

1-1-2013

Bioengineering of articular cartilage: past, present and future

Ken Ye
University of Melbourne

Raed Felimban
The University Of Melbourne

Simon E. Moulton
University of Wollongong, smoulton@uow.edu.au

Gordon G. Wallace
University of Wollongong, gwallace@uow.edu.au

Claudia Di Bella
University of Melbourne

See next page for additional authors

Follow this and additional works at: <https://ro.uow.edu.au/aiimpapers>



Part of the [Engineering Commons](#), and the [Physical Sciences and Mathematics Commons](#)

Recommended Citation

Ye, Ken; Felimban, Raed; Moulton, Simon E.; Wallace, Gordon G.; Di Bella, Claudia; Traianedes, Kathy; Choong, Peter F. M; and Myers, Damian E., "Bioengineering of articular cartilage: past, present and future" (2013). *Australian Institute for Innovative Materials - Papers*. 698.
<https://ro.uow.edu.au/aiimpapers/698>

Bioengineering of articular cartilage: past, present and future

Abstract

The treatment of cartilage defects poses a clinical challenge owing 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 decellularization of biological tissue. Although promising, this technique often results in poor biomechanical strength of the graft. However, biomechanical strength could be improved if biomaterials could be incorporated back into the decellularized tissue to overcome this limitation.

Keywords

present, cartilage, past, articular, future, bioengineering

Disciplines

Engineering | Physical Sciences and Mathematics

Publication Details

Ye, K., Felimban, R., Moulton, S. E., Wallace, G. G., Bella, C., Traianedes, K., Choong, P. F. & Myers, D. E. (2013). Bioengineering of articular cartilage: past, present and future. *Regenerative Medicine*, 8 (3), 333-349.

Authors

Ken Ye, Raed Felimban, Simon E. Moulton, Gordon G. Wallace, Claudia Di Bella, Kathy Traianedes, Peter F. M Choong, and Damian E. Myers

Bioengineering of articular cartilage: past, present and future

Ye K^{1,2}, Felimban R^{1,2}, Moulton SE³, Wallace GG³, Di Bella C^{1,2}, Traianedes K¹, Choong PFM^{1,2} and Myers DE^{1,2}

¹ Department of Surgery, St Vincent's Hospital, University of Melbourne

Fitzroy, Victoria, 3065, Australia

² Department of Orthopaedics, St Vincent's Hospital

Fitzroy, Victoria, 3065, Australia

³ Intelligent Polymer Research Institute, University of Wollongong

Squires Way, North Wollongong, New South Wales 2552, Australia

Corresponding author: Dr Ken Ye

Department of Orthopaedics, Level 3 Daly Wing, St Vincent's Hospital, Melbourne

41 Victoria Parade, Fitzroy 3065

Email: mr.yekken@gmail.com or ken.ye@svhm.org.au

Phone: +61 3 9288 3960

^{2nd} Corresponding author: A/Prof. Damian Myers

Department of Orthopaedics, Level 3 Daly Wing, St Vincent's Hospital, Melbourne

41 Victoria Parade, Fitzroy 3065

Email: damian.myers@svhm.org.au or damianem@unimelb.edu.au

Phone: +61 3 9288 3990

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.

Word count: 4437 (excluding summary, tables, figures, references)

Keywords: Bioengineering, Cartilage, Regeneration, Defects, Review, Tissue Scaffolds, Stem Cells

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 differentiate 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 tumorigenicity 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 tumorigenicity [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 advantages 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- β 1, TGF- β 3, 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- β 1 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- β 3 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 acellular 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-accumulation 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- β 3. They found TGF- β 3 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 acellular 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.

Financial disclosure / Acknowledgements

This work was funded through the Australian Orthopaedic Association and NHMRC Postgraduate Scholarship for author KY.

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.

References

1. Tuan RS. A second-generation autologous chondrocyte implantation approach to the treatment of focal articular cartilage defects. *Arthritis Res Ther*, 9(5), 109 (2007).
2. Vinatier C, Bouffi C, Merceron C *et al.* Cartilage tissue engineering: towards a biomaterial-assisted mesenchymal stem cell therapy. *Curr Stem Cell Res Ther*, 4(4), 318-329 (2009).
3. Kheir ESD. Management of Articular Cartilage Defects. *Orthopaedics and Trauma*, 23(4), 266-273 (2009).
4. Knutsen G, Engebretsen L, Ludvigsen TC *et al.* Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. *J Bone Joint Surg Am*, 86-A(3), 455-464 (2004).
5. Knutsen G, Drogset JO, Engebretsen L *et al.* A randomized trial comparing autologous chondrocyte implantation with microfracture. Findings at five years. *J Bone Joint Surg Am*, 89(10), 2105-2112 (2007).
6. Gudas R, Kalesinskas RJ, Kimtys V *et al.* A prospective randomized clinical study of mosaic osteochondral autologous transplantation versus microfracture for the treatment of osteochondral defects in the knee joint in young athletes. *Arthroscopy*, 21(9), 1066-1075 (2005).
7. Dozin B, Malpeli M, Cancedda R *et al.* Comparative evaluation of autologous chondrocyte implantation and mosaicplasty: a multicentered randomized clinical trial. *Clin J Sport Med*, 15(4), 220-226 (2005).
8. Bentley G, Biant LC, Carrington RW *et al.* A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. *J Bone Joint Surg Br*, 85(2), 223-230 (2003).
9. Saris DB, Vanlauwe J, Victor J *et al.* Characterized chondrocyte implantation results in better structural repair when treating symptomatic cartilage defects of the knee in a randomized controlled trial versus microfracture. *Am J Sports Med*, 36(2), 235-246 (2008).
10. Hubbard MJ. Articular debridement versus washout for degeneration of the medial femoral condyle. A five-year study. *J Bone Joint Surg Br*, 78(2), 217-219 (1996).
11. Moseley JB, O'Malley K, Petersen NJ *et al.* A controlled trial of arthroscopic surgery for osteoarthritis of the knee. *N Engl J Med*, 347(2), 81-88 (2002).
12. Breinan HA, Martin SD, Hsu HP, Spector M. Healing of canine articular cartilage defects treated with microfracture, a type-II collagen matrix, or cultured autologous chondrocytes. *J Orthop Res*, 18(5), 781-789 (2000).
13. Bae DK, Yoon KH, Song SJ. Cartilage healing after microfracture in osteoarthritic knees. *Arthroscopy*, 22(4), 367-374 (2006).
14. Mithoefer K, Williams RJ, 3rd, Warren RF *et al.* The microfracture technique for the treatment of articular cartilage lesions in the knee. A prospective cohort study. *J Bone Joint Surg Am*, 87(9), 1911-1920 (2005).
15. Clouet J, Vinatier C, Merceron C *et al.* From osteoarthritis treatments to future regenerative therapies for cartilage. *Drug Discov Today*, 14(19-20), 913-925 (2009).
16. Clair BL, Johnson AR, Howard T. Cartilage repair: current and emerging options in treatment. *Foot Ankle Spec*, 2(4), 179-188 (2009).
17. Gudas R, Gudaite A, Pocius A *et al.* Ten-year follow-up of a prospective, randomized clinical study of mosaic osteochondral autologous transplantation versus microfracture for the treatment of osteochondral defects in the knee joint of athletes. *Am J Sports Med*, 40(11), 2499-2508 (2012).
18. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med*, 331(14), 889-895 (1994).

19. Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Relat Res*, (374), 212-234 (2000).
20. Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous chondrocyte implantation: a long-term follow-up. *Am J Sports Med*, 38(6), 1117-1124 (2010).
21. Vijayan S, Bartlett W, Bentley G *et al.* Autologous chondrocyte implantation for osteochondral lesions in the knee using a bilayer collagen membrane and bone graft: a two-to eight-year follow-up study. *J Bone Joint Surg Br*, 94(4), 488-492 (2012).
22. Vanlauwe J, Saris DB, Victor J, Almqvist KF, Bellemans J, Luyten FP. Five-year outcome of characterized chondrocyte implantation versus microfracture for symptomatic cartilage defects of the knee: early treatment matters. *Am J Sports Med*, 39(12), 2566-2574 (2011).
23. Bedi A, Feeley BT, Williams RJ, 3rd. Management of articular cartilage defects of the knee. *J Bone Joint Surg Am*, 92(4), 994-1009 (2010).
24. Lane JG, Massie JB, Ball ST *et al.* Follow-up of osteochondral plug transfers in a goat model: a 6-month study. *Am J Sports Med*, 32(6), 1440-1450 (2004).
25. Gooding CR, Bartlett W, Bentley G, Skinner JA, Carrington R, Flanagan A. A prospective, randomised study comparing two techniques of autologous chondrocyte implantation for osteochondral defects in the knee: Periosteum covered versus type I/III collagen covered. *Knee*, 13(3), 203-210 (2006).
26. Lange J, Follak N, Nowotny T, Merk H. [Results of SaluCartilage implantation for stage IV chondral defects in the knee joint area]. *Unfallchirurg*, 109(3), 193-199 (2006).
27. Meyer C, Horas U, Horbelt R, Schnettler R. [Dislocation of artificial cartilage (SaluCartilage)]. *Unfallchirurg*, 108(2), 163-166 (2005).
28. Martin I, Miot S, Barbero A, Jakob M, Wendt D. Osteochondral tissue engineering. *J Biomech*, 40(4), 750-765 (2007).
29. Poole AR, Kojima T, Yasuda T, Mwale F, Kobayashi M, Laverty S. Composition and structure of articular cartilage: a template for tissue repair. *Clin Orthop Relat Res*, (391 Suppl), S26-33 (2001).
30. Chung C, Burdick JA. Engineering cartilage tissue. *Adv Drug Deliv Rev*, 60(2), 243-262 (2008).
31. McDevitt CA. Biochemistry of articular cartilage. Nature of proteoglycans and collagen of articular cartilage and their role in ageing and in osteoarthritis. *Ann Rheum Dis*, 32(4), 364-378 (1973).
32. Darling EM, Athanasiou KA. Retaining zonal chondrocyte phenotype by means of novel growth environments. *Tissue Eng*, 11(3-4), 395-403 (2005).
33. Goessler UR, Bugert P, Bieback K *et al.* Expression of collagen and fiber-associated proteins in human septal cartilage during in vitro dedifferentiation. *Int J Mol Med*, 14(6), 1015-1022 (2004).
34. Goessler UR, Bugert P, Bieback K *et al.* In vitro analysis of differential expression of collagens, integrins, and growth factors in cultured human chondrocytes. *Otolaryngol Head Neck Surg*, 134(3), 510-515 (2006).
35. Buschmann MD, Gluzband YA, Grodzinsky AJ, Kimura JH, Hunziker EB. Chondrocytes in agarose culture synthesize a mechanically functional extracellular matrix. *J Orthop Res*, 10(6), 745-758 (1992).
36. Homicz MR, Chia SH, Schumacher BL *et al.* Human septal chondrocyte redifferentiation in alginate, polyglycolic acid scaffold, and monolayer culture. *Laryngoscope*, 113(1), 25-32 (2003).
37. Lin Z, Willers C, Xu J, Zheng MH. The chondrocyte: biology and clinical application. *Tissue Eng*, 12(7), 1971-1984 (2006).
38. Freed LE, Hollander AP, Martin I, Barry JR, Langer R, Vunjak-Novakovic G. Chondrogenesis in a cell-polymer-bioreactor system. *Exp Cell Res*, 240(1), 58-65 (1998).

39. Kurz B, Domm C, Jin M, Sellckau R, Schunke M. Tissue engineering of articular cartilage under the influence of collagen I/III membranes and low oxygen tension. *Tissue Eng*, 10(7-8), 1277-1286 (2004).
40. Mandl EW, van der Veen SW, Verhaar JA, van Osch GJ. Serum-free medium supplemented with high-concentration FGF2 for cell expansion culture of human ear chondrocytes promotes redifferentiation capacity. *Tissue Eng*, 8(4), 573-580 (2002).
41. Dominici M, Le Blanc K, Mueller I *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*, 8(4), 315-317 (2006).
42. Tuan RS, Boland G, Tuli R. Adult mesenchymal stem cells and cell-based tissue engineering. *Arthritis Res Ther*, 5(1), 32-45 (2003).
43. French MM, Rose S, Canseco J, Athanasiou KA. Chondrogenic differentiation of adult dermal fibroblasts. *Ann Biomed Eng*, 32(1), 50-56 (2004).
44. Lee KH, Song SU, Hwang TS *et al.* Regeneration of hyaline cartilage by cell-mediated gene therapy using transforming growth factor beta 1-producing fibroblasts. *Hum Gene Ther*, 12(14), 1805-1813 (2001).
45. Mackay AM, Beck SC, Murphy JM, Barry FP, Chichester CO, Pittenger MF. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng*, 4(4), 415-428 (1998).
46. Muraglia A, Martin I, Cancedda R, Quarto R. A nude mouse model for human bone formation in unloaded conditions. *Bone*, 22(5 Suppl), 131S-134S (1998).
47. Winter A, Breit S, Parsch D *et al.* Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells. *Arthritis Rheum*, 48(2), 418-429 (2003).
48. Ogawa R, Mizuno S. Cartilage regeneration using adipose-derived stem cells. *Curr Stem Cell Res Ther*, 5(2), 129-132 (2010).
49. Coleman RM, Case ND, Guldberg RE. Hydrogel effects on bone marrow stromal cell response to chondrogenic growth factors. *Biomaterials*, 28(12), 2077-2086 (2007).
50. Huang CY, Reuben PM, D'Ippolito G, Schiller PC, Cheung HS. Chondrogenesis of human bone marrow-derived mesenchymal stem cells in agarose culture. *Anat Rec A Discov Mol Cell Evol Biol*, 278(1), 428-436 (2004).
51. Indrawattana N, Chen G, Tadokoro M *et al.* Growth factor combination for chondrogenic induction from human mesenchymal stem cell. *Biochem Biophys Res Commun*, 320(3), 914-919 (2004).
52. Zuk PA, Zhu M, Ashjian P *et al.* Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*, 13(12), 4279-4295 (2002).
53. Chiou M, Xu Y, Longaker MT. Mitogenic and chondrogenic effects of fibroblast growth factor-2 in adipose-derived mesenchymal cells. *Biochem Biophys Res Commun*, 343(2), 644-652 (2006).
54. Awad HA, Wickham MQ, Leddy HA, Gimble JM, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials*, 25(16), 3211-3222 (2004).
55. Mauck RL, Yuan X, Tuan RS. Chondrogenic differentiation and functional maturation of bovine mesenchymal stem cells in long-term agarose culture. *Osteoarthritis Cartilage*, 14(2), 179-189 (2006).
56. Toh WS, Lee EH, Guo XM *et al.* Cartilage repair using hyaluronan hydrogel-encapsulated human embryonic stem cell-derived chondrogenic cells. *Biomaterials*, 31(27), 6968-6980 (2010).

57. Hwang NS, Varghese S, Lee HJ *et al.* In vivo commitment and functional tissue regeneration using human embryonic stem cell-derived mesenchymal cells. *Proc Natl Acad Sci U S A*, 105(52), 20641-20646 (2008).
 58. Bilousova GJ, DH. King, KB. Langhe, SD. Chick, WS. Torchia, EC. Chow, KS. Klemm, DJ. Roop, DR. Majka, SM. Osteoblasts Derived from Induced Pluripotent Stem Cells Form Calcified Structures in Scaffolds Both in Vitro and in Vivo. *Stem Cells*, 29, 206-216 (2011).
 59. Wei Y, Zeng W, Wan R *et al.* Chondrogenic differentiation of induced pluripotent stem cells from osteoarthritic chondrocytes in alginate matrix. *Eur Cell Mater*, 23, 1-12 (2012).
 60. Koyama N, Miura M, Nakao K *et al.* Human Induced Pluripotent Stem Cells Differentiated into Chondrogenic Lineage Via Generation of Mesenchymal Progenitor Cells. *Stem Cells Dev*, (2012). *
- * This study demonstrates the potential role of induced pluripotent stem cells from human osteoarthritic chondrocytes in cartilage tissue engineering.**
61. Yu J, Hu K, Smuga-Otto K *et al.* Human induced pluripotent stem cells free of vector and transgene sequences. *Science*, 324(5928), 797-801 (2009).
 62. Jia F, Wilson KD, Sun N *et al.* A nonviral minicircle vector for deriving human iPS cells. *Nat Methods*, 7(3), 197-199 (2010).
 63. Okita K, Matsumura Y, Sato Y *et al.* A more efficient method to generate integration-free human iPS cells. *Nat Methods*, 8(5), 409-412 (2011).
 64. Warren L, Ni Y, Wang J, Guo X. Feeder-free derivation of human induced pluripotent stem cells with messenger RNA. *Sci Rep*, 2, 657 (2012).
 65. Darling EM, Athanasiou KA. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. *J Orthop Res*, 23(2), 425-432 (2005).
 66. Bonaventure J, Kadhon N, Cohen-Solal L *et al.* Reexpression of cartilage-specific genes by dedifferentiated human articular chondrocytes cultured in alginate beads. *Exp Cell Res*, 212(1), 97-104 (1994).
 67. Domm C, Schunke M, Christesen K, Kurz B. Redifferentiation of dedifferentiated bovine articular chondrocytes in alginate culture under low oxygen tension. *Osteoarthritis Cartilage*, 10(1), 13-22 (2002).
 68. Malda J, Martens DE, Tramper J, van Blitterswijk CA, Riesle J. Cartilage tissue engineering: controversy in the effect of oxygen. *Crit Rev Biotechnol*, 23(3), 175-194 (2003).
 69. Bryant SJ, Anseth KS. Hydrogel properties influence ECM production by chondrocytes photoencapsulated in poly(ethylene glycol) hydrogels. *J Biomed Mater Res*, 59(1), 63-72 (2002).
 70. Bhardwaj T, Pilliar RM, Grynblas MD, Kandel RA. Effect of material geometry on cartilagenous tissue formation in vitro. *J Biomed Mater Res*, 57(2), 190-199 (2001).
 71. Woodfield TB, Malda J, de Wijn J, Peters F, Riesle J, van Blitterswijk CA. Design of porous scaffolds for cartilage tissue engineering using a three-dimensional fiber-deposition technique. *Biomaterials*, 25(18), 4149-4161 (2004).
 72. Li WJ, Danielson KG, Alexander PG, Tuan RS. Biological response of chondrocytes cultured in three-dimensional nanofibrous poly(epsilon-caprolactone) scaffolds. *J Biomed Mater Res A*, 67(4), 1105-1114 (2003).
 73. Miyata T, Taira T, Noishiki Y. Collagen engineering for biomaterial use. *Clin Mater*, 9(3-4), 139-148 (1992).
 74. Stenzel KH, Miyata T, Rubin AL. Collagen as a biomaterial. *Annu Rev Biophys Bioeng*, 3(0), 231-253 (1974).
 75. Schoof H, Apel J, Heschel I, Rau G. Control of pore structure and size in freeze-dried collagen sponges. *J Biomed Mater Res*, 58(4), 352-357 (2001).
 76. Brown RA, Phillips JB. Cell responses to biomimetic protein scaffolds used in tissue repair and engineering. *Int Rev Cytol*, 262, 75-150 (2007).

77. Randolph MA, Anseth K, Yaremchuk MJ. Tissue engineering of cartilage. *Clin Plast Surg*, 30(4), 519-537 (2003).
 78. Crawford DC, DeBerardino TM, Williams RJ, 3rd. NeoCart, an autologous cartilage tissue implant, compared with microfracture for treatment of distal femoral cartilage lesions: an FDA phase-II prospective, randomized clinical trial after two years. *J Bone Joint Surg Am*, 94(11), 979-989 (2012).
 79. Tognana E, Borrione A, De Luca C, Pavesio A. Hyalograft C: hyaluronan-based scaffolds in tissue-engineered cartilage. *Cells Tissues Organs*, 186(2), 97-103 (2007).
 80. Laurent TC, Fraser JR. Hyaluronan. *FASEB J*, 6(7), 2397-2404 (1992).
 81. Allison DD, Grande-Allen KJ. Review. Hyaluronan: a powerful tissue engineering tool. *Tissue Eng*, 12(8), 2131-2140 (2006).
 82. Prestwich GD, Kuo JW. Chemically-modified HA for therapy and regenerative medicine. *Curr Pharm Biotechnol*, 9(4), 242-245 (2008).
 83. Prestwich GD, Marecak DM, Marecek JF, Vercruyse KP, Ziebell MR. Controlled chemical modification of hyaluronic acid: synthesis, applications, and biodegradation of hydrazide derivatives. *J Control Release*, 53(1-3), 93-103 (1998).
 84. Toh WS, Lim TC, Kurisawa M, Spector M. Modulation of mesenchymal stem cell chondrogenesis in a tunable hyaluronic acid hydrogel microenvironment. *Biomaterials*, 33(15), 3835-3845 (2012).
 85. Grigolo B, Lisignoli G, Desando G *et al.* Osteoarthritis treated with mesenchymal stem cells on hyaluronan-based scaffold in rabbit. *Tissue Eng Part C Methods*, 15(4), 647-658 (2009).
 86. Solchaga LA, Dennis JE, Goldberg VM, Caplan AI. Hyaluronic acid-based polymers as cell carriers for tissue-engineered repair of bone and cartilage. *J Orthop Res*, 17(2), 205-213 (1999).
 87. Nehrer S, Dorotka R, Domayer S, Stelzeneder D, Kotz R. Treatment of full-thickness chondral defects with hyalograft C in the knee: a prospective clinical case series with 2 to 7 years' follow-up. *Am J Sports Med*, 37 Suppl 1, 81S-87S (2009). *
- * **Clinical case series reporting on long term results of an engineered product, Hyalograft C, which shows improvements in young patients with single chondral defects. However the patient with more advanced disease and osteoarthritic changes did not show improvement.**
88. Gunatillake PA, Adhikari R. Biodegradable synthetic polymers for tissue engineering. *Eur Cell Mater*, 5, 1-16; discussion 16 (2003).
 89. Middleton JC, Tipton AJ. Synthetic biodegradable polymers as orthopedic devices. *Biomaterials*, 21(23), 2335-2346 (2000).
 90. Barrows TH. Degradable implant materials: a review of synthetic absorbable polymers and their applications. *Clin Mater*, 1, 233-257 (1986).
 91. Pulapura S, Kohn J. Trends in the development of bioresorbable polymers for medical applications. *J Biomater Appl*, 6(3), 216-250 (1992).
 92. Li WJ, Cooper JA, Jr., Mauck RL, Tuan RS. Fabrication and characterization of six electrospun poly(alpha-hydroxy ester)-based fibrous scaffolds for tissue engineering applications. *Acta Biomater*, 2(4), 377-385 (2006).
 93. Freed LE, Marquis JC, Nohria A, Emmanuel J, Mikos AG, Langer R. Neocartilage formation in vitro and in vivo using cells cultured on synthetic biodegradable polymers. *J Biomed Mater Res*, 27(1), 11-23 (1993).
 94. Daniels AU, Chang MK, Andriano KP. Mechanical properties of biodegradable polymers and composites proposed for internal fixation of bone. *J Appl Biomater*, 1(1), 57-78 (1990).
 95. Athanasiou KA, Agrawal CM, Barber FA, Burkhart SS. Orthopaedic applications for PLA-PGA biodegradable polymers. *Arthroscopy*, 14(7), 726-737 (1998).

96. Katz AR, Turner RJ. Evaluation of tensile and absorption properties of polyglycolic acid sutures. *Surg Gynecol Obstet*, 131(4), 701-716 (1970).
97. Athanasiou KA, Niederauer GG, Agrawal CM. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials*, 17(2), 93-102 (1996).
98. O'Shea TM, Miao X. Bilayered scaffolds for osteochondral tissue engineering. *Tissue Eng Part B Rev*, 14(4), 447-464 (2008).
99. Zhu G, Mallery SR, Schwendeman SP. Stabilization of proteins encapsulated in injectable poly(lactide-co-glycolide). *Nat Biotechnol*, 18(1), 52-57 (2000).
100. Ueda H, Tabata Y. Polyhydroxyalkanoate derivatives in current clinical applications and trials. *Adv Drug Deliv Rev*, 55(4), 501-518 (2003).
101. Cai Q, Bei J, Wang S. Synthesis and properties of ABA-type triblock copolymers of poly(glycolide-co-caprolactone) (A) and poly(ethylene glycol) (B). *Polymer*, 43(13), 3585-3591 (2002).
102. Fabre T, Schappacher M, Bareille R *et al.* Study of a (trimethylenecarbonate-co-epsilon-caprolactone) polymer--part 2: in vitro cytocompatibility analysis and in vivo ED1 cell response of a new nerve guide. *Biomaterials*, 22(22), 2951-2958 (2001).
103. Schappacher M, Fabre T, Mingotaud AF, Soum A. Study of a (trimethylenecarbonate-co-epsilon-caprolactone) polymer part 1: preparation of a new nerve guide through controlled random copolymerization using rare earth catalysts. *Biomaterials*, 22(21), 2849-2855 (2001).
104. Edlund U, Albertsson AC. Polyesters based on diacid monomers. *Adv Drug Deliv Rev*, 55(4), 585-609 (2003).
105. Saad B, Hirt TD, Welte M, Uhlschmid GK, Neuenschwander P, Suter UW. Development of degradable polyesterurethanes for medical applications: in vitro and in vivo evaluations. *J Biomed Mater Res*, 36(1), 65-74 (1997).
106. Shi J, Zhang X, Zeng X *et al.* One-step articular cartilage repair: combination of in situ bone marrow stem cells with cell-free poly(L-lactic-co-glycolic acid) scaffold in a rabbit model. *Orthopedics*, 35(5), e665-671 (2012).
107. Melton JT, Wilson AJ, Chapman-Sheath P, Cossey AJ. TruFit CB bone plug: chondral repair, scaffold design, surgical technique and early experiences. *Expert Rev Med Devices*, 7(3), 333-341 (2010).
108. Carmont MR, Carey-Smith R, Saithna A, Dhillon M, Thompson P, Spalding T. Delayed incorporation of a TruFit plug: perseverance is recommended. *Arthroscopy*, 25(7), 810-814 (2009).
109. Williams RJ, Gamradt SC. Articular cartilage repair using a resorbable matrix scaffold. *Instr Course Lect*, 57, 563-571 (2008).
110. Scherer K, Schunke M, Sellckau R, Hassenpflug J, Kurz B. The influence of oxygen and hydrostatic pressure on articular chondrocytes and adherent bone marrow cells in vitro. *Biorheology*, 41(3-4), 323-333 (2004).
111. Hu JC, Athanasiou KA. The effects of intermittent hydrostatic pressure on self-assembled articular cartilage constructs. *Tissue Eng*, 12(5), 1337-1344 (2006).
112. Mauck RL, Nicoll SB, Seyhan SL, Ateshian GA, Hung CT. Synergistic action of growth factors and dynamic loading for articular cartilage tissue engineering. *Tissue Eng*, 9(4), 597-611 (2003).
113. Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF-beta and osteoarthritis. *Osteoarthritis Cartilage*, 15(6), 597-604 (2007).
114. Danisovic L, Varga I, Polak S. Growth factors and chondrogenic differentiation of mesenchymal stem cells. *Tissue Cell*, 44(2), 69-73 (2012).

115. Schmidt MB, Chen EH, Lynch SE. A review of the effects of insulin-like growth factor and platelet derived growth factor on in vivo cartilage healing and repair. *Osteoarthritis Cartilage*, 14(5), 403-412 (2006).
116. Longobardi L, O'Rear L, Aakula S *et al.* Effect of IGF-I in the chondrogenesis of bone marrow mesenchymal stem cells in the presence or absence of TGF-beta signaling. *J Bone Miner Res*, 21(4), 626-636 (2006).
117. An C, Cheng Y, Yuan Q, Li J. IGF-1 and BMP-2 induces differentiation of adipose-derived mesenchymal stem cells into chondrocytes-like cells. *Ann Biomed Eng*, 38(4), 1647-1654 (2010).
118. Ge Z, Hu Y, Heng BC *et al.* Osteoarthritis and therapy. *Arthritis Rheum*, 55(3), 493-500 (2006).
119. Moore EE, Bendele AM, Thompson DL *et al.* Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. *Osteoarthritis Cartilage*, 13(7), 623-631 (2005).
120. Fedele AO, Whitelaw ML, Peet DJ. Regulation of gene expression by the hypoxia-inducible factors. *Mol Interv*, 2(4), 229-243 (2002).
121. Robins JC, Akeno N, Mukherjee A *et al.* Hypoxia induces chondrocyte-specific gene expression in mesenchymal cells in association with transcriptional activation of Sox9. *Bone*, 37(3), 313-322 (2005).
122. Wang Y, Kim UJ, Blasioli DJ, Kim HJ, Kaplan DL. In vitro cartilage tissue engineering with 3D porous aqueous-derived silk scaffolds and mesenchymal stem cells. *Biomaterials*, 26(34), 7082-7094 (2005).
123. Chen FH, Rousche KT, Tuan RS. Technology Insight: adult stem cells in cartilage regeneration and tissue engineering. *Nat Clin Pract Rheumatol*, 2(7), 373-382 (2006).
124. Betre H, Ong SR, Guilak F, Chilkoti A, Fermor B, Setton LA. Chondrocytic differentiation of human adipose-derived adult stem cells in elastin-like polypeptide. *Biomaterials*, 27(1), 91-99 (2006).
125. Gemmiti CV, Guldberg RE. Fluid flow increases type II collagen deposition and tensile mechanical properties in bioreactor-grown tissue-engineered cartilage. *Tissue Eng*, 12(3), 469-479 (2006).
126. Saini S, Wick TM. Concentric cylinder bioreactor for production of tissue engineered cartilage: effect of seeding density and hydrodynamic loading on construct development. *Biotechnol Prog*, 19(2), 510-521 (2003).
127. Lu CH, Lin KJ, Chiu HY *et al.* Improved chondrogenesis and engineered cartilage formation from TGF-beta3-expressing adipose-derived stem cells cultured in the rotating-shaft bioreactor. *Tissue Eng Part A*, 18(19-20), 2114-2124 (2012).
128. Baker B, Spadaro J, Marino A, Becker RO. Electrical stimulation of articular cartilage regeneration. *Ann N Y Acad Sci*, 238, 491-499 (1974).
129. Brighton CT, Wang W, Clark CC. The effect of electrical fields on gene and protein expression in human osteoarthritic cartilage explants. *J Bone Joint Surg Am*, 90(4), 833-848 (2008).
130. Mollenhauer JA. Perspectives on articular cartilage biology and osteoarthritis. *Injury*, 39 Suppl 1, S5-12 (2008).
131. Ulrich-Vinther M, Maloney MD, Schwarz EM, Rosier R, O'Keefe RJ. Articular cartilage biology. *J Am Acad Orthop Surg*, 11(6), 421-430 (2003).
132. Akizuki S, Mow VC, Muller F, Pita JC, Howell DS, Manicourt DH. Tensile properties of human knee joint cartilage: I. Influence of ionic conditions, weight bearing, and fibrillation on the tensile modulus. *J Orthop Res*, 4(4), 379-392 (1986).
133. Warman ML. Human genetic insights into skeletal development, growth, and homeostasis. *Clin Orthop Relat Res*, (379 Suppl), S40-54 (2000).

134. Kim TK, Sharma B, Williams CG *et al.* Experimental model for cartilage tissue engineering to regenerate the zonal organization of articular cartilage. *Osteoarthritis Cartilage*, 11(9), 653-664 (2003).
135. Kon E, Delcogliano M, Filardo G, Busacca M, Di Martino A, Marcacci M. Novel nano-composite multilayered biomaterial for osteochondral regeneration: a pilot clinical trial. *Am J Sports Med*, 39(6), 1180-1190 (2011). *
- * In vivo study using biomimetic multilayered gradient nano-composite collagen scaffold reinforced with hydroxyapatite nanoparticles in femoral condyles of sheep. Defects with scaffold and/or cells showed better regeneration of cartilage tissue. An interesting finding is that the main mode of action of scaffold is based on recruitment of local cells rather than exogenous cell implantation.**
136. Coburn J, Gibson M, Bandalini PA *et al.* Biomimetics of the Extracellular Matrix: An Integrated Three-Dimensional Fiber-Hydrogel Composite for Cartilage Tissue Engineering. *Smart Struct Syst*, 7(3), 213-222 (2011).
137. Hodde J. Naturally occurring scaffolds for soft tissue repair and regeneration. *Tissue Eng*, 8(2), 295-308 (2002).
138. Sarig U, Au-Yeung GC, Wang Y *et al.* Thick acellular heart extracellular matrix with inherent vasculature: a potential platform for myocardial tissue regeneration. *Tissue Eng Part A*, 18(19-20), 2125-2137 (2012).
139. Zajicek R, Mandys V, Mestak O, Sevcik J, Konigova R, Matouskova E. Human keratinocyte growth and differentiation on acellular porcine dermal matrix in relation to wound healing potential. *ScientificWorldJournal*, 2012, 727352 (2012).
140. Loai Y, Yeger H, Coz C *et al.* Bladder tissue engineering: tissue regeneration and neovascularization of HA-VEGF-incorporated bladder acellular constructs in mouse and porcine animal models. *J Biomed Mater Res A*, 94(4), 1205-1215 (2010).
141. Wang B, Borazjani A, Tahai M *et al.* Fabrication of cardiac patch with decellularized porcine myocardial scaffold and bone marrow mononuclear cells. *J Biomed Mater Res A*, 94(4), 1100-1110 (2010).
142. Guo SZ, Ren XJ, Wu B, Jiang T. Preparation of the acellular scaffold of the spinal cord and the study of biocompatibility. *Spinal Cord*, 48(7), 576-581 (2010).
143. Cheng NC, Estes BT, Young TH, Guilak F. Engineered cartilage using primary chondrocytes cultured in a porous cartilage-derived matrix. *Regen Med*, 6(1), 81-93 (2011). *
- * The use of articular cartilage derived scaffolds can support the formation of neocartilage without exogenous growth factors.**
144. Yang Z, Shi Y, Wei X *et al.* Fabrication and repair of cartilage defects with a novel acellular cartilage matrix scaffold. *Tissue Eng Part C Methods*, 16(5), 865-876 (2010). *
- * Methods paper describing the fabrication and decellularisation of bovine articular cartilage to form acellular cartilage matrix (ACM) through a sequence of trypsin, nuclease solution, hypotonic buffer, Triton x100 solution with freeze-dry molding and ultraviolet irradiation cross-linking.**
145. Cheng NC, Estes BT, Young TH, Guilak F. Genipin-crosslinked cartilage-derived matrix as a scaffold for human adipose-derived stem cell chondrogenesis. *Tissue Eng Part A*, 19(3-4), 484-496 (2013). *
- * Genipin-crosslinkage manipulation of cartilage-derived matrix reduces shrinkage of the acellular graft.**
146. Schwarz S, Koerber L, Elsaesser AF *et al.* Decellularized cartilage matrix as a novel biomatrix for cartilage tissue-engineering applications. *Tissue Eng Part A*, 18(21-22), 2195-2209 (2012).
147. Schwarz S, Elsaesser AF, Koerber L *et al.* Processed xenogenic cartilage as innovative biomatrix for cartilage tissue engineering: effects on chondrocyte differentiation and function. *J Tissue Eng Regen Med*, (2012).

148. Kang H, Peng J, Lu S *et al.* In vivo cartilage repair using adipose-derived stem cell-loaded decellularized cartilage ECM scaffolds. *J Tissue Eng Regen Med*, (2012). *
- * Use of adipose stem cells on a decellularised cartilage ECM scaffold in a rabbit in vivo model to repair 4mm defects in the patellar groove. At 6 months cell-scaffold constructs were filled with repair tissue compared with partial filling of scaffold alone group.**
149. Park KP, Do SH, Han KC *et al.* Induction of re-differentiation of passaged rat chondrocytes using a naturally obtained extracellular matrix microenvironment. *Tissue Eng Part A*, (2012).
150. Lee CH, Cook JL, Mendelson A, Moiola EK, Yao H, Mao JJ. Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. *Lancet*, 376(9739), 440-448 (2010). *
- * Repair of 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 through recruitment and differentiation of host cells.**
151. Hoemann CD, Sun J, McKee MD *et al.* Chitosan-glycerol phosphate/blood implants elicit hyaline cartilage repair integrated with porous subchondral bone in microdrilled rabbit defects. *Osteoarthritis Cartilage*, 15(1), 78-89 (2007).
152. Biomet I. Biomet announces acquisition of the assets of cartilage repair company Cartilix. (Ed.^(Eds) (Warsaw, Indiana, 2009)
153. Regentis. GelrinC - Synchronized with Nature. (Ed.^(Eds) (Haifa, Israel, 2012)
154. TiGenix. TiGenix announces the grant of a US patent for ChondroMimetic. (Ed.^(Eds) (Leuven, Belgium, 2010)
155. De Bie C. Genzyme: 15 years of cell and gene therapy research. *Regen Med*, 2(1), 95-97 (2007).
156. Haddo O, Mahroof S, Higgs D *et al.* The use of chondrocyte membrane in autologous chondrocyte implantation. *Knee*, 11(1), 51-55 (2004).
157. Farr J, Cole BJ, Sherman S, Karas V. Particulated articular cartilage: CAIS and DeNovo NT. *J Knee Surg*, 25(1), 23-29 (2012).
158. Hatic SO, 2nd, Berlet GC. Particulated juvenile articular cartilage graft (DeNovo NT Graft) for treatment of osteochondral lesions of the talus. *Foot Ankle Spec*, 3(6), 361-364 (2010).
159. Kleinman HK, Klebe RJ, Martin GR. Role of collagenous matrices in the adhesion and growth of cells. *J Cell Biol*, 88(3), 473-485 (1981).
160. Sams AE, Minor RR, Wootton JA, Mohammed H, Nixon AJ. Local and remote matrix responses to chondrocyte-laden collagen scaffold implantation in extensive articular cartilage defects. *Osteoarthritis Cartilage*, 3(1), 61-70 (1995).
161. Lee CR, Grodzinsky AJ, Hsu HP, Spector M. Effects of a cultured autologous chondrocyte-seeded type II collagen scaffold on the healing of a chondral defect in a canine model. *J Orthop Res*, 21(2), 272-281 (2003).
162. Abedi G, Sotoudeh A, Soleymani M, Shafiee A, Mortazavi P, Aflatoonian MR. A Collagen-Poly(vinyl alcohol) Nanofiber Scaffold for Cartilage Repair. *J Biomater Sci Polym Ed*, (2010).
163. Chen WC, Yao CL, Wei YH, Chu IM. Evaluating osteochondral defect repair potential of autologous rabbit bone marrow cells on type II collagen scaffold. *Cytotechnology*, 63(1), 13-23 (2011).
164. Wu CH, Ko CS, Huang JW, Huang HJ, Chu IM. Effects of exogenous glycosaminoglycans on human chondrocytes cultivated on type II collagen scaffolds. *J Mater Sci Mater Med*, 21(2), 725-729 (2010).
165. Yan LP, Wang YJ, Ren L *et al.* Genipin-cross-linked collagen/chitosan biomimetic scaffolds for articular cartilage tissue engineering applications. *J Biomed Mater Res A*, 95(2), 465-475 (2010).

166. Zheng L, Fan HS, Sun J *et al.* Chondrogenic differentiation of mesenchymal stem cells induced by collagen-based hydrogel: an in vivo study. *J Biomed Mater Res A*, 93(2), 783-792 (2010).
167. Pulkkinen HJ, Tiitu V, Valonen P, Jurvelin JS, Lammi MJ, Kiviranta I. Engineering of cartilage in recombinant human type II collagen gel in nude mouse model in vivo. *Osteoarthritis Cartilage*, 18(8), 1077-1087 (2010).
168. Ko CS, Huang JP, Huang CW, Chu IM. Type II collagen-chondroitin sulfate-hyaluronan scaffold cross-linked by genipin for cartilage tissue engineering. *J Biosci Bioeng*, 107(2), 177-182 (2009).
169. Shields KJ, Beckman MJ, Bowlin GL, Wayne JS. Mechanical properties and cellular proliferation of electrospun collagen type II. *Tissue Eng*, 10(9-10), 1510-1517 (2004).
170. Ahmed TA, Dare EV, Hincke M. Fibrin: a versatile scaffold for tissue engineering applications. *Tissue Eng Part B Rev*, 14(2), 199-215 (2008).
171. Fortier LA, Lust G, Mohammed HO, Nixon AJ. Coordinate upregulation of cartilage matrix synthesis in fibrin cultures supplemented with exogenous insulin-like growth factor-I. *J Orthop Res*, 17(4), 467-474 (1999).
172. Nixon AJ, Fortier LA, Williams J, Mohammed H. Enhanced repair of extensive articular defects by insulin-like growth factor-I-laden fibrin composites. *J Orthop Res*, 17(4), 475-487 (1999).
173. Brittberg M, Sjogren-Jansson E, Lindahl A, Peterson L. Influence of fibrin sealant (Tisseel) on osteochondral defect repair in the rabbit knee. *Biomaterials*, 18(3), 235-242 (1997).
174. Ahmed TA, Giulivi A, Griffith M, Hincke M. Fibrin glues in combination with mesenchymal stem cells to develop a tissue-engineered cartilage substitute. *Tissue Eng Part A*, 17(3-4), 323-335 (2011).
175. Paige KT, Cima LG, Yaremchuk MJ, Vacanti JP, Vacanti CA. Injectable cartilage. *Plast Reconstr Surg*, 96(6), 1390-1398; discussion 1399-1400 (1995).
176. Selmi TA, Verdonk P, Chambat P *et al.* Autologous chondrocyte implantation in a novel alginate-agarose hydrogel: outcome at two years. *J Bone Joint Surg Br*, 90(5), 597-604 (2008).
177. Diduch DR, Jordan LC, Mierisch CM, Balian G. Marrow stromal cells embedded in alginate for repair of osteochondral defects. *Arthroscopy*, 16(6), 571-577 (2000).
178. Knudson W, Casey B, Nishida Y, Eger W, Kuettner KE, Knudson CB. Hyaluronan oligosaccharides perturb cartilage matrix homeostasis and induce chondrocytic chondrolysis. *Arthritis Rheum*, 43(5), 1165-1174 (2000).
179. Solchaga LA, Yoo JU, Lundberg M *et al.* Hyaluronan-based polymers in the treatment of osteochondral defects. *J Orthop Res*, 18(5), 773-780 (2000).
180. Wu SC, Chang JK, Wang CK, Wang GJ, Ho ML. Enhancement of chondrogenesis of human adipose derived stem cells in a hyaluronan-enriched microenvironment. *Biomaterials*, 31(4), 631-640 (2010).
181. Stok KS, Lisignoli G, Cristino S, Facchini A, Muller R. Mechano-functional assessment of human mesenchymal stem cells grown in three-dimensional hyaluronan-based scaffolds for cartilage tissue engineering. *J Biomed Mater Res A*, 93(1), 37-45 (2010).
182. Suh JK, Matthew HW. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. *Biomaterials*, 21(24), 2589-2598 (2000).
183. Jin R, Moreira Teixeira LS, Dijkstra PJ *et al.* Injectable chitosan-based hydrogels for cartilage tissue engineering. *Biomaterials*, 30(13), 2544-2551 (2009).
184. Nettles DL, Elder SH, Gilbert JA. Potential use of chitosan as a cell scaffold material for cartilage tissue engineering. *Tissue Eng*, 8(6), 1009-1016 (2002).
185. Griffon DJ, Sedighi MR, Schaeffer DV, Eurell JA, Johnson AL. Chitosan scaffolds: interconnective pore size and cartilage engineering. *Acta Biomater*, 2(3), 313-320 (2006).

186. Chen J, Chen H, Li P *et al.* Simultaneous regeneration of articular cartilage and subchondral bone in vivo using MSCs induced by a spatially controlled gene delivery system in bilayered integrated scaffolds. *Biomaterials*, 32(21), 4793-4805 (2011).
187. Neves SC, Moreira Teixeira LS, Moroni L *et al.* Chitosan/poly(epsilon-caprolactone) blend scaffolds for cartilage repair. *Biomaterials*, 32(4), 1068-1079 (2011).
188. Qi J, Chen A, You H, Li K, Zhang D, Guo F. Proliferation and chondrogenic differentiation of CD105-positive enriched rat synovium-derived mesenchymal stem cells in three-dimensional porous scaffolds. *Biomed Mater*, 6(1), 015006 (2011).
189. Abarrategi A, Lopiz-Morales Y, Ramos V *et al.* Chitosan scaffolds for osteochondral tissue regeneration. *J Biomed Mater Res A*, 95(4), 1132-1141 (2010).
190. Chen YL, Lee HP, Chan HY, Sung LY, Chen HC, Hu YC. Composite chondroitin-6-sulfate/dermatan sulfate/chitosan scaffolds for cartilage tissue engineering. *Biomaterials*, 28(14), 2294-2305 (2007).
191. Muller FA, Muller L, Hofmann I, Greil P, Wenzel MM, Staudenmaier R. Cellulose-based scaffold materials for cartilage tissue engineering. *Biomaterials*, 27(21), 3955-3963 (2006).
192. Jin CZ, Park SR, Choi BH, Park K, Min BH. In vivo cartilage tissue engineering using a cell-derived extracellular matrix scaffold. *Artif Organs*, 31(3), 183-192 (2007).
193. Chang CH, Lin FH, Lin CC, Chou CH, Liu HC. Cartilage tissue engineering on the surface of a novel gelatin-calcium-phosphate biphasic scaffold in a double-chamber bioreactor. *J Biomed Mater Res B Appl Biomater*, 71(2), 313-321 (2004).
194. Gellynck K, Verdonk PC, Van Nimmen E *et al.* Silk worm and spider silk scaffolds for chondrocyte support. *J Mater Sci Mater Med*, 19(11), 3399-3409 (2008).
195. Wang Y, Blasioli DJ, Kim HJ, Kim HS, Kaplan DL. Cartilage tissue engineering with silk scaffolds and human articular chondrocytes. *Biomaterials*, 27(25), 4434-4442 (2006).
196. Cao Y, Rodriguez A, Vacanti M, Ibarra C, Arevalo C, Vacanti CA. Comparative study of the use of poly(glycolic acid), calcium alginate and pluronics in the engineering of autologous porcine cartilage. *J Biomater Sci Polym Ed*, 9(5), 475-487 (1998).
197. Chen JP, Su CH. Surface modification of electrospun PLLA nanofibers by plasma treatment and cationized gelatin immobilization for cartilage tissue engineering. *Acta Biomater*, 7(1), 234-243 (2011).
198. Xue D, Zheng Q, Zong C *et al.* Osteochondral repair using porous poly(lactide-co-glycolide)/nano-hydroxyapatite hybrid scaffolds with undifferentiated mesenchymal stem cells in a rat model. *J Biomed Mater Res A*, 94(1), 259-270 (2010).
199. Toyokawa N, Fujioka H, Kokubu T *et al.* Electrospun synthetic polymer scaffold for cartilage repair without cultured cells in an animal model. *Arthroscopy*, 26(3), 375-383 (2010).
200. Wang W, Li B, Li Y, Jiang Y, Ouyang H, Gao C. In vivo restoration of full-thickness cartilage defects by poly(lactide-co-glycolide) sponges filled with fibrin gel, bone marrow mesenchymal stem cells and DNA complexes. *Biomaterials*, 31(23), 5953-5965 (2010).
201. Sha'ban M, Kim SH, Idrus RB, Khang G. Fibrin and poly(lactic-co-glycolic acid) hybrid scaffold promotes early chondrogenesis of articular chondrocytes: an in vitro study. *J Orthop Surg Res*, 3, 17 (2008).
202. Pulliainen O, Vasara AI, Hyttinen MM *et al.* Poly-L-D-lactic acid scaffold in the repair of porcine knee cartilage lesions. *Tissue Eng*, 13(6), 1347-1355 (2007).
203. Nagura I, Fujioka H, Kokubu T, Makino T, Sumi Y, Kurosaka M. Repair of osteochondral defects with a new porous synthetic polymer scaffold. *J Bone Joint Surg Br*, 89(2), 258-264 (2007).
204. Shin HJ, Lee CH, Cho IH *et al.* Electrospun PLGA nanofiber scaffolds for articular cartilage reconstruction: mechanical stability, degradation and cellular responses under mechanical stimulation in vitro. *J Biomater Sci Polym Ed*, 17(1-2), 103-119 (2006).

205. Moroni L, Poort G, Van Keulen F, de Wijn JR, van Blitterswijk CA. Dynamic mechanical properties of 3D fiber-deposited PEOT/PBT scaffolds: an experimental and numerical analysis. *J Biomed Mater Res A*, 78(3), 605-614 (2006).
206. Mercier NR, Costantino HR, Tracy MA, Bonassar LJ. Poly(lactide-co-glycolide) microspheres as a moldable scaffold for cartilage tissue engineering. *Biomaterials*, 26(14), 1945-1952 (2005).
207. Ushida T, Furukawa K, Toita K, Tateishi T. Three-dimensional seeding of chondrocytes encapsulated in collagen gel into PLLA scaffolds. *Cell Transplant*, 11(5), 489-494 (2002).
208. Schagemann JC, Chung HW, Mrosek EH *et al.* Poly-epsilon-caprolactone/gel hybrid scaffolds for cartilage tissue engineering. *J Biomed Mater Res A*, 93(2), 454-463 (2010).
209. Mrosek EH, Schagemann JC, Chung HW *et al.* Porous tantalum and poly-epsilon-caprolactone biocomposites for osteochondral defect repair: preliminary studies in rabbits. *J Orthop Res*, 28(2), 141-148 (2010).
210. Martinez-Diaz S, Garcia-Giralt N, Lebourg M *et al.* In vivo evaluation of 3-dimensional polycaprolactone scaffolds for cartilage repair in rabbits. *Am J Sports Med*, 38(3), 509-519 (2010).
211. Xie J, Han Z, Naito M *et al.* Articular cartilage tissue engineering based on a mechano-active scaffold made of poly(L-lactide-co-epsilon-caprolactone): In vivo performance in adult rabbits. *J Biomed Mater Res B Appl Biomater*, 94(1), 80-88 (2010).
212. Valonen PK, Moutos FT, Kusanagi A *et al.* In vitro generation of mechanically functional cartilage grafts based on adult human stem cells and 3D-woven poly(epsilon-caprolactone) scaffolds. *Biomaterials*, 31(8), 2193-2200 (2010).
213. Moutos FT, Guilak F. Functional properties of cell-seeded three-dimensionally woven poly(epsilon-caprolactone) scaffolds for cartilage tissue engineering. *Tissue Eng Part A*, 16(4), 1291-1301 (2010).
214. Mahmood TA, Shastri VP, van Blitterswijk CA, Langer R, Riesle J. Evaluation of chondrogenesis within PEGT: PBT scaffolds with high PEG content. *J Biomed Mater Res A*, 79(1), 216-222 (2006).
215. Riley SL, Dutt S, De La Torre R, Chen AC, Sah RL, Ratcliffe A. Formulation of PEG-based hydrogels affects tissue-engineered cartilage construct characteristics. *J Mater Sci Mater Med*, 12(10-12), 983-990 (2001).
216. You M, Peng G, Li J *et al.* Chondrogenic differentiation of human bone marrow mesenchymal stem cells on polyhydroxyalkanoate (PHA) scaffolds coated with PHA granule binding protein PhaP fused with RGD peptide. *Biomaterials*, 32(9), 2305-2313 (2011).
217. Spiller KL, Holloway JL, Gribb ME, Lowman AM. Design of semi-degradable hydrogels based on poly(vinyl alcohol) and poly(lactic-co-glycolic acid) for cartilage tissue engineering. *J Tissue Eng Regen Med*, 5(8), 636-647 (2011).
218. Stenhamre H, Nannmark U, Lindahl A, Gatenholm P, Brittberg M. Influence of pore size on the redifferentiation potential of human articular chondrocytes in poly(urethane urea) scaffolds. *J Tissue Eng Regen Med*, (2010).
219. Lee CR, Grad S, Gorna K, Gogolewski S, Goessl A, Alini M. Fibrin-polyurethane composites for articular cartilage tissue engineering: a preliminary analysis. *Tissue Eng*, 11(9-10), 1562-1573 (2005).

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	<i>BST-CarGel</i> [®] [151]	Chitosan-glycerol phosphate based hydrogel	Piramal Healthcare, Laval, Quebec, Canada
		<i>ChonDux</i> [™] [152]	Photopolymerized hydrogel combined with a biological adhesive	Biomet, Inc., Warsaw, IN, USA
		<i>Gelrin C</i> [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	<i>Salucartilage</i> [26,27]	Biodegradable hydrogel implant	Salumedica, Smyrna, GA, USA
		<i>Chondromimetic</i> [154]	Multilayer triple co-precipitate of collagen, glycosaminoglycans and calcium phosphate	TiGenix, Leuven, Belgium
		<i>Tru-Fit Plug</i> [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	<i>Carticel</i> [155]	Porcine-derived type I and type II collagen scaffolds	Genzyme Inc, Cambridge, MA, USA
	biomaterials used in scaffold	<i>Chondrogide</i> [156]	Porcine-derived type I and type II collagen scaffolds	Geistlich Biomaterials, Wolhausen, Switzerland
		<i>Hyalograft-C</i> [87]	Hyaluronic acid based scaffold	Fidia Advanced Biopolymers, Abano Terma, Italy), and Neocart (Histogenics, Waltham, MA
	Use of bioreactor to enhance in vitro culture	<i>Neocart</i> [78]	type I collagen matrix	Histogenics, Waltham, MA
	Morselized cartilage	<i>Cartilage Autograft Implantation System (CAIS)</i> [157]	Morselized cartilage	DePuy/Mitek, Raynham, MA
		<i>DeNovo Natural</i>	Morselized cartilage	Zimmer, Inc., Warsaw, IN, USA

Table 2:

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

Material	Advantages	Disadvantages	Example References
<i>Natural Materials</i>			
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]	Concerns of biocompatibility [176]	[177]; [176]
	In vivo injectable options [175]		
	Abundant and low cost		
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]	Limited solubility [183]	[184]; [185]; [186]; [187]; [188]; [189]; [182]; [190]
	Degradation products non-toxic and are involved in the synthesis of articular cartilage	Certain cross-linkage can result in poor biocompatibility	
	- Chondroitin sulphate,		

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

	chondrocyte layer in multilayered scaffold	structures	
	Uniform porosity allows better cell growth and proliferation		
Silk	Supports growth of chondrocytes	Issues with biocompatibility and allergic reactions with certain types of silk	[194]; [195]
	Good tensile strength		
Synthetic Materials			
Poly(α -hydroxy esters)	Satisfactory	Degradation by-products	[197];[198]; [199]; [197]; [200];
<ul style="list-style-type: none"> • Poly(lactic acid) • Poly(glycolic acid) • Poly(lactic-co-glycolic) • Poly(caprolactone) 	biocompatibility [97]	has been shown to elicit inflammatory response and decreased pH level [98]	[201]; [202]; [203]; [204]; [205];; [92]; [206]; [207]; [208]; [209]; [210]; [211]; [212]; [209]; [213]
	Good mechanical properties	Mechanical stiffness can sometimes be undesirable	
	Flexibility in degradation rates	[196]	
Poly(ethylene glycol)	Hydrophilicity	Hydrophobicity [196]	[214]; [215]
	Biocompatibility		

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