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Tissue Engineering of Articular Cartilage

REVIEW

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ABSTRACT

Articular cartilage (AC) is the tissue that wraps moving joints in the body. As a tissue, it provides lubrication as well as load bearing in some joints. The tissue is subject to harsh chemical and mechanical environments in vivo and is characterized by being devoid of blood, nerve and lymph nodes. Upon injury, the tissue is incapable of healing. Damaged AC tissues mark the global disease of osteoarthritis (OA). As a pandemic, OA is prevalent worldwide and is ranked first for which patients seek treatments. Unfortunately, the disease has no current disease modifying drugs and it is only managed for symptomatic relief using pain killers, physical therapy and surgical procedures. In search for less invasive treatments, tissue engineering has been sought to provide the alternative. Tissue engineering refers to creation of eventually personalized tissues that can be used to replace damaged tissues in vitro. As an approach, it relies on seeding cells on scaffolds and incorporating the two in bioreactors that mimic the joint environment. Cells are then fed a medium with growth factors in it for a period of time until they form tissues. The tissues are then characterized to check if they represent the native tissue in structure and function. If not, the parameters used to engineer the tissue are revisited and the loop is repeated again. Here, tissue engineering of AC will be introduced. After that, the four pillars of tissue engineering (cells, scaffolds, growth factors, and bioreactors) will be discussed with questions that remain to be addressed. A section that discusses how engineered tissues are characterized will follow. Finally, the review ends with a summary of where the field is heading to realized quality AC tissues. This review is not meant to be comprehensive of existing literature.

Keywords: Articular cartilage,tissue engineering, and osteoarthritis

Introduction

Articular cartilage (AC) is the tissue that wraps moving joints (Bhosale and Richardson 2008). As a tissue, it provides lubrication (Guilak and Mow 2000) as well as load bearing in some joints like the knee (Wong and Carter 2003). To facilitate its function, the tissue is characterized by complex heterogeneous anatomy (Klein et al. 2007). As a tissue, AC is multiphasic, anisotropic, and viscoelastic (Mow et al. 1984, Mow and Guo 2002, Shieh and Athanasiou 2006). The tissue has three distinct zones. These are the superficial zone (SZ), the intermediate zone (IMZ) and the deep zone (DZ) (Shieh and Athanasiou 2006). The three zones gradate spatially in properties as a function of depth (Klein et al. 2007). For example, the density of chondrocytes, the specific AC cell type, is highest in the SZ and lowest in the DZ (Wong et al. 1996). Similarly, the properties of the extracellular matrix (ECM) are also depth dependent, with the lowest aggrecan and highest collagen concentrations in the SZ (Maroudas 1974). Furthermore, the surface zone protein (SZP) responsible for lubrication is abundant in the SZ and sparse in the IMZ and DZ (AR et al. 2001, TJ et al. 2003, EM et al. 2004, Yunsup et al. 2018). Collagen fibrils are primarily parallel to the surface in the SZ, isotopically intermingled in the IMZ, and vertically aligned parallel to cellular columns in the DZ (LC et al. 2005).

Based on their locations in the body, the majority of AC tissues are subject to significant mechanical stresses including compression, oscillating hydrostatic pressures (OHP), tensile stresses and shear forces

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(Bachrach et al. 1998, Guilak and Mow 2000, Abusharkh et al. 2021, Abusharkh et al. 2022). Furthermore, AC tissues are subject to chemical stresses including hypoxia (Lafont 2010). Compared to other body tissues, AC has least cell density (Wong and Carter 2003). The tissue gains its white color from deprivation of blood (Amr et al. 2020; Mallah et al. 2021). Finally, the tissue is devoid of nerves and lymph nodes (Fox et al. 2009).

When injured, the tissue can't repair itself (Fox et al. 2009) resulting in osteoarthritis (OA). OA is the most common joint disease and is characterized by pain, inflammation, swelling of the joint, bone remodeling and sclerosis, cartilage breakdown, ligament dysfunction, synovial hypertrophy, muscle atrophy and disability (Cui et al. 2020). The probability of developing OA increases with systemic factors such as age, race, genetics and local factors such as obesity, sex and injury (Zhang and Jordan 2010). OA is a global pandemic with approximately 654.1 million individuals living with knee OA in 2020 worldwide (Cui et al. 2020; Jacobs 2021). OA imposes a huge socioeconomical burden on the society costing more than $185.5 billion annually in the U.S. (Bhosale 2008). There currently exists no therapies for OA. The disease is largely managed using uptake of oral non-steroidal anti-inflammatory drugs (NSAIDs) to kill the pain, anti-inflammatory or hyaluronic acid injections, topical medications, physical therapy, weight loss, use of structural support devices or braces, use of comparative alternative medicinal drugs or nutraceuticals and eventually, a total knee replacement (TKR) surgery is needed (Chen et al. 2017; Amr et al. 2020; Mallah et al. 2021). A TKR takes place every minute in the US (Shmerling 2018).

The lack of disease modifying drugs for OA calls for alternatives. Regenerative medicine approaches can provide a platform through which personalized AC tissues can be engineered. Tissue engineering refers to forming mimetic tissues to these present *in vivo* in structure, properties and function. That is achieved through enabling cells to proliferate and grow on a scaffold with the use of growth factors in a bioreactor that mimics the *in vivo* environment of the tissue to be replaced. When AC tissue's formation is concerned, many complex parameters come into play. To address them briefly, in the text below, these factors are divided into cells, scaffolds, growth factors, bioreactors, and characterizing resulting tissues.

Cells

When cells are concerned, one would want them to be easily obtained in high yields, possess hyaline cartilage phenotype or can commit to it, do not elicit immune responses, and do not dedifferentiate *in vitro.* When the criteria above are considered, autologous chondrocytes native to the AC of the patient represents the most desired cell type to use (Hubka et al. 2014). However, isolating chondrocytes from a healthy joint in the body results in a local site morbidity. In comparison, chondrocytes isolated from a diseased joint are often stressed and tend to dedifferentiate *in vitro*; resulting in fibrillated cartilage growth that is mechanically inferior to AC (Dehne et al. 2009). Other sources of cells including adipose derived stem cells (ADSCs) (Hamid et al. 2018), bone -marrow mesenchymal stem cells (BMSCs)(Hubka et al. 2014), or induced pluripotent stem cells (iPSCs) (Diederichs et al. 2019) provide alternative options. When compared, ADSCs can be obtained in higher yields than BMSCs while the latter differentiate into chondrocytes at a better rate (Hamid et al. 2018, Khurshid et al. 2018). The use of iPSCs comes with ethical concerns to be addressed. Irrespective of the type of stem cell to be used, the use of specific growth factors to induce chondrogenic differentiation such as transforming growth factor beta (TGF-β) is needed (Bian et al. 2011; Nazempour et al. 2016). However, TGF-β is also a common inducing factor of osteogenesis; making it extremely hard to control the fate of stem cells (Chen et al. 2012). Finally, co-cultures of chondrocytes and stem cells have also been proposed with the idea that chondrocytes assess stem cells in committing a chondrogenic lineage while stem cells help chondrocytes in proliferating (Acharya et al. 2012; Abusharkh et al. 2021). The ratio at which both cell types are to be mixed at is yet to be determined.

Scaffolds

Equally important to the choice of cells is the choice and design of a scaffold to which cells grow on. Scaffolds should be biocompatible such as they do not elicit foreign immune responses, biodegradable/bioresorbable to be replaced by ECM formed upon implantation, porous to allow for diffusion of nutrients, mechanically sound to support cellular proliferation and differentiation to produce ECM, have appropriate chemistry to enable cell adhesion and growth, and not cytotoxic (Bertrand and Hellmich 2009; Amr et al. 2021). Scaffolds used in AC tissue engineering can be divided into natural and synthetic scaffolds. Natural scaffolds include decellularized ECM, collagen, fibrin, agarose, gelatin, hyaluronan, and silk fibers (Awad et al. 2004). Synthetic scaffolds includes Poly(glycolic acid), Poly(lactic acid), Poly(ethylene glycol), Poly(vinyl alcohol), and copolymers (Bosworth and Downes 2010; Luczynski et al. 2013). When compared to natu-

ral scaffolds, synthetic scaffolds offer reproducibility and controllable superior mechanical properties, but usually lack cell-specific bioactivities such as cell adhesion, migration and biodegradation. Natural polymers on the other hand provide better cell adhesion and degradation but usually lack sound mechanical properties and processability (Mouw et al. 2005). Irrespective of the material of scaffold to be used, the scaffold properties need to be tailored to enable the engineering of AC tissues that are multilayered (Woodfield et al. 2002). With that in mind, many studies attempted to vary the mechanical and chemical proporties of scaffolds to enable the growth of AC tissues that are zonal (Steele et al. 2014; Amr et al. 2022). Recent emphasis devoted to the potential of using emerging three-dimensional printing technologies to fabricate heterogeneous cell-laden scaffolds which are capable of supporting cellular growth to yield mimicking AC tissues (Amr et al. 2021). Despite efforts, a complex, multizonal, heterogenous, and multiphasic AC tissue does not exist. All existing engineered AC tissues available in the market lack to the ability recapitulate the complex structure and mechanical function of native AC and as such provide limited clinical benefits.

Growth Factors

Next, key to success in any tissue's formation is the choice of appropriate growth factors, nutrients and supplements (Ren et al. 2020). This is especially important when stem cells are to be guided to commit a chondrogenic lineage (Danisovic et al. 2012). Growth factors are molecules critical for different processes taking place during tissue formation, repair and regeneration (Ren et al. 2020). The supplementation of growth factors to enable cellular growth is an art that is not well investigated in the literature. Most studies utilize a cocktail of desired growth factors and supplement cells with it on a frequently basis. While this is the norm practice, many questions critical to utilizing growth factors in optimized fashion are yet to be addressed. Example questions include: 1) should different growth factors be supplemented at different time points during culture?; 2) should different phenotypes of cells be complemented with different growth factors?; 3) should growth factors be augmented additively or sequentially to culture?; 4) What concentrations of growth factors should be used?; 5) should the concentration of growth factors be changed as a function of time of culture?; and 6) Do growth factors work synergistically or not? In summary, determining the frequency, duration, concentration and temporal times at which cells are to be fed by given growth factors are vital to obtaining desired outcomes for AC tissue engineering and largely unexplored.

Example commonly used growth factors in AC tissue engineering include TGF-β1, Bone morphogenetic proteins (BMP), insulin-like growth factor (IGF-1, FGF-2), non-essential amino acids, L-Proline, dexamethasone, ascorbate, and sodium pyruvate (Trippel 1995; Nazempour et al. 2016). In addition to common growth factors, 45% of OA patients utilize off-shelf medications in hopes for pain relief (Amr et al. 2020, Mallah et al. 2021; Abusharkh et al. 2022). These come in the form of nutraceuticals which are food-derived additives that have a pharmaceutical value. The Orthopaedic Research Society (ORS) recommends patients to try a nutraceutical for two months and if felt benefits, use should be continued and vice versa if no benefits were attained. Available nutraceuticals for uptake by OA patients are not US Food and Drug Administration (FDA) approved. Validating the efficacy of nutraceuticals in reducing inflammation associated with OA and promoting chondrogenesis is the responsibility of the scientific community.

Bioreactors

To successfully engineer an AC tissue, the cells and the scaffolds have to be housed in an environment that simulates that present *in vivo*. This is often done in a bioreactor. Bioreactors should enable controlled environments, be scalable and allow for high throughput experiments or screening for drugs to be carried out (Fu et al. 2021). For example, to engineer an AC tissue capable of replacing a damaged AC tissue of the knee, a bioreactor that operates at 37 ^{o}C, 5% CO_2, 2% O_2 to represent hypoxia as well as capable of exposing cells to loads of oscillating compression, shear, and hydrostatic pressure often experienced in the knee will be ultimate. Designing a bioreactor that is able to capture the native environment of a joint for the variable OA phenotypes is an extreme challenge as each individual will experience different loads and environments from others. The ultimate goal will be to customize the design of bioreactors to suit personalized needs.

Bioreactors used in engineering AC tissues are divided into static and dynamic categories based on whether they enable mechanical stimuli to be introduced into the reactor or not (Martin et al. 2004). While several types of dynamic bioreactors have been developed for use in AC tissue engineering including spinner flasks, rotating wall vessels, perfusion, magnetic reactors, compression and stretching, membrane, and ultrasonic bioreactors (Cicek 2003, Nazempour et al. 2016; Nazempour et al. 2017; Abusharkh et al. 2021;

Abusharkh et al. 2022), none of the bioreactors that exist in the market allow for the native AC environment to be fully captured.

Characterization of Resulting AC Engineered Tissue

Once a tissue has been engineered, it is critical to assess how does it mimic the native tissue in structure, propoerties and function and is it suitable for *in vivo* implantation (Cohen et al. 1998; Bhosale and Richardson 2008). The structure of the AC is complex as described earlier. A successful engineered tissue should capture its zonal complexity to enable appropriate desired functions. The main two functions of AC are to provide lubrication as joints move and to withstand load in certain joints such as the knee (Becerra et al. 2010). To check if the engineered AC tissue mimics a native AC in structure, histological staining for cell distribution, the key markers of AC (glycosaminoglycans (GAGs) and collagen formation, density and distributions) are carried out using an array of microscopic investigations (confocal microscopy, fluorescence microscopy, and scanning electron microscopy, atomic force microscopy (AFM))(Amr et al. 2022). Immunohistochemistry is also used to track the expression of key markers desired in AC such as collagen II (Amr et al. 2022). AFM's, nanoindentation and other tribological techniques are also frequently used to check the lubrication capacity and the mechanical integrity of the AC tissue (Iscru et al. 2008; Lee et al. 2010; Sakai et al. 2012; Amr et al. 2022). Success in engineering the tissue is marked by mimicking the lubrication and zonal properties of the native healthy tissues. Furthermore, engineered tissues are characterized for the kinetics of degradation. Ideally, the degradation of the tissue should match the rate of tissue's formation and integration *in vivo* (Akhtar et al. 2017). Furthermore, quantitative real time polymerase chain reaction (PCR) is commonly used to assess the expression of key markers (desired or to be avoided) upon tissue's growth. Commonly, PCR is used to assess the expression of genes responsible for coding for aggrecan, collagen II, lubricin, SRY-Box Transcription Factor 9 (SOX9), and assess markers responsible for inflammation, differentiation of stem cells into other lineages such as osteocytes and adipocytes (Amr et al. 2020; Mallah et al. 2021). Finally, experiments using animal models that aim at assessing the ability of an engineered tissue to be integrated within the injured AC *in vivo* are needed (Olivos-Meza et al. 2017).

The Outlook of Articular Cartilage Tissue Engineering

In summary, while tissue engineering approaches of AC are promising, realizing a functional AC tissue *in vitro* that is mimetic of the native AC tissues in structure, function and properties is yet to be apprehended. This is largely influenced by the complex interplay between all the parameters that are to be optimized to engineer such tissue. Scientists and researchers have a way to go to address key questions that range all the way from what types of cells to be used to how to design appropriate scaffolds that match the anatomy of the AC tissue. Bioreactors that mimic *in vivo* conditions and allow for high throughput and scaled up production of tissues are yet to be designed. Animal models that represent the human tissues closely are largely lacking. Small animals lack sufficient tissues to be explored and large animals are costly prohibitive. Efforts have to be merged in order for the scientific community to move forward and bring us closer to mimetic AC tissue of native AC. This is much needed to offer mobility and life quality to approximately one third of the World's population above the age of 40 who are suffering or will suffer from OA.

Glossary of Acronyms Used in the Review

Adipose derived stem cells (ADSCs)
Articular cartilage (AC)
Atomic force microscopy (AFM)
Bone-marrow mesenchymal stem cells (BMSCs)
Bone morphogenetic proteins (BMP)
Deep zone (DZ)
Extracellular matrix (ECM)
Glycosaminoglycans (GAGs)
Induced pluripotent stem cells (iPSCs)
Insulin-like growth factor (IGF)
Intermediate zone (IMZ)
Non-steroidal anti-inflammatory drugs (NSAIDs)
Oscillating hydrostatic pressures (OHP)
Orthopaedic Research Society (ORS)
Osteoarthritis (OA)
Polymerase chain reaction (PCR)
SRY-Box Transcription Factor 9 (SOX9)
Superficial zone (SZ)
Surface zone protein (SZP)
Total knee replacement (TKR)
Transforming growth factor beta (TGF-β)
US Food and Drug Administration (US-FDA)

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