Tissue Engineered Cartilage Constructs for Cartilage Regeneration

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Abstract

Cartilage is a unique tissue with structures specifically designed for its intended functions. It is a vascular, aneural and alymphatics, thus severely limiting its self repairing capabilities. Current treatment options are insufficient in treatment of cartilage diseases. Therefore, researchers are exploring the use of tissue engineered cartilage constructs to treat cartilage diseases and for cartilage regeneration. However, a good cartilage construct should have some prerequisite requirements, and physical architectures such as pore size, distribution, geometry, pore interconnectivity and porosity can determine feasibility of construct. In addition, advancement in biomaterials development and biofabrication techniques have enabled biofabrication of more complex constructs that are able to better mimic native cartilages. Current ideal physical architectures of cartilage constructs, commonly used biomaterials and synthetic materials and several biofabrication techniques are reviewed in this review. In addition, current problems faced in cartilage biofabricated are also highlighted in this review.

ABBREVIATIONS

3D: 3-dimensional; ECM: Extracellular Matrix; Gel MA: Gelatin Methacryloyl

INTRODUCTION

Cartilage is a specially designed tissue specially meant for withstanding frictional forces, compression stress and protection of underlying structures [1,2]. Therefore, it has a highly specific and has limited nerves, blood vessels and lymphatic supplies. Owing to these factors, any damage to these cartilaginous tissues is a clinical problem as their self regeneration is severely limited and impaired when compared to other tissues [3]. Due to their inert metabolic activities, healing only occurs when the damage reaches the subchondral bone, which then triggers a series of inflammation process and invoking fibro vascular repairing processes such as releasing of transforming growth factor β and bone morphogenetic proteins to stimulate stimulation and migration of mesenchymal stem cells for repair [4,5]. However, it is to note that self regenerated cartilage do not possess similar functional properties as native cartilage due to additional surface fibrillation during this repair process which results in a composition with higher amounts of collagen I [6].

Current treatment pedagogy usually depends on the severity of conditions. Pharmacotherapy and physical therapy are the preferred treatments for patients with mild conditions. Non-cell regenerative therapies such as microfracture and mosaicoasty are the next line of treatment if the prior fails to achieve the intended expectations. In addition, these treatments have highly unpredictable outcomes coupled with inevitable limitations such as lack of donor sites and quality of grafts.

These limitations prompted for the emergence of cell based regenerative therapies such as autologous chondrocyte implantations and other similar implantation techniques. However, these approaches do not have much superiority over non-cell regenerative therapies and they are generally costly and non-cost effective [7,8]. The main limitation of microfracture, mosaicoasty and autologous chondrocyte implantation often led to formation of a fibrocartilage instead of a collagen type 2 cartilages, which is physically inferior to native cartilage [9]. Inability to prevent further degeneration and difficulty in enhancing regeneration has brought scientists and physicians of various specialties together to figure out and to solve the problems encompassing cartilage regeneration [10].

TISSUE ENGINEERED CARTILAGE CONSTRUCTS AS AN APPROACH FOR CARTILAGE REGENERATION

Since its inclusion into regenerative medicine, tissue engineered cartilage constructs has been widely anticipated to revolutionize cartilage regeneration due to its ability to allow specific distribution of different cells in supporting biomaterials with high resolutions that are able to closely mimic the
microstructure of native cartilages [11]. Precise control and fine tuning of microstructure, mechanical and physical properties of scaffolds by adjusting biomaterials concentration and modification capabilities, sources of cells and biological signaling factors are some of the factors that could potentially affect the results of our intended constructs [12,13].

PHYSICAL ARCHITECTURES OF CONSTRUCTS FOR CARTILAGE REGENERATION

A good construct should have controlled biodegradability, good biocompatibility, and ability to attach to wound sites and be able to regulate cellular activities. In addition, a good cartilage construct must be able to weight bear in a harsh microenvironment. Therefore, the construct must have similar mechanical properties as native cartilages. Tissue engineering allows us to constructs with precise microstructures and yet able to manipulate physical architectures of constructs. By manipulating physical architectures, we are able to influence biodegradability, cellular activities and mechanical properties of constructs. 3-dimensional (3D) scaffolds are reported to enhance cellular activities, cellular interactions and also promote production of extracellular matrix (ECM) as chondrocytes exist in a 3D microenvironment in cartilages [14,15].

Physical architectures such as pore size, distribution, geometry, pore interconnectivity and porosity are factors that would greatly affect and influence properties of constructs [16]. Pore sizes between 200 µm and 500 µm are found to be optimal in enhancing chondrocytes activities, with smaller pore sizes affecting differentiation and larger pore sizes affecting ECM production and formation. With this, scaffolds with increasing or decreasing pore sizes gradients have been biofabricated and are reported to promote both differentiation and ECM production [17,18]. Size and arrangement of fibers are also found to influence cellular activities. Fiber sizes of less than 100 µm stacked in a honey-combed arrangement is found to up-regulate cellular activities as such architectures provide three or more contact points for cells to adhere to [19,20]. Scaffolds with higher porosity and pore interconnectivity are found to allow efficient and even diffusion of nutrients which in turn promote cellular activities and cartilage regeneration [21]. However, there is a need to strike a balance between porosity, pore interconnectivity; mechanical property and cellular proliferation. Increasing the porosity would decrease the mechanical strength, a factor which is essential for cartilage scaffolds. On the other hand, low porosity causes uneven growth of tissues due to improper diffusion of nutrients [22,23]. In addition, poor pore interconnectivity is found to cause uneven cell growth, with cells reported to be growing only on the peripheral side of the scaffold [24]. For cartilage constructs, it is important to factor the overall distribution of pore architectures so as to better mimic the different zonal structural properties of native cartilages.

Degradation rates of scaffolds must be constant and proportional to ECM production to ensure adequate structural support throughout the healing process. Uncontrolled degradation rates can impact the quality of tissue formation and invoke unnecessary immune responses [25]. Degradation affects the mechanical, structural and surface properties of the scaffolds and thus determines the overall feasibility of constructs. Controlled degradation is required to allow integration of ECM with surrounding tissues [26,27]. Also, by-products released during degradation should be non-toxic and should not illicit any unnecessary immune responses.

In addition to design of constructs, application of different mechanical stimulation strategies such as dynamic deformational loading or sliding loading has been tested and proven to be able to maintain chondrocyte structure and function and to enhance mechanical and physical properties of the constructs [28]. Mechanically generated signals seem to have a positive role in the differentiation and maturation into chondrogenic phenotype [29,30]. Similarly, mechanical factors play a huge role in the development of in-vivo articular cartilage. A recent review has shown that mechanical compression of constructs with an optimal parameter of 1 Hz loading frequency, loading amplitude of 5-10% strain and duration of at least 7 days is able to better mimic native conditions and is effective in promoting non-hypertrophic chondrogenesis [31].

SCAFFOLD MATERIALS FOR CARTILAGE TISSUE ENGINEERED CONSTRUCTS

Material suitability can also influence properties of constructs. The chosen material must have suitable mechanical properties and yet able to provide a good microenvironment for cells to thrive in. Currently, numerous natural biopolymers and synthetic biomaterials have been widely investigated and some have been proved to be potential applicants for cartilage tissue engineering [32]. A summary of commonly used biopolymers and synthetic biomaterials for cartilage tissue engineered constructs is listed in Table 1.

Natural biopolymers consist of protein, polysaccharides or a mixture of both. One of their main advantages is that natural polymers have highly hydrated 3D fibrous structure in which it highly mimics the native microenvironment or ECM that cells commonly resides in. With this, cells are able to interact specifically which then up-regulates cellular activities [33]. Biopolymer hydrogels generally allow even cell distribution and high cell encapsulation but they tend to have weaker mechanical properties when compared to synthetic materials and hydrogels do not allow biofabrication of scaffolds with specific physical architectures. In addition, there is a problem of reproducibility with hydrogel scaffolds. Therefore, pre and post modification strategies such as additional cross linking, surface modification and modification of biopolymer bonds have been developed to improve the mechanical properties of hydrogels [34-37].

On the other hand, even though synthetic biomaterials have superior mechanical properties and calls for easier biofabrication and greater control over scaffold designs, they usually possess a rather inert surface or component that limit or inhibit the biological activities of cells [38]. Furthermore, there is presence of foreign body giant cells response reported during implantations [39]. Even for biocompatible synthetic scaffolds that do not induce any inflammatory response, their degraded by-products are commonly acidic which would affect the pH of local environment. Especially so for chondrocytes, whom are very sensitive to pH changes [40].
BIOFABRICATION TECHNIQUES

Cells, scaffolds and growth factors are key components of biofabrication or otherwise termed as the triads of tissue engineering [41]. Traditional processes such as particulate leaching, electro spinning and phase separation techniques generally harness the thermal, mechanical, physical and chemical properties of the materials in biofabricating a scaffold [42,43]. Successful biofabrication of biomimetic cartilage structures with these techniques have been reported [44-47]. However, these methods provide us with very limited control over design of constructs. Textile scaffolds are another type of scaffolds biofabricated from nanofibers which can be woven, braided or knitted to meet specific requirements, of which knitted fibers are commonly used to biofabricate cartilage scaffolds due to its achievable mechanical property and nontoxic biodegradability [48,49]. Recent technological advances have brought about additive manufacturing technology, a kind of automated manufacturing that builds an object in a layer-by-layer manner. Successful biofabrication of 3D cartilage scaffolds have been reported to be biofabricated via means of 3D printing technology such as stereo lithography, selective laser sintering, fused deposition modeling and digital light process [50-52]. These additive manufacturing have caused a paradigm shift in cartilage biofabrication as they allow us to construct complex scaffolds with high resolutions and specificity, yet bringing about reproducibility and having a wider range of material selections. With current technologies, we are able to incorporate living cells into our biomaterials. Hydrogels are gaining popularity in the recent years as they can be extruded while supporting a wide variety of viable cells, growth factors and genetic materials [53]. However, hydrogels have low mechanical strength. Therefore, researchers have tried to develop strategies to improve mechanical properties of hydrogels such as additional functionalization of gelatin with Methacryloyl groups (GelMA)which creates irreversible covalent kinetic chain cross-linking polymers when exposed to UV light [34]. In addition, hydrogels with mixtures of multiple polymers stacked in independent networks or integrated networks are also reported to improve mechanical properties of hydrogels and mimic the design of native cartilage. These hydrogels exhibit higher mechanical properties and superior tissue integration properties when compared to traditional scaffolds [54,55]. Currently, multi-layered construct seems to be most promising for cartilage constructs. This type of biofabrication aims to mimic the exact zonal structures and properties of native cartilages with individual desired functions. Till date, tri-layered scaffolds have been biofabricated and are shown to have similar structures as native cartilages[56].

CONCLUSION & FUTURE PERSPECTIVE

There have been significant advances in biofabrication techniques and materials development. Biofabrication for cartilage regeneration is considered to be one of the most complex specialties due to its complex structure and functions and current
results seem promising [57-65]. However, some problems remain to be solved. Firstly, there are no common evaluation criteria for assessment of constructs feasibility. Therefore, there is a need for a standardized evaluation methodology to allow evaluation of constructs. Secondly, current literatures show that there are vast differences in results between in vivo and in vitro studies. Therefore, there is a need for more in vivo studies for more validities results [66-70].

Lastly, biofabricated constructs are still short of resembling a complete native cartilage. Currently, researchers are trying to integrate different techniques and materials to biofabricate scaffolds with optimal parameters. With the current integration of additive manufacturing and discovery of novel biomaterials, biofabricated cartilage construct for cartilage regeneration might not be too far a goal and it certainly can be explored as a promising treatment methodology for cartilage diseases in the future [71-73].

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