MECHANICS IN ARTICULAR CARTILAGE REGENERATION

Articular cartilage is an avascular load-bearing tissue lining the surface of long bones where it serves for the absorbance of shocks as well as the lubrication of joints. Treatments to repair cartilage defects mainly consist of cell therapies, which do not yield biomechanically sound tissue. Therefore tissue engineering has been proposed as a viable alternative. In order to meet biomechanical demands tissue engineered constructs require specific architecture, which could be accomplished by 3D deposition of hydrogels. Unfortunately, hydrogels are not mechanically compatible with native articular cartilage. The use of reinforced hydrogels in biofabrication allows tailoring of mechanical properties with a retained biocompatibility. Mechanical loading in a bioreactor can contribute to the improvement of tissue engineered constructs by mimicking *in vivo* conditions. Mechanical tuning in biofabrication as well as mechanical training could contribute to a clinically applicable tissue.

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Index

1. Introduction

Repair of articular cartilage defects for degenerative diseases such as osteoarthritis are a main focus for orthopedic research. Articular cartilage has limited self-regenerating capacity, which increases the demand for a natural substitute (Mollon et al., 2013). Different strategies such as microfracture or autologous chondrocyte implantation aim at restoring the defect, but these clinical examples generate tissue that lacks the specific architecture of native articular cartilage, ultimately leading to biomechanical failure (Kock et al., 2012).

Engineering a construct which resembles native tissue architecture is proposed as a functional alternative for articular cartilage repair (Huey et al., 2012). Tissue engineering as a replacement for dysfunctional tissue is a large goal within regenerative medicine. The tissue engineering process has been clearly indicated in six phases, the first phase of which is described as the fabrication of a scaffold which is degradable over time (Hutmacher, 2000). Taken together the mechanical impairment of current repair treatments and the development of scaffolds, mechanical demands could be implemented within this phase to influence the qualitative outcome of a tissue engineered construct.

This review will focus on the mechanics in articular cartilage regeneration. For proper understanding, knowledge on the existing features of articular cartilage structure and biomechanical functioning is vital. In light of these functions, biofabrication in tissue engineering will be discussed as well as the mechanical testing of biofabricated constructs. A discussion on influence of the biomechanical parameters in both biofabrication and tissue expansion will be given, after which some concluding remarks and prospects in future articular cartilage tissue engineering are reported.

2. Native articular cartilage

2.1 Articular cartilage is essential to joint function – macro level

Articular cartilage is an avascular tissue lining long bones and is usually situated on opposing joints. It provides a barrier to absorb shocks and to reduce the conduction of stress to the bone it covers. The contact between opposite cartilage surfaces during loading causes exudation of fluid from the tissue. The thin layer of fluid, called the interstitial fluid, lubricates joint surface (Wong & Carter, 2003). Articular cartilage has therefore often been described as viscoelastic comprising both fluidic and solid properties.

During normal loading, articular cartilage is subjected to compression due to weight bearing, tension because of joint translation and shear stresses (Silver & Bradica, 2002). Articular cartilage needs to process large amounts of loading during which it has to react within seconds and has to endure fast compression and shear. Compressive loads on the articular cartilage of for example a hip vary from three to eleven MegaPascal (MPa) for regular activities to an increase of a twenty fold when running (Mansour, 2013).

2.2 The extracellular matrix of articular cartilage

Mechanical behavior of articular cartilage is dependent on its structure. One of the main components of articular cartilage extracellular matrix (ECM) are the proteoglycans, which consist of a protein core with covalently bound glycosaminoglycan (GAG) chains such as chondroitin sulphate and keratan sulphate (Wong & Carter, 2003). The most abundant form of proteoglycans is aggrecan. Proteoglycans are negatively charged, which allows them to bind water and positive charged ions. The attraction of water and other electrolytes causes swelling of the tissue therefore creating an

osmotic pressure (Halloran et al., 2012). Other components of the ECM are bundles of collagen fibers. These fibers are primarily made up of collagen type II, which accounts for 90 to 95% of the total content. Collagen fibers predominantly resist tensile forces, whereas proteoglycans cause swelling. The collagen fibers also restrict swelling resulting in the mechanical balance of shock absorbance and prevention of permanent deformation (Halloran et al., 2012).

The specific structure has resulted in the so called biphasic theory to describe the mechanical properties according to the material properties. As has been described before, cartilage is viscoelastic comprising both fluidic and solid components. The interaction between the two compartments results in the functions it exerts (Mansour, 2013).

2.3 The zonal organization of articular cartilage

Articular cartilage can be divided into different zones, each with different properties to minimize stress for the underlying bone and to transduce forces (Wong & Carter, 2003). It can be divided into the superficial, the middle and the deep zone. The zone closest to the cartilage surface is the superficial zone. This zone is important in resisting tensile and compressive forces due to arrangement of densely packed collagen fibers parallel to the joint surface (Wong & Carter, 2003). The matrix within this zone is very porous in comparison to the other zones, which facilitates the exudation of interstitial fluid during loading. On the long term, the superficial zone can be affected due to large compressive forces and shear from joint loading (Silver & Bradica, 2002). This can lead to cartilage thinning, which is a feature of osteoarthritis.

The middle and deep zone have limited fluid passage due to a decreased porosity of the matrix. The confinement of both the adjacent cartilage and the calcified cartilage and subchondral bone also contribute to the limited fluid flow. The limited fluid passage causes a large fluid pressure within this zone. The collagen fibers are randomly dispersed in the middle zone and are perpendicular to the joint surface in the deep zone. Both zones are therefore important in the loading capacity of cartilage (Silver & Bradica, 2002).

It has been indicated that the superficial zone is the leading structural component in resistance of compressive loads and stresses, because of both ECM structure and chondrocyte density and activity. However, recent findings by Buckley *et al.* suggest a more profound role for the transitional zone in withstanding shear, although this zone has previously been seen to be important in shock absorption (Buckley et al., 2013).

A visual representation of the different zones within articular cartilage and its collagen architecture is depicted in figure 1.

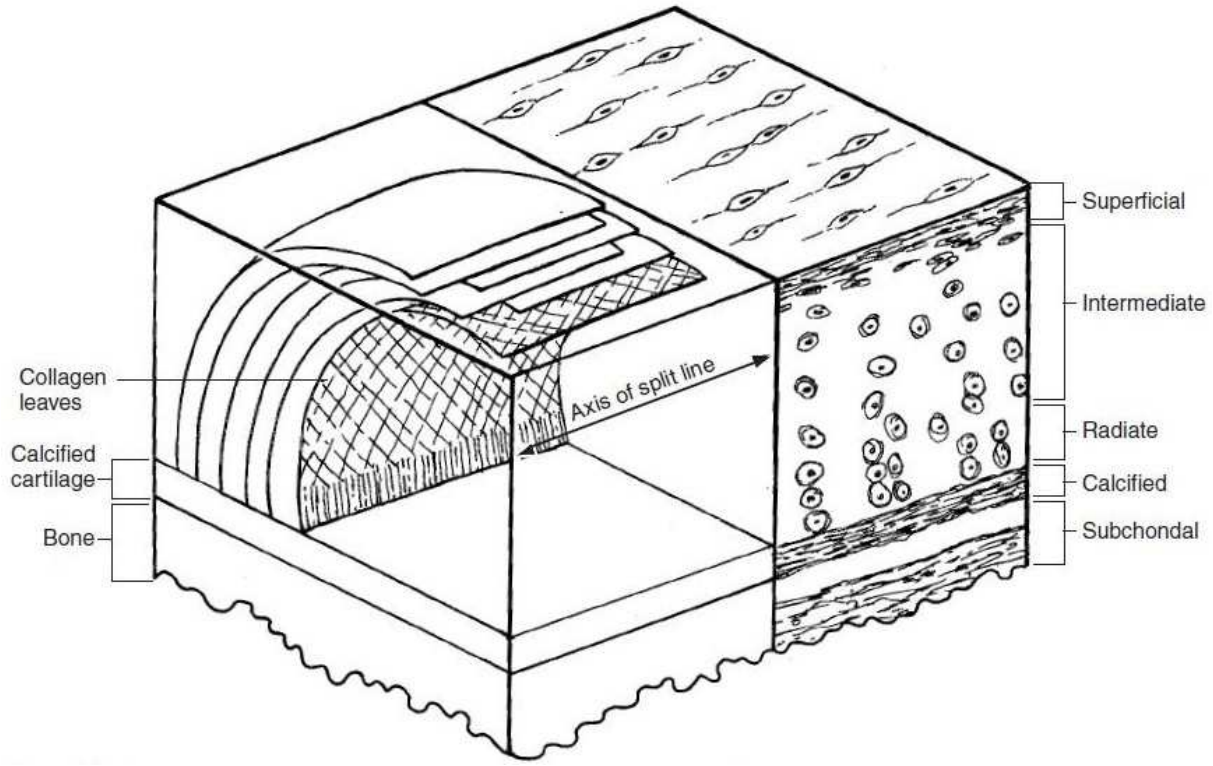

Figure 1 Side view of articular cartilage
Articular cartilage is divided into zones. The superficial zone is densely packed with chondrocytes. The intermediate or middle zone contains less chondrocytes, which are also less densely packed. The deep or radial zone is connected to the calcified cartilage and the adjacent subchondral bone. Collagen has a specific architecture which is different in each zone, varying from perpendicular fibers to the joint surface in the middle zone to parallel fibers in the superficial zone. Adapted from Mansour (2013).

2.4 Functional unit of articular cartilage: the chondron

The zones accordingly differ not only in structure but also in function. The functional cells of articular cartilage, chondrocytes, are also dispersed in a distinctive way across the different zones. Chondrocytes are essential to cartilage function as they are needed to synthesize matrix and other proteins important in cartilage maintenance and repair (Wong & Carter, 2003). Chondrocytes are the only cell type to be found in articular cartilage.

The pericellular matrix is a unique feature for chondrocytes compared to other eukaryotic cells. Together with the chondrocytes it forms the chondron. This thin layer around the chondrocyte consists of regular ECM proteins, which differ from the ECM proteins surrounding the chondrocyte (Guilak et al., 2006). The main type in the pericellular matrix is collagen type VI which has been postulated to be pivotal in anchoring the chondrocyte to the ECM. The pericellular matrix enhances the transduction of mechanical forces to the chondrocyte which ultimately leads to influence on cell behavior (Guilak et al., 2006). Because the pericellular matrix is important in maintenance of the chondrocytes, it has been investigated intensively. However, the exact pathways involved in its function have not yet been fully elucidated. Practical considerations play a role, since the pericellular matrix is a delicate tissue and experiments are often destructive to the thin coat. McLane *et al.* attempted to elucidate the structure and mechanical properties by using optical force probe assays in which probes can be tracked in real time. They postulate an incoherent structure for the pericellular matrix with varying mesh size which greatly influences transport of molecules to and

from the pericellular matrix (McLane et al., 2013). It is however debatable to which extent the results presented represent physiological values, since chemical interactions were excluded from the experiments to precisely examine the microstructure. Therefore more investigation on the pericellular matrix is necessary to elucidate the structure and mechanotransduction pathways.

2.5 Articular cartilage – micro level

The structural components of articular cartilage counteract loads and stresses, while transduction of the forces by the experienced load occurs on a microscale. It has been postulated that interactions between chondrocytes play an important role in this process, as well as interactions between chondrocytes and the extracellular matrix. One recently discovered player is the primary cilium. The primary cilium is abundant in most eukaryotic cell types and acts as a mechanosensor (Wann et al., 2012). In chondrocytes primary cilia have been postulated to be involved in exogenous ATP reception, where it initiates calcium signaling in order to enhance matrix component synthesis.

However, cell-matrix and cell-cell interactions have been investigated to be important factors in mechanosensing and mechanotransducing properties. An interplay between matrix and chondrocytes provides proper downstream signaling. Especially the chondrocyte-collagen fibril interface contributes to this downstream signaling pathway via specific cell-membrane associated integrins (Silver & Bradica, 2002). Integrins are connected to the cytoskeleton which directly induces downstream signaling. Binding of chondrocytes to the collagen fibrils leads to clustering of the integrins, forming focal adhesions. Focal adhesions have been indicated to be mechanotransducers (Wong & Carter, 2003). Cell-cell interactions are no less important. Cadherines and the adherens junctions have been theorized to be transducers of mechanical forces through activation of mechanosensitive calcium channels, which leads to increased actin polymerization. Stretching of gap junctions and their associated connexins during mechanical load causes cellular tensile forces. These tensile forces allow intercellular communication via secondary messengers.

Interactions between chondrocytes and extracellular matrix mostly induce downstream signaling. Mechanical cues can lead to conformational changes in cell membrane receptors, activating protein cascades or enhance or inhibit gene expression. Activity of matrix degrading proteins such as matrix metalloproteinases are decreased in response to mechanical load, whereas matrix synthesizing components are increased after load (Silver & Bradica, 2002).

2.6 Current research

Even though cartilage function and structure are clarified, many features still need to be elucidated. The pericellular matrix is an example, but also the precise role of collagen type II in the mechanical output of articular cartilage. It has been observed that superficial collagen fibrils in the superficial zone recruit the collagen fibril from deeper zones within the cartilage to distribute loads which aids in the load bearing properties (Hosseini et al., 2013). Knowledge on the exact mechanism of collagen within cartilage is not only useful for cartilage regeneration studies, but might also provide more information for pathology of disease such as intervertebral disc degeneration (Römgens et al., 2013).

3. Biofabrication

Tissue engineering for articular cartilage comprises different production techniques, cell types and materials (Johnstone et al., 2013)(Kock et al., 2012) . Biofabrication is rapidly developing within the field of tissue engineering and can be described as the assembly of various biological building blocks to create a well-tuned biomaterial (Malda et al., 2013). In light of this review, it concerns the development of a bio- and mechanical compatible scaffold material for articular cartilage which in

time can be replaced by neo-tissue. The choice of material and technique are critical in scaffold production.

3.1 Hydrogels

In cartilage tissue engineering different groups have attempted to create scaffolds that are both cell compatible and support mechanical functions. Suitable materials for that would be hydrogels. Hydrogels are polymers that are very biocompatible due to their high water content. Hydrogels enable the inclusion of cells, proteins and other signaling molecules as well as practically limitless processing possibilities (Malda et al., 2013). Hydrogels can be chemically modified to enhance mechanical properties. Since hydrogels can be derived from polymers that are abundant in the extracellular matrix, the endogenous tissue environment can be mimicked (Malda et al., 2013). In this way, the specific architecture of articular cartilage with the different zones could be resembled. The biocompatibility and mimicry of the natural environment, favor the use of hydrogels for articular cartilage tissue engineering.

3.2 3D fiber deposition

To precisely mimic native cartilage architecture, specific architecture of the biomaterial is likely to benefit the formation of tissue and the resulting biomechanical parameters. A technique that has been developed in order to accomplish that is 3d fiber deposition. 3D fiber deposition, or 3D printing, is based on the layered deposition of polymer in three dimensions allowing specific architecture of a scaffold in tissue engineering (Woodfield et al., 2004). A computer model can be translated into a 3D design. 3D printing employs different dispensing systems, varying from inkjet printing for small volumes to robotic dispensing for polymer strands (Seliktar, 2012). The accuracy of 3D printing allows spatial control over the printed scaffold. The translation of a specific design to a construct combined with the biocompatibility of hydrogels, makes an excellent combination for articular cartilage engineering. An example of a 3D printed construct is given in figure 2.

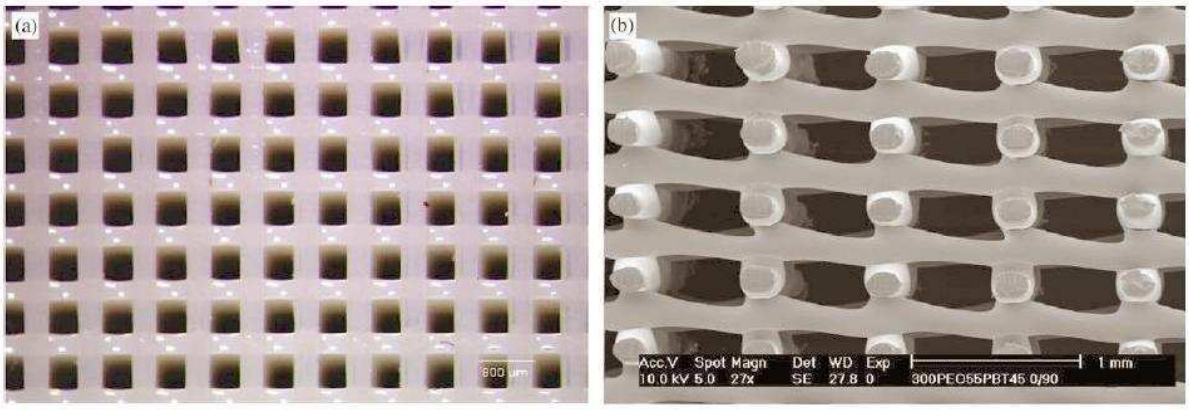

Figure 2. 3D printed structure of thermoplastic polymers
(a) Optical microscopy picture of a 3D deposited structure in a 0/90 configuration, consisting of polyethyleneoxide-terephtalate (PEOT)/ polybutylene-terephtalate (PBT) block copolymers. (b) Scanning Electron Microscopy picture of a 3D deposited structure in a 0/90 configuration, consisting of PEOT/PBT block copolymers. Adapted from Moroni *et al.*

3.3 Design of a biofabricated construct

The outcome of a 3D printed design is highly dependent on the properties of the hydrogel. The scaffold architecture, stiffness and therefore mechanical output are greatly influenced. Hydrogel properties need to be carefully considered in scaffold design, but the translation to a printed scaffold is even more challenging (Guillotin & Guillemot, 2011). Viscosity in light of bioprinting is an important parameter, since it promotes structure preservation but also increases difficulty in the deposition of the hydrogel strands (Malda et al., 2013). The resulting shear causes thinning of the gel and limits preservation of scaffold structure. The shear stress causes damage to encapsulated cells. Furthermore, high viscosity materials tend to limit cell migration and proliferation (Malda et al., 2013).

Due to hydrophilic properties of the hydrogel, the gel swells when immersed into a solution (Anseth et al., 1996). This is often beneficial for the cells encapsulation, but may interfere with scaffold architecture. The swelling can be predicted to implement in scaffold design, which may inversely affect mechanical strength (Anseth et al., 1996). The gelation time of the hydrogel used is important in for preservation of the construct. The gelation time should not take too long, since the deposition of layers is time-consuming and the structure needs to be preserved during that time frame (Malda et al., 2013).

Pore size is very important in scaffold design and its mechanical capabilities. Pores in the direction of compression are mechanically weaker (Moroni et al., 2006). Regarding the quantity of pores, more contact points between fibers in the scaffold lead to a decreased experiences load for the construct. A configuration of 0/90 with two layers in each direction seemed to be most beneficial for mechanical strength, as well as thin layers with small fiber spacing (Moroni et al., 2006). It should be noted that characteristics as layer configuration and thickness as mentioned above have been studied in block copolymers.

3.4 Mechanical testing

The evaluation of mechanical properties by testing is an important parameter in predicting future possibilities in tissue production and construct quality. Several quantitative output methods have been developed to determine the strength of a scaffold and to compare with the native tissue. When it comes to testing hydrogel and reinforced hydrogel structures, many different tests can be applied to provide information on the construct. One way is to look at the integrity of the hydrogel and its implications on the amount of viscosity and elasticity of the compound as can be measured with rheology. Rheology studies the flow of matter and yields storage and loss moduli which are values for elasticity and viscosity respectively (Malda et al., 2013). A viscoelastic material such as cartilage encompassed both and hydrogels must therefore meet these requirements.

Compression of cylindrical hydrogel samples between two plates, confined or unconfined, yields a stress-strain curve after which the elastic modulus can be derived (Wong & Carter, 2003). The compressive modulus for articular cartilage is 0.7 to 0.8 MPa (Mansour, 2013).

The elastic modulus is a value to which extent the material can be compressed until failure. The elastic modulus is also called the Young's modulus, which is 4.1 MPa for cartilage (Schuurman et al., 2011). The elasticity of a material can also be determined by a creep recovery test, in which the recovery of a material is measured after a certain load is applied. A mechanical value that is more applied to cartilage is the aggregate modulus, which is correlated to the stiffness when there is no fluid flow. The aggregate modulus can be compared to the cartilage after compression, when the interstitial fluid is exudated. This value can be derived from the Young's modulus. For articular cartilage, this value lies between 0.5 and 0.9 MPa (Mansour, 2013).

Although multiple types of testing exist to measure hydrogels for their mechanical properties, it has been argued that also tests of fracture might give more insight into the mechanical mimicry of hydrogels since they differ from native cartilage, which has to withstand large loads (Xiao et al., 2013).

While hydrogels have been indicated to be suitable for the fabrication of tissue engineered articular cartilage constructs, several studies argue against the use of hydrogels as the sole scaffold material. Hydrogels are cell compatible and promote cellular migration and differentiation. However, the stiffness of the hydrogel does not compare to either articular cartilage or less biocompatible polymers (Seliktar, 2012). Since articular cartilage is very important in weight bearing and other load distributing functions, the scaffold for the target tissue must be of compatible mechanical strength. Therefore different methods have been presented to combine the strong polymer with a cell compatible hydrogel. In such a hybrid construct tissue production, mechanical stability and scaffold degradation are balanced.

4. Reinforcement of biofabricated constructs

Recently, the technique of reinforcing biological or synthetic hydrogels with another material gained increased attention. Moutos *et al.* combined a hydrogel and a synthetic polymer in woven technique in order to produce a scaffold mimicking the native articular cartilage. The porous structure was build up out of intelligent biodegradable yarns, woven in three different directions to obtain an anisotropic scaffold (Moutos et al., 2007). A fibrin or 2% agarose gel was part of the woven construct in which chondrocytes were seeded. The construct mimicked the viscoelastic anisotropic properties of native articular cartilage although cell viability was not reported.

4.1 Fiber reinforcement

Fiber reinforcement is a technique that has been developed in the twentieth century, but has been postulated for articular cartilage tissue engineering from the early 2000s. In a research conducted by Slivka *et al.* two polymers were combined. By using different mixtures of Poly-(Lactic-Co-Glycolic) Acid (PLGA) and Polyglycolide different mechanical properties could be obtained. Even though the scaffold architecture was simple, this study revealed that the manufacturing technique was important in the orientation of the fibers. Fibers in return are important for the mechanical properties (Slivka et al., 2001). Pore geometry was found to be associated with the amount of fibers within the scaffold.

Fiber reinforcement has been previously used with simple scaffold architecture. Since articular cartilage is a complex tissue with a specific architecture which is important in its function a tailored scaffold design is likely to aid in the production and differentiation of tissue.

By combining 3d fiber deposition and the use of both synthetic polymers and a hydrogel the best of both worlds could be combined to create a well-tuned biofabricated scaffold. Schuurman *et al.* showed by combining an alginate hydrogel with a PolyCaproLactone (PCL) scaffold increased mechanical properties equal to the mechanical properties of a thermoplastic polymer (Schuurman et al., 2011). Constructs showed good biocompatibility as cell viability was compatible with survival in non-reinforced hydrogels. This study demonstrated the exchangeability of hydrogels, which enables other hydrogel-polymer mixtures to be used and tested to create more specific scaffolds.

Visser *et al.* extended the possibilities of creating scaffolds by both the production of reinforced hydrogels as well as complex hydrogel structures using sacrificial polymers (Visser et al., 2013). As in the study of Schuurman, these procedures did not seem to affect cell viability. Further 3D fiber deposition studies might incorporate more polymer combinations for scaffold architecture design. Sacrificial polymers might aid in supporting hydrogel structure without affecting biocompatibility. An example of fiber reinforcement is depicted in figure 3.

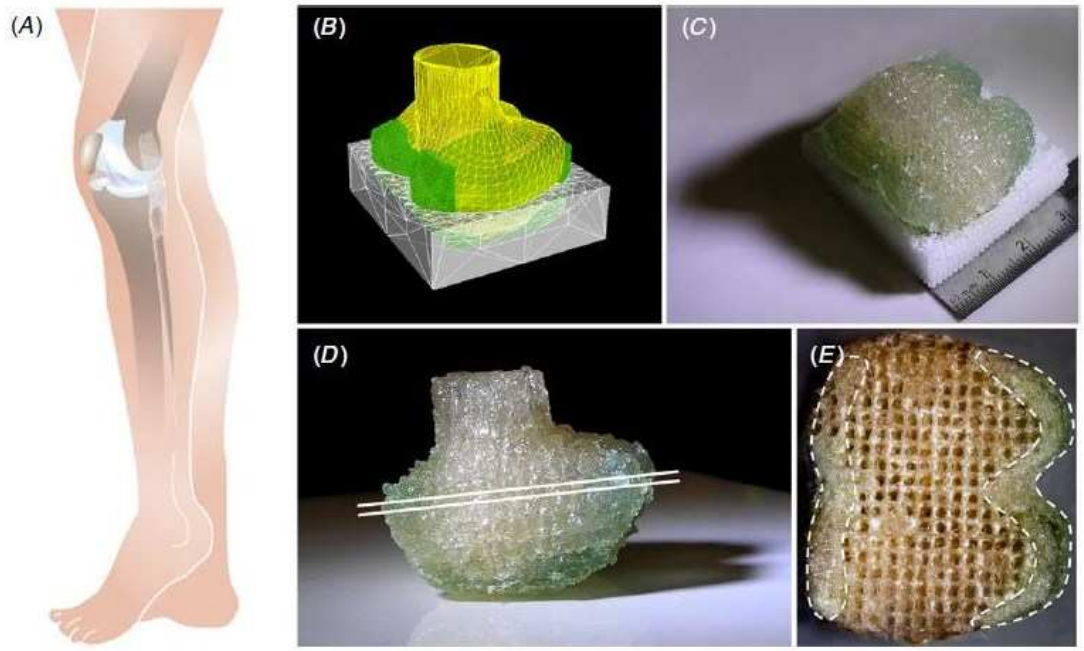

Figure 3 Possible clinical application of 3D fiber deposition with fiber reinforced hydrogels
(A) Model of distal human knee femur (B) Computer design of scaffold with bone indicated in yellow , cartilage in green and support structure in white (C) 3D deposited hydrogel in a PCL coating on support structure (D) 3D deposited construct without support structure (E) Clear distinction between multiple components; the porous bone and surrounding cartilage (dashed lines). Adapted from Visser *et al.*

4.2 Interpenetrating Polymer Networks (IPNs)

Fiber reinforcement is not the only method that focuses on combining different polymers for constructs. Interpenetrating polymer networks or IPNs can be described as a combination of two different polymers which create a network with more features than the polymers alone (Myung *et al.*, 2009). Polymers can become stronger due to the effect of the other, or one can enforce the other polymer when it comes to swelling and polymerization.

Double networks are a specific type of IPN which is mostly focused on increasing the mechanical strength of water based constructs (Shin et al., 2012). They can be particularly interesting for engineering of load bearing tissues since it can combine different hydrogels without using any other synthetic polymers. Furthermore, mechanical properties have been shown to increase in double network hydrogels (Shin et al., 2012). This eliminates the necessity for using and breaking down of the synthetic compound within the construct and limits any harmful effects to the tissue produced and the other surrounding tissue.

Double networks resemble IPN and fiber reinforced structures since both a rigid and soft structure are used, modeling the soft structure within the rigid structure. Gellan Gum Methacrylate (GGMA) was employed by Shin *et al.* to create a firm network achieved by photocrosslinking. Gelatin Methacrylamide was dispersed throughout the network and then photocrosslinked to create the double network (Shin et al., 2012). Many hydrogel double networks have been developed, but most of these contain toxic residues, which limits the possibilities of encapsulating cells.

Also with hydrogels the balance between mechanical strength and cellular compatibility is important. Although higher molecular weight and higher polymer concentration promote mechanical function by density, cell viability and GAG production significantly decrease with higher concentration. Therefore aggrecan was incorporated in the same type of IPN with increased chondrocyte activity as a result (Ingavle et al., 2013) .

4.3 Other strategies

Besides fiber reinforcement, other strategies can be employed in order to increase the strength of the hydrogel building blocks. As has been mentioned before, the increase of the viscosity is likely to result in a stronger structure. A higher viscosity can be accomplished by increasing polymer concentration. Strength is also correlated to crosslinking density, as more crosslinks are likely to result in a stronger material (Malda et al., 2013).

Electrospinning is also another strategy for reinforced constructs, which utilizes a current to deposit liquid fibers into complex structures . it has been shown that the mechanically strong fibers support chondrocyte viability (Seyednejad et al., 2011). More recently, electrospinning in combination with a hydrogel is proposed as an improvement of present electrospun structures. The carefully designed fibers in combination with a biocompatible gel mimics extracellular matrix while maintaining mechanical properties (Bosworth et al., 2013). Electrospinning has recently been combined with inkjetprinting. A PCL scaffold was electrospun together with an inkjet deposited fibrin-collagen hydrogel with encapsulated chondrocytes to make one hybrid construct. Chondrocytes showed high viability, as well as GAG production and collagen type II synthesis (Xu et al., 2013).

4.4 Implications for mechanics and cell compatibility

Reinforcing hydrogels as well as electrospun scaffolds have been observed to increase mechanical outcome. A stiffer material has an increased Young's modulus which means that it deforms less under high load. The influence of material properties can have significant effects on cell viability. A higher polymer concentration as well as an increased crosslink density affect cell viability negatively and hamper cell migration and matrix production.

Since cells respond to their environment, they also react to the stiffness of the substrate they adhere to. An increasing stiffness is likely to influence cell differentiation. It has been postulated that the differentiation of multipotent stromal cells within a hydrogel is promoted towards a certain lineage due to viscoelasticity of the hydrogel (Guvendiren & Burdick, 2012). Furthermore, cells tend to migrate towards increased stiffness (Ehrbar et al., 2011). Since the zones of articular cartilage comprise different properties and thus different strengths, a zonal scaffold architecture might interfere with cell migration.

5. Beyond biofabrication

Stages in tissue engineering as postulated in Hutmachers review encompass more steps to engineer a clinically applicable construct (Hutmacher, 2000). Phases include the seeding of cells into the

scaffold, which is often performed in current studies. Also the production and maturation of the tissue are important steps in the tissue engineering process. The production and maturation of tissue occurs in a dynamic environment that mimics *in vivo* conditions such as temperature, flow and nutrient supply. Such an environment is created in a bioreactor.

5.1 Bioreactors

The use of a bioreactor is essential in providing the construct with the necessary nutrients and flow to mimic the natural tissue environment (Schulz & Bader, 2007). The hydrostatic pressure, tension and shear maturate the tissue to eventually promote matrix synthesis and production of other components to withstand the forces and mechanically enhance the cartilage (L. Kock et al., 2012). Motions and loads are simulated while maintaining a suitable environment for the tissue to produce matrix. Different types of bioreactors are suited for 3D deposited structures but all types try to mimic *in vivo* conditions.

Applying compression increases the compressive properties of constructs, which are important in native articular cartilage for shock absorbance. Compression causes a hydrostatic pressure encouraging the proteoglycans and collagen to withstand the forces and prevent deformation (Schulz & Bader, 2007). Furthermore, compression in a bioreactor promotes matrix production. Systems that apply hydrostatic pressure are also often used in mechanical loading to mimic *in vivo* conditions. Together with compression systems, hydrostatic pressure systems are very abundant in tissue engineering studies (L. Kock et al., 2012). Examples of mechanical loading is depicted in figure 4.

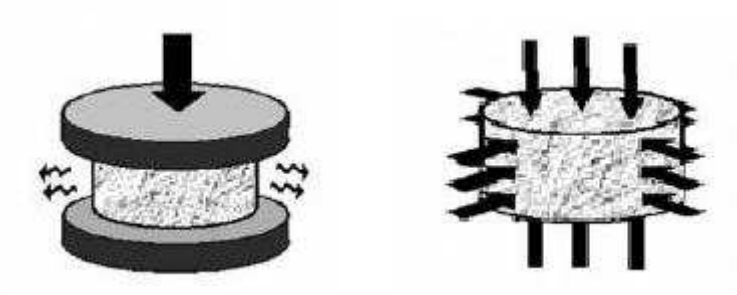

Figure 4. Examples of the most abundant loading protocols in bioreactors
Compression is often applied to tissue engineered cartilage constructs to maturate the tissue (left). Hydrostatic pressure has also often been used in bioreactors to enhance mechanical properties of the neo tissue (right). Pictures depicted are based on cartilage explants, adapted from Wong *et al.*

Upregulation of collagen type II markers has been observed in flow perfusion systems, as well as cell proliferation (Kock et al., 2013). A downside of flow perfusion is the exposure to shear. Shear stresses are also encountered during regular loading, but can affect cells negatively in culture (Linda M Kock et al., 2013). Bioreactors can also apply stretch to 3D cell constructs. As can be derived from the function of native articular cartilage, stretching promotes mechanotransduction during loading (Riehl, 2012).

A sliding indentation bioreactor system has recently been developed in order to increase chondrogenic capacities and collagen synthesis (Kock et al., 2010). An increased collagen synthesis might be beneficial for tissue engineered constructs. Explants between agarose scaffolds were subjected to strains resulting from a sliding indenter. Mechanical stimulation seemed to increase collagen type II production after the inclusion of Transforming Growth Factor Beta 1, a known enhancer of chondrogenesis (Kim et al., 2011).

5.2 Recent developments

Besides mechanical stimulation and the addition of signaling molecules, many other systems are in development to increase mechanical properties and general quality of tissue engineered constructs. Electromagnetic stimulation is one of those upcoming methods, which is believed to increase cartilage regeneration in current treatments (Hilz et al., 2013). The authors argue an increased effect of the electromagnetic based on GAG and DNA content but do not report on the mechanical properties of the produced tissue (Hilz et al., 2013). Furthermore, long term effects were not reported.

The development of suitable bioreactors is also an upcoming field all aiming at closely resembling the conditions of articular cartilage in vivo. That taken into account, recent findings suggest the use of a bioreactors in which a gradient of nutrients exist in order to resemble *in vivo* zonal perfusion conditions. By a combination of cyclic loading and nutrient diffusion from both vertical directions a gradient for oxygen and glucose was induced (Spitters et al., 2013).

5.3 Bioreactors and 3D printed scaffolds

The application of a bioreactor to 3D printed hydrogel constructs has not been reported extensively thus far, although there are studies that use other 3D deposited constructs in a bioreactor (Kock et al., 2013). It has been postulated to employ a bioreactor to promote tissue formation for 3D deposited hydrogels (Malda et al., 2013). Future studies are needed to investigate this possibility.

6. Concluding remarks

This review attempts at looking at mechanics in both biofabrication and tissue production for articular cartilage regeneration. As can be read, mechanics are vital in the qualitative outcome of a tissue engineered construct. Further steps need to be taken in order to gain a clinically applicable construct which exceeds the positive outcome of current therapies such as autologous chondrocyte implantation. Tissue engineering is a rapidly evolving and promising field but does not yield clinical applicable examples thus far.

Both in the biofabrication process and the tissue production process, mechanics has proven itself to be a vital component in the qualitative outcome of a tissue engineered construct. Mechanical output can be influenced via biofabrication in the design of a scaffold. The implementation of loading protocols in the bioreactor stage can also contribute to improved mechanical properties of a tissue engineered construct. Which one is key is not yet fully elucidated. Thus far it has not yet been proven which approach leads to the most promising results. Therefore further studies, both fundamental and applied need to be conducted in order to determine the best cartilage regeneration method.

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Laymen summary

Articular cartilage is important in the absorbance of shocks that result from movement. The specific architecture reacts to the shock by decreasing in volume. This decrease is smaller during walking than during running and is not permanent. Articular cartilage is also important in the lubrication of joints, which means that it provides a small fluid layer between cartilage surfaces that lie opposite of each other to facilitate easy movement.

Defects in cartilage can occur as a result of disease or trauma. Because cartilage cannot repair itself fully, a different approach is needed. The tissue that is the replacement should be mechanically related to the natural cartilage to be functional. It has also has to be accepted by the body. Tissue engineering aims at the creation of a functional tissue by combining natural materials with an intelligent design. For articular cartilage, 3D printed hydrogels are a promising possibility. The hydrogels provide a cell-friendly environment, while printing in 3D allows the creation of a specific architecture.

However, the hydrogels that are currently used are not strong enough to match with the mechanical properties of cartilage. Therefore, they are reinforced by other materials to increase these properties while maintaining the cell-friendly environment. These enhancements are promising for possible clinical applications. Next to that, newly formed tissue can be mechanically trained in a bioreactor. This is a culture environment that resembles the conditions in the body and provides forces that are naturally occurring. Mechanical training is another way to enhance mechanical properties of tissue engineered constructs.

Both 3D printed reinforced hydrogels and mechanical training in a bioreactor could contribute to tissue that is mechanically resembled to natural cartilage. Future research needs to point out which approach will lead to the best clinical application.