microarchitecture of the material to mimic the natural environment [76].

However, in some cases the use of collagen has resulted in a foreign body reaction and poor integration with surrounding tissue [2,77].

Recently, a two year randomised clinical trial of NeoCart, a collagen type I based bioscaffold seeded with autologous chondrocytes cultured in a bioreactor, showed improved clinical outcomes compared with baseline and microfracture groups [78]. Adverse events related to the study were consistent with those associated with knee arthroscopy. Whilst the results are promising, larger studies over longer periods are required before definitive conclusions can be drawn on the efficacy, safety and benefit of novel therapies.

Hyaluronan is an important component of the extracellular matrix of cartilage. Not only does it hold water to give compressive strength to cartilage, it also interacts with binding proteins, proteoglycans and growth factors which help maintain the ECM structure [79]. Hyaluronic acid (HA) is useful in the development of hydrogels due to its negative charge and water-trapping properties [80]. HA has been used extensively in tissue engineering not only for bone and cartilage but also in liver, cardiac, vascular, dermal, ophthalmic and neural tissue [81]. Mechanical, degradation rates and biological function can often be modified and controlled through modification of the HA molecule via chemical derivatisation and/or crosslinking with different molecules [82,83]. Toh et al found that lower cross-linking improved chondrogenesis of mesenchymal stem cells in a HA based hydrogel with increases in the percentage of cells with chondrocytic morphology and improved biosynthesis of collagen type II and glycosaminoglycans. Increasing hydrogel cross-linking improved matrix stiffness but promoted fibrocartilage formation [84]. HA also exists as fibrous scaffolds in the form of Hyaff. Hyaff scaffolds have been shown to allow growth of chondrocytes and support the chondrogenic and osteogenic differentiation of mesenchymal stem cells [85,86]. Hyalograft C autograft is composed of autologous chondrocytes grown in a 3D Hyaff scaffold, and was first introduced into the clinical setting in 1999 for the repair of full thickness cartilage defects [79]. Recent prospective clinical case series, with 2 and 7 year follow-up, showed clinical improvement in

young patients with single defects, however for patients with more advanced disease or with generalised osteoarthritis the results were poor [87].

Synthetic polymers

Synthetic biodegradable polymers offer an alternative to natural materials for the purposes of tissue engineering. These materials offer certain advantages in recreating the complex and dynamic nature of native ECM. The key advantage include increased mechanical strength, degradation kinetics, versatility of fabrication methods with excellent control over shape, size and porosity, as well as the ability to add functional chemical groups to enhance the biological effect of the material [88]. Biodegradation has proven to be important in clinical use. Biodegradable polymers such as poly(glycolic acid), poly(lactic acid) and their copolymers have been in clinical use since the 1960s such as in resorbable sutures [89]. Since then many other materials such as poly(dioxanone), poly(trimethylene carbonate) copolymers, and poly(ε-caprolactone) have been used in many medical devices [90,91]. The ideal polymer must consist of the appropriate mechanical properties to match the native ECM whilst allowing sufficient degradation time for tissue healing or regeneration to occur. However it must not cause inflammation or toxicity from the material itself or its degradation products and ideally be fully metabolized by the body after use [89]. A number of materials have been used for cartilage tissue engineering listed in Table 2.

Poly(α-hydroxy esters) include poly(lactic acid) (PLA), poly(glycolic acid) (PGA), the copolymer poly(lactic-co-glycolic acid) (PLGA), and poly(ε-caprolactone) [92]. They are the most commonly used synthetic biodegradable polymers for cartilage tissue engineering [93]. PLA exists in three isomer forms: poly(L-lactic acid), poly(D-lactic acid), and poly(DL-lactic acid) depending on the position of the methyl group [92]. Amongst these poly(L-lactic acid) and poly(DL-lactic acid) are used more often as biomaterials. Poly(L-lactic acid) is a semicrystalline polymer exhibiting high tensile strength and low elongation making it suitable for load bearing applications such as sutures and orthopaedic fixation devices [94,95]. Poly(DL-lactic acid) is an amorphous polymer consisting of a

random distribution of each isomer and therefore has lower tensile strength and higher elongation and more rapid degradation, therefore making it more useful in a drug delivery system [89].

PGA is a highly crystalline polymer with high tensile strength used to develop the synthetic absorbable suture known as DEXON® in the 1970 [96]. However PGA also exhibits a high degradation rate and low solubility in most organic compounds due to its highly crystalline structure. This can result in the accumulation of degradation products which can cause inflammatory reactions [97,98].

One major issue with the use of synthetic materials is the acidic degradation by-product of the polyester materials. This has been implication in the stimulation of inflammatory reactions as well as deactivation of proteins in the surrounding tissue [99]. Therefore, this has led to the development of copolymers of the lactides/glycolides with other monomers to form poly(ether esters), poly(ester carbonates), poly(ester amides) and poly(ester urethanes) [100-105].

Shi et al (2012) used a 3D fibrous poly(L-lactic-co-glycolic acid) (PLLGA) scaffold to repair femoral trochlear lesions in rabbit knees. They showed when combined with microfracture the repair of full thickness defects was more rapid and efficient when compared to either microfracture or scaffold alone. There was positive staining of collagen type II and toluidine blue with good integration of repair tissue at 24 weeks [106]. Tru-Fit Plug (Smith & Nephew, Andover, MA, USA) is a synthetic resorbable biphasic implant from polyactide-coglycolide copolymer, calcium sulphate and polyglycolide. In early results, it has shown formation of fibrocartilage with inferior biomechanical stability when subject to high shear forces in the knee, ongoing articular surface irregularity resulting in subsequent arthritic change and delayed integration [107-109].

Techniques to accelerate chondrogenesis

Stimulating factors modulate cell behaviour and this may be by direct biochemical interaction or induced by mechanical stimulation. Growth factors commonly used to induce chondrogenesis of various cell types. Articular cartilage is subjected to mechanical pressure under physiological conditions. Mechanical stimulation such as hydrostatic pressure and dynamic

compression techniques have been used to mimic intra-articular conditions and do improve chondrogenesis in vitro [110,111]. Furthermore, the addition of growth factors with mechanical stimulation seems to produce synergistic effects [112].

Growth factors

Multiple growth factors play an important role in the chondrogenesis of stem cells. The transforming growth factor-beta (TGF-β) superfamily contains many which promote chondrogenesis, including TGF- β1, TGF- β3, BMP-2, BMP-4, BMP-7, and GDF-5 have been shown to promote cartilaginous ECM production [113]. Whilst they all promote cartilaginous ECM production, TGF- β1 and BMP-2 also down-regulate collagen type I production [114]. Insulin-like growth factor 1 (IGF-1) is the main anabolic growth factor in cartilage and controls proteoglycan synthesis and breakdown, and induces expression of chondrocyte phenotype [115]. Its effect is independent to the TGF-β signalling pathway and therefore when combined leads to additive effects on cartilage matrix synthesis [116,117]. Fibroblast growth factor (FGF)-2 and FGF-18 promotes the proliferation of chondrocytes and helps to prevent cartilage against damage [118,119].

Oxygen tension

Articular cartilage is avascular with oxygen and nutrients being delivered via passive diffusion from synovial fluid [68]. Therefore, articular cartilage exists naturally in a low oxygen environment. Hypoxia inducible factor (HIF) mediates transcription factors to allow chondrocytes to adapt to low oxygen tension [120]. Hypoxia has been shown to increase the synthesis of ECM proteins in vitro in both chondrocytes as well as hypoxia-induced chondrogenic differentiation of MSCs [67,121,122]. Hypoxia has also been shown to inhibit the expression of collagen Type X, present in fibrocartilage and a marker of chondrocyte hypertrophy [123,124]. Therefore it seems hypoxia is an important environmental factor to be considered for cartilage regeneration.

Bioreactor

Bioreactors are used to improve nutrient transport and provide a fluid-induced shear stress to tissues to promote chondrogenesis. Current bioreactors used for cartilage tissue engineering include parallel-plate bioreactors, rotating wall bioreactors, and concentric cylinder bioreactors [38,125,126]. Lu et al (2012) showed increased deposition of collagen II and glycosaminoglycans leading to the formation of cartilage like tissue in a rotating-shaft bioreactor using TGF-β3 expressing adipose stem cells[127].

Electrical stimulation

Electrical stimulation has also been employed to induce cartilage and bone repair. In 1974, Baker et al. attempted to enhance cartilage repair stimulation of articular cartilage repair by electrical means using bimetallic devices inserted into full-thickness articular cartilage defects [128]. They demonstrated enhancement of latent potential for repair with hyaline cartilage. The repair response appeared to derive from proliferating chondrocytes at the defect margin, with encroachment over the surface of the central defect. More recently Brighton et al. reported that capacitatively coupled electrical signal resulted in significant up-regulation of cartilage matrix protein expression and production while simultaneously significantly attenuating the up-regulation of metalloproteinase expression [129]. These results support the contention that delivery of a specific, defined electrical field to articular cartilage could result in matrix preservation. They concluded that the use of electrical stimulation to both increase matrix production and diminish matrix destruction has the promising potential to treat osteoarthritic patients in a non-invasive manner.

Recreating the microarchitecture of articular cartilage

The biomechanical function of articular cartilage results from the structure of the extracellular matrix. The dense network of collagen and proteoglycans in the ECM not only support chondrocyte attachment but also transmits mechanical force within the ECM to allow cells to respond to mechanical stress [130]. The collagen network provides tensile strength and the

proteoglycans, due to their negative charge, maintains high levels of approximately 70% water content to resist compressive forces [131]. The intrinsic structure of articular cartilage is further organized into three distinct zones: superficial or tangential, middle or transition, deep or radial zone. This sits above a layer of calcified cartilage. Each zone has distinct ECM composition, organisation and cellular phenotype. Towards the superficial layer the chondrocytes are smaller, thinner, and orientated parallel to the articulating surface along with the orientation of the collagen network to provide resistance to shear forces [132]. Here chondrocytes also secrete lubricin, otherwise known as superficial zone protein, which acts to reduce friction resistance of the cartilage [29,133]. The middle zone consists of larger rounded chondrocytes with random collagen orientation with high levels of proteoglycans [134]. The deep zone consists of oval chondrocytes with collagen fibres forming a vertical or perpendicular alignment. Deep zone cells produce more collagen and proteoglycans than the superficial layer however has a lower cell density [131].

Biomimetic scaffolds

Most attempts to date at bioengineering cartilage have focused on using natural and synthetic biomaterials, as mentioned previously, to mimic the natural microarchitecture and biomechanical properties of native cartilage. Recent examples of such an approach include Kon et al, where a multilayered gradient nano-composite scaffold using collagen type I fibrils with hydroxyapatite nanoparticles were used in a pilot trial of thirty patients with chondral and osteochondral knee lesions [135]. Others have used fibre-hydrogel composite materials to mimic the native extracellular structure [136]. More examples are listed in the references of table 2 and many have been discussed throughout the course of this review. The advantages and disadvantages of each scaffold relate to the materials used. However in general composite materials attempt to harness the strengths of each material used.

Acellular biological scaffolds

Acellular scaffolds consist of noncellular parts of a tissue such that collagen and carbohydrate structures are maintained in their natural state. Therefore they should maintain the appropriate environment for cellular re-attachment, migration, differentiation and proliferation to enhance tissue regeneration when transplanted, whilst maintaining, in theory, a perfect microarchitecture for the repair tissue (Figure 1) [137]. In recent years decellularised biological matrices has been used to regeneration various tissue types including skin, cartilage, bladder, spinal cord, and myocardium[138-142].

A number of studies to date have described the use of acellular cartilage matrices in the repair of chondral and osteochondral defects [143,144]. Cheng et al showed acellular porcine cartilage-derived matrix was able to support the growth of neocartilage formation in the absence of exogenous growth factors [143]. Recently the same group was able to induce chondrogenic differentiation of human adipose-derived stem cells without exogenous growth factors on an acelluar cartilage matrix crosslinked with genipin to prevent scaffold contraction [145].Schwarz et al have shown the successful decellularisation and sterilization of porcine knee and nasal cartilage and human nasal cartilage. They also show the ability to remove proteoglycan content whilst maintaining the collagen structure. However the decellularisation process also increased the amount of denatured collagen compared with native cartilage. Overall there was significant decrease of biomechanical loading stress, which the acellular matrix showing reduced stiffness by about 69.5% [146]. The matrix did however support the growth of chondrocytes and re-acculumation of proteoglycans in the process of in vitro culture [147]. Kang et al also reported the use of decellularized cartilage ECM scaffold loaded with adipose stem cells [148]. They used a rabbit osteochondral defect model to show adipose stem cell loaded ECM scaffold induced cartilage repair tissue comparable to native cartilage in both mechanical and biochemical properties at 6 months.

Other types of cell-derived matrix (CDM) including fibroblast-derived matrix, preosteoblast-derived matrix and chondrocyte-derived matrix have been explored and found to support and

enhance the growth of chondrocytes and provide a chondro-inductive microenvironment for re-differentiation of dedifferentiated chondrocytes [149].

The primary concern with decellularised extracellular matrix is the loss of biomechanical strength and stability during the process of decellularisation. All studies so far have demonstrated a loss of mechanical strength as a result of reducing or removing certain components of the extracellular matrix in order to achieve decellularisation.

Lee et al was able to regenerate an entire joint surface of the rabbit proximal humeral joint using an acellular bioscaffold created from composite poly-ε-caprolactone and hydroxyapatite infused with TGF-β3. They found TGF-β3 infused scaffolds yielded uniform chondrocyte distribution across the surface of the bioscaffold and form hyaline-like cartilage expressing collagen type II and aggrecan. Furthermore complex microarchitecture of cartilage was recreated as exemplified by the formation of stratified avascular cartilage and vascularised bone [150]. This study indicates that using acellular scaffolds to provide a suitable environment for endogenous cell recruitment and differentiation may be a viable alternative.

Conclusion and future perspectives

Injuries to articular cartilage are common, affect people of all ages and cause significant morbidity. Cartilage tissue has limited capacity for self-repair and regeneration of fibrous cartilage post injury results in numerous attempts at repair. Current approaches may provide adequate long-term solutions for certain patient groups; however results can often be inconsistent and comparable to basic techniques such as microfracture. The implementation of tissue engineering techniques to improve traditional methods has culminated in many products being taken to clinical trials for use in clinical practice. Early results for some products show some promise; however, results have been inconsistent and poor histological repair and complications have been reported.

Regeneration-based tissue engineering approaches should provide better management of articular cartilage defects. However, our complete understanding of the nature of articular cartilage

and the processes which govern tissue regeneration are still not completely understood. The optimal combination of cells, biomaterials and stimulatory factors to mimic the natural articular environment are yet to be defined.

In our opinion tissue engineering strategies could be improved in the areas of source of cells as well as the nature of biomaterials. Recently, the use of iPSCs in the regeneration of bone and cartilage tissue in vitro and in vivo has demonstrated a potential role in regenerative orthopaedic medicine [58,59]. iPSCs may prove to have a greater capacity for expansion and differentiation. However, this technology is in its formative stages and requires development to the stage where iPSCs may be used safely in clinical settings.

We believe that the key to successful regeneration of osteochondral tissue lies with recreating not only the composition of the extracellular matrix such as collagen type II and proteoglycans, but more importantly creating the complex nano-structure and microarchitecture of cartilage tissue itself. Acellular tissue matrix such as acelluar cartilage matrix may provide the best possible chance of recapitulating the native microarchitecture of hyaline cartilage in a transplantable form for tissue regeneration. However the process of decellularisation may cause destruction of microarchitecture resulting in weaker biomechanical strength than expected. This limitation may be overcome by augmenting decellularised cartilage with, for example, additional collagen content via nanofabrication techniques to improve biomechanical strength and stability. Such hybrid scaffolds may benefit from retaining a natural microarchitecture environment whilst improving biomechanical strength lost during the decellularisation process.

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Executive summary

Background

- Cartilage damage is a significant clinical problem and management is difficult due to lack of intrinsic regenerative capacity of cartilage tissue.
- New products aim to improve existing technique through the use of tissue engineering strategies.

Tissue Engineering

- Tissue engineering combines cells, biomaterials and stimulatory factors to regenerate tissue.
- Cell sources for cartilage tissue engineering include chondrocytes, mesenchymal stem cells, embryonic stem cells and induced pluripotent stem cells.
- Many scaffold materials have been used to support chondrogenesis, and these materials often include the use of natural and/or synthetic materials.
- The advantages of natural scaffold materials include increased biodegradability, biocompatibility, however biomechanical strength can be weaker compared with synthetic materials.
- Synthetic materials such as poly(lactic acid), poly(glycolic acid), poly(caprolactone) and their various copolymers provide an alternative to natural scaffold materials, often providing greater biomechanical strength. However biodegradation and biocompatibility can be an issue which limits their use.
- Often hybrid natural and synthetic scaffolds are used to complement the strengths and weaknesses of each material.

- Stimulatory factors for chondrogenesis include growth factors such as TGF-β, FGF, BMP and IGF, mechanical stimulation, hypoxic environments, bioreactors, and electrical stimulation.

Recreating the microarchitecture of articular cartilage

- Recreating the microarchitecture of articular cartilage is crucial to achieving normal biomechanical function of engineered cartilage.
- Natural and synthetic materials have been manufactured to mimic the microarchitecture of articular cartilage.
- Acellular cartilage matrix is a viable alternative to preserving the microarchitecture environment, thereby creating a scaffold with enhance regenerative capacity
- The major drawback with acellular cartilage matrix is the loss of biomechanical strength that exists with the decellularisation process.

Conclusion and future perspectives

- Supplementing decellularized tissue with natural and/or synthetic materials through the use of nanofabrication methods could improve the biomechanical properties of decellularized tissue while maintaining its natural architecture and biocompatibility properties.

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Figure Legends

Figure 1: Acellular cartilage matrix retains the natural microarchitecture thereby maintain the appropriate environment for cellular re-attachment, migration, differentiation and proliferation to enhance tissue regeneration when transplanted.

Tables

Table 1: Products which enhance traditional methods of repair using tissue engineering approaches

Traditional method	Enhancements	Product name	Material	Company
Marrow Stimulation (e.g. microfracture)	Scaffold-guided microfracture	*BST-CarGel®* [151]	Chitosan-glycerol phosphate based hydrogel	Piramal Healthcare, Laval, Quebec, Canada
		ChonDux™ [152]	Photopolymerized hydrogel combined with a biological adhesive	Biomet, Inc., Warsaw, IN, USA
		Gelrin C [153]	Polyethylene glycol diacrylate (PEG-DA) and denatured fibrinogen hydrogel	Regentis Biomaterials, Regentis, Haifa, Israel
Osteochondral graft (e.g. mosaicplasty)	Replacement of osteochondral plug with natural and synthetic biomaterial graft	*Salucartilage* [26,27]	Biodegradable hydrogel implant	Salumedica, Smyna, GA, USA
		Chondromimetic [154]	Multilayer triple co-precipitate of collagen, glycosaminoglycans and calcium phosphate	TiGenix, Leuven, Belgium
		Tru-Fit Plug [107-109]	Synthetic resorbable biphasic implant from polylactide-coglycolide copolymer, calcium	Smith & Nephew, Andover, MA, USA

			sulphate and polyglycolide	
Autologous chondrocyte implantation (ACI) / Matrix-assisted chondrocyte implantation (MACI)	Changes to biomaterials used in scaffold	*Carticel* [155]	Porcine-derived type I and type II collagen scaffolds	Genzyme Inc, Cambridge, MA, USA
		Chondrogide [156]	Porcine-derived type I and type II collagen scaffolds	Geistlich Biomaterials, Wolhausen, Switzerland
		Hyalograft-C [87]	Hyaluronic acid based scaffold	Fidia Advanced Biopolymers, Abano Terma, Italy), and Neocart (Histogenics, Waltham, MA
	Use of bioreactor to enhance in vitro culture	*Neocart* [78]	type I collagen matrix	Histogenics, Waltham, MA
	Morselized cartilage	*Cartilage Autograft Implantation System (CAIS)* [157]	Morselized cartilage	DePuy/Mitek, Raynham, MA
		DeNovo Natural	Morselized cartilage	Zimmer, Inc., Warsaw, IN, USA

Tissue (NT) [158]

Table 2:

Table 2: Scaffold materials used for tissue engineering of articular cartilage

Material	Advantages	Disadvantages	Example References
Natural Materials			
Collagen	Biocompatible Contains ligands that aid in cell adhesion, migration and differentiation [159]	Some cases of poor integration [77]	[160]; [161]; [162]; [163]; [39]; [164]; [165]; [166]; [167]; [168]; [169]
Fibrin	Biodegradable Fibrin glue can be used to enhance integration of engineered tissue with native cartilage and bone	Weak mechanical strength [170] Rapid degradation [170]	[171]; [172]; [173]; [174]

Alginate	Aids re-differentiation of de-differentiated chondrocytes [66] In vivo injectable options [175] Abundant and low cost	Concerns of biocompatibility [176]	[177]; [176]
Hyaluronan	Hyaluronan hydrogels can supplement matrices with cells and other biomimetics [81]	Products of biodegradation can induce chondrolysis [178]	Hyalograft C [79]; [179]; [180]; [181]
Chitosan	Structurally shares some characteristics with various GAGs and hyaluronic acid[182] Degradation products non-toxic and are involved in the synthesis of articular cartilage - Chondroitin sulphate,	Limited solubility [183] Certain cross-linkage can result in poor biocompatibility	[184]; [185]; [186]; [187]; [188]; [189]; [182]; [190]

	Dermatan sulphate, Hyaluronic acid, Keratin sulphate, Glycosylated type II collagen		
Bacterial Cellulose	Biocompatibility Match of mechanical properties with hard and soft tissue Implantable in gel form	Lack of direct bond between cellulose and bone	[191]
Cartilage Derived Matrix	Biocompatibility Support neocartilage formation in absence of exogenous growth factors Contains entrapped bioactive molecules that interact with cells	Lower mechanical strength and higher rates of degradation compared with synthesized scaffolds Chemical cross-linking to improve strength can cause issues with biocompatibility	[143]; [144]; [192]
Gelatin	Supports growth of	Poor integration with bony	[193]

	chondrocyte layer in multilayered scaffold Uniform porosity allows better cell growth and proliferation	structures	
Silk	Supports growth of chondrocytes Good tensile strength	Issues with biocompatibility and allergic reactions with certain types of silk	[194]; [195]
Synthetic Materials			
Poly(α-hydroxy esters) • Poly(lactic acid) • Poly(glycolic acid) • Poly(lactic-co-glycolic) • Poly(caprolactone)	Satisfactory biocompability [97] Good mechanical properties Flexibility in degradation rates	Degradation by-products has been shown to elicit inflammatory response and decreased pH level [98] Mechanical stiffness can sometimes be undesirable [196]	[197];[198]; [199]; [197]; [200]; [201]; [202]; [203]; [204]; [205];; [92]; [206]; [207]; [208]; [209]; [210]; [211]; [212]; [209]; [213]
Poly(ethylene glycol)	Hydrophilicity Biocompatibility	Hydrophobicity [196]	[214]; [215]

Poly(hydroxyalkanoate)	Good biodegradability Minimal inflammatory reaction in vivo Pizoelectric properties	Cellular Dedifferentiation	[216]
Poly(vinyl alcohol)	Biocompatible PVA hydrogels have similar properties to native cartilage	Poor integration with surrounding cartilage	[162,217]
Poly (urethane urea)	Excellent mechanical and biochemical properties	Polyurethanes using polyester diols are hydrolytically unstable	[218]; [219]