A Review Study: Using Stem Cells in Cartilage Regeneration and Tissue Engineering

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ABSTRACT

Articular cartilage, the load-bearing tissue of the joint, has limited repair and regeneration ability. The scarcity of treatment modalities for large chondral defects has motivated researchers to engineer cartilage tissue constructs that can meet the functional demands of this tissue in vivo. Cartilage tissue engineering requires 3 components: cells, scaffold, and environment. Owning to their easy isolation, expansion, and multilineage differentiation, adult stem cells, specifically multipotential mesenchymal stem cells, are considered the proper candidate for tissue engineering. Successful outcome of cell-based cartilage tissue engineering ultimately depends on the proper differentiation of stem cells into chondrocytes and assembly of the appropriate cartilaginous matrix to achieve the load-bearing capabilities of the natural articular cartilage. Furthermore, multiple parameters such as growth factors, signaling molecules, and physical conditions must be considered. Adult mesenchymal stem-cell-based tissue engineering is a promising technology for creating a transplantable cartilage replacement to improve joint function.

1. Introduction

Osteoarthritis (OA) and related degenerative joint diseases result in a heavy burden to the health system and suffer millions of people annually. Once damaged, articular cartilage lacks the ability to properly repair and regenerate itself. In this regard, various surgical interventions and procedures are used to relieve pain and restore joint function. Among these surgical interventions, relatively minimal procedures include lavage, shaving, laser abrasion, and microfracture of the subchondral bone. More extensive surgical procedures comprise autogenic or allogenic osteochondral

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transplantation, autologous perichondral and periosteal grafts, and autologous chondrocyte implantation [1].

Today, the drastic operation of total joint replacement is the treatment of choice for extensive lesions or joint destruction. Total joint replacement, which has its inherent risks and a finite life expectancy, although successful to varying degrees in treating articular defects, is only effective for chondral defects of limited sizes [2]. Furthermore, availability of tissue graft material and donor site morbidity have remained the major challenges for this procedure. Therefore, we still need improved cartilage-repair modalities and suitable engineered-tissue constructs for transplantation. Using chondrocytes in cartilage tissue engineering is restricted due to the limited availability of these cells and their intrinsic tendency to lose their phenotype during expansion. Adult stem cells, however, because of their easy isolation, capacity to self-replicate, and ability to differentiate along multiple connective-tissue lineages, have become the cell type of choice for engineering of cartilage tissue.

In this review article, the current knowledge of cartilage tissue engineering and regeneration are discussed and summarized [3]. We first review the structure and function of cartilage, as a foundation to guide cartilage tissue engineering and regeneration. This section is followed by describing the critical components for cartilage tissue engineering and discussing the advantages of using adult stem cells for this process. Then, important factors and signaling molecules for chondrogenic differentiation of stem cells are reviewed. Finally some examples of cartilage regeneration and tissue engineering are presented to highlight the current knowledge and limitations of this process, and ongoing research in this area.

2. Structure and Functional Properties of Cartilage Tissue

A clear understanding of the function and structure of articular cartilage is essential for engineering rationally-designed, biomimetic cartilage constructs. The most important function of articular cartilage is supporting large loads during motion [4]. This ability is attributed to its highly organized extracellular matrix (ECM). Chondrocytes, the only cell type resident in articular cartilage, are responsible for the production, organization, and maintenance of the articular cartilage ECM and are, therefore, ultimately responsible for the integrity of the cartilage. Articular cartilage ECM contains a fluid part of water (68%-85% of total weight) and a solid ECM organic proteins part. Interstitial fluid support can account for more than 90% of the load-bearing capacity of the joint. The articular cartilage ECM comprises 3 classes of proteins: collagens (60%–86% of dry weight), proteoglycans (15%–40% of dry weight), and other noncollagenous proteins, including link proteins, fibronectin, cartilage oligomeric matrix proteins, and the smaller proteoglycans, biglycan, decorin and fibromodulin.

The ability of articular cartilage to resist compression is primarily due to proteoglycan aggregates. The high density of the fixed negative charges of the sulfated glycosaminoglycan chains of proteoglycans draws water into cartilage, resulting in high osmotic pressure, which is restrained by the collagen fiber network, thus providing the compressive behavior of cartilage. The tensile resilience and strength of the cartilage, on the other hand, is imparted primarily by the network of type II collagen fibers. It is also important for the dynamic and functional properties of cartilage [3].

The biomechanical properties of articular cartilage thus depend on the maintenance of its high proteoglycan and collagen contents within the matrix, which comprise the current standards for measuring the functionality of engineered cartilage constructs [5].

3. Cartilage Tissue Engineering

The emerging field of tissue engineering combines the principles of engineering, biology, and medicine for the creation of functional tissue and cell substitutes [6]. In this regard, there are 2 basic approaches: ex vivo tissue engineering, in which the tissue is generated entirely in vitro with full functionality before transplantation; and in vivo tissue engineering, in which the construct is implanted with or without prior partial in vitro cultivation and allowed to mature in vivo for tissue repair and regeneration. In both approaches, 3 components critically influence the outcome of a tissue-engineered construct; scaffold, responsive cells, and environment [7,8].

Scaffold

The scaffold shapes the form and guides the orderly differentiation and development of the replacement tissue. It should be biocompatible and noncytotoxic too. In addition, its biomaterials should preferably have a balanced bioresorbable and biodegradable rate matching the rate of new ECM formation. It should be sufficiently porous to permit cell penetration and also be permeable to facilitate nutrient delivery, waste removal, and gas exchange, while maintaining adequate biomechanical strength. Moreover, an ideal scaffold should be chondroinductive and chondroconduc-

tive and guide the cells to differentiate along the right lineage [9].

The various materials under investigation for use in cartilage tissue engineering have been reviewed extensively [5]. So far, none of the currently available scaffolds fulfills all of the requirements, and highly variable effects are observed during growth, differentiation, and maintenance of the cells. Thus, their ability to differentiate and produce cartilage ECM is variable as well [10].

Responsive cells

Long-term stability and survival of the engineered tissue construct is critically dependent on the proper matrix structure and assembly, similar to that of native ECM. Thus, although scaffolds have been used for cartilage repair, in most cases, cartilage repair is improved with cell-seeded scaffolds [8]. In principle, fully differentiated chondrocytes are the ideal cell source [11]. Carticel® (Genzyme, Cambridge, MA), the only FDA-approved cell-based cartilage repair product in the USA, involves the harvesting and dissociation of cartilage from a less weight-bearing site, followed by in vitro expansion of the isolated chondrocytes, and their implantation into the damaged site. Although good to excellent patient's satisfaction has been reported [9], the long-term benefits of this procedure are debatable [10].

Additional drawbacks in using chondrocytes for cartilage tissue engineering include limited availability of source material, potential donor site morbidity (complications of donor site healing), and poor replicative capacity of chondrocytes that show rapid differentiation in monolayer expansion. Stem cells, defined by their ability to undergo self-renewal, replication, and differentiation upon stimulation, are emerging as a more attractive alternative for cartilage tissue engineering. With their pluripotential to differentiate into multiple cell lineages, including connective-tissue cells [12-13], embryonic stem cells are potential candidates for cartilage tissue engineering.

The difficulty of directing their differentiation along a specific lineage, their potential to form teratomas and associated legal, ethical, and political issues; however, hampered the development of embryonic stem cells for use in tissue engineering. On the other hand, adult stem cells sidestep the potential sociological issues. Because of their ease of isolation and expansion [14], also their multipotential differentiation into cells of connective tissue lineages, they are increasingly being considered as a promising alternative to differentiated chondrocytes for use in cell-based cartilage repair strategies, as discussed later [15].

4. Adult Stem Cells

Mesenchymal stem cells (MSCs) are uncommitted, nonhematopoietic progenitor cells characterized by their ability (in response to appropriate stimuli [16-18]) to differentiate along various mesenchymal lineages. Originally isolated from bone marrow [19], MSCs are now known to reside in many adult tissues like adipose tissue, periosteum, synovial membrane, muscle, trabecular bone, articular cartilage, and deciduous teeth (Figure 1) [20]. Although the exact tissue origin and function of these various adult stem cells are still unclear [21], it is speculated that they participate in adult tissue remodeling and repair. MSCs can differentiate into different lineages (Figure 1) upon stimulation by specific signaling molecules [22].

The potential for chondrogenesis, adipose tissue showed that contained the highest number of hMSCs to change into cartilage tissue. [23]. These cells can be obtained by a less invasive procedure than is required to collect human MSCs from bone marrow. Adipose-tissue-derived MSCs, however, seem to have an inferior chondrogenic potential compared with bone-marrow-derived human MSCs. In addition, comparison of human MSCs derived from bone marrow [24], periosteum, synovium, skeletal muscle, and adipose tissue revealed that synovium-derived human MSCs exhibited the highest capacity for chondrogenesis, followed by bone-marrow and periosteum-derived human MSCs. To complicate more the inherent difficulties of choosing an appropriate cell source for tissue-engineering techniques is the issue of different responses of MSCs to a multitude of factors that affect MSC differentiation, as will discuss below [25-27].

5. Factors Influencing Adult Stem Cell Differentiation

Current attempts of in vitro chondrogenic differentiation of MSCs are based on the studies on

the developmental cartilage formation and cartilage homeostasis and function, which involve soluble factors, mechanical stimulation, and ECM components [28]. The standard systems to stimulate chondrogenic differentiation in vitro consist of growth factor supplementation, high density micromass, pellet culture, or 3D cultures of MSCs in scaffolds. Transforming growth factor (TGF)-β superfamily members (TGF-βs, bone mor-

phogenetic proteins [BMPs], and growth differentiation factors [GDFs]), as well as insulin-like growth factors, Wnt proteins, and fibroblast growth factors (FGFs) are functionally implicated in mesenchymal chondrogenesis (Figure 1) [29]. In vitro successful chondrogenic differentiation is characterized by upregulation and production of cartilage-specific matrix components, including type II collagen and aggrecan, as well as cartilaginous histology. TGF-β1, TGF-β2, and TGF-β3 have all been shown to induce in vitro chondrogenic differentiation of MSCs [30].

With regard to human MSCs, TGF-β2 and TGF-β3 were superior to TGF-β1 in promoting chondrogenesis [31]. Bone morphogenic proteins (BMPs), known for their involvement in cartilage formation, act alone or in combination with other growth factors to induce or enhance MSCs chondrogenic differentiation, albeit in a manner that is dependent on species and tissue source. For example, any kind of BMP2, BMP4, and BMP6, combined with TGF-β3, induces the chondrogenic phenotype in cultured bone-marrow-derived human MSC pellets, with BMP2 being the most effective one [32]. While TGF-β3 alone was sufficient for chondrogenesis in human MSCs derived from bone marrow [33], for synovium-derived human MSCs, it was necessary to combine BMP2 with TGF-β3 and dexamethasone for optimal chondrogenic differentiation [34]. The chondrogenic ability of BMP2 is well-established, however, it has also been implicated in terminal chondrocyte differentiation. For example, the mouse mesenchymal cell line C3H10T1/2 undergoes chondrogenesis when treated with BMP2 or TGF-β1 as high-density micromass cultures [35-36] but undergoes chondrogenesis followed by osteogenesis when treated with BMP2 and BMP7 [35].

By contrast, Schmitt et al. [37] showed that BMP2 alone or in combination with TGF-β3 induced chondrogenesis, but not osteogenesis, in human MSC pellet cultures. Interestingly, BMP13 does not promote terminal chondrocyte differentiation as BMP2 does. Another BMP family member, GDF5 (also known as cartilage-

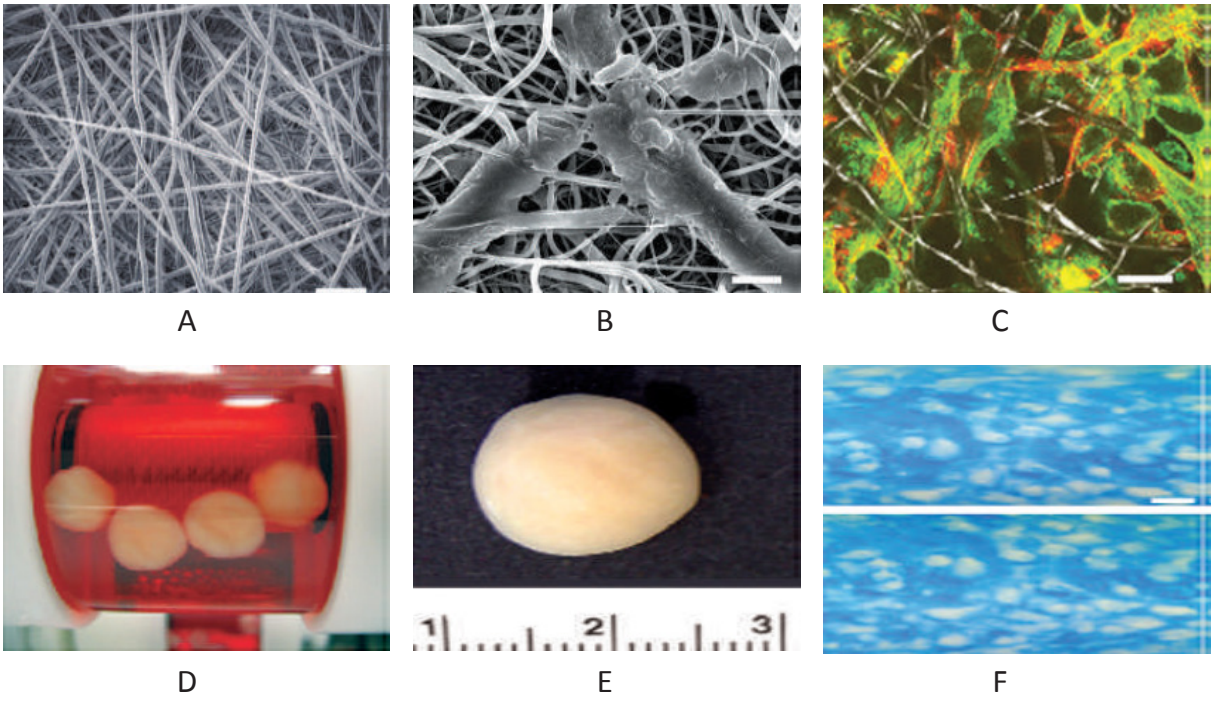

Figure 1. Cartilage tissue engineering using biodegradable polymeric nanofibrous scaffold.
(A) Ultrastructure of nanofibers. (B) Seeded mesenchymal stem cells (arrow) accommodated within the nanofibrous matrix. (C) Seeded chondrocytes interspersed within nanofibrous scaffold visualized by confocal laser scanning microscopy; the cytoskeleton was stained (red=actin; green=tubulin). (D) Preparation of 3D, tissue-engineered cartilage constructs using a horizontal axis rotating bioreactor. (E) Macroscopic view of engineered cartilage, showing its smooth surface and substantial size. Scale is in cm. (F) Alcian-blue-stained histology of engineered cartilage construct generated using human mesenchymal stem cells, showing abundant cartilaginous matrix surrounding large chondrocytes. Apparent zonal cell morphologies are visible (brackets): a top layer of large, flattened cells (*), a matrix-rich middle zone (**), and a deep zone of small, flat cells (***). Bar = 10 µm. (A) and (F) are reprinted from Biomaterials, 26, Li et al., A 3D nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells, 599–609, copyright (2005), with permission from Elsevier [43] (B), (D), and (E) are from WJ Li and RS Tuan, unpublished data.

derived morphogenetic protein-1) is critical for mesenchymal aggregation and differentiation [38]. In the presence of TGF-β3, GDF5-transfected rabbit MSCs enhance in vivo repair of full-thickness articular cartilage defects in rabbits. The promitotic activity of the FGFs has also been exploited for cell expansion purposes. Surprisingly, FGF2-supplemented human MSCs proliferate more rapidly, and exhibite greater chondrogenic potential, than untreated controls [39].

Wnt proteins have recently been implicated in the progression of rheumatoid arthritis and OA. In the developing limb, members of the Wnt family are expressed in spatiotemporally specific profiles, and have been shown to be critical in regulating the timing, extent, and pattern of cartilage formation. Canonical Wnts have been shown to be involved in MSC differentiation [40], and Wnt signaling in chondrogenesis has been shown to crosstalk with TGF-β signaling. Evidence suggests that both Wnt signaling pathways (canonical and noncanonical) also actively crosstalk in the regulation of MSC differentiation (D Baksh and RS Tuan, unpublished observations) [41]. Clearly, the optimal combination and temporal administration of growth factors, along with other appropriate environmental stimuli, is necessary for the stable differentiation of MSCs into chondrocytes and successful cartilage repair [42].

6. Discussion

Cartilage tissue engineering using adult stem cells, although still at a developmental stage with some hurdles, is promising to bring hope and eventually end the patients' and physicians' quest for functional cartilage tissue replacements. The outcome of this approach will critically depend on our understanding of fundamental stem cell biology and then our ability to develop approaches to control and direct complete and stable chondrogenic differentiation. Relying merely on the detection of cartilage-specific molecules is not enough and markers or quantitative measurements of mature articular cartilage are also required. Furthermore, systematic investigations into the behavior of MSCs on engineered scaffolds under the correct environmental control are warranted, with outcomes measured against standardized parameters of cartilage function, such as mechanical properties or cartilage ECM composition.

The ideal targets in vivo scenario of cell-based cartilage repair are complete integration of the grafted construct with the host tissue and the long-term survival and maintenance of structural and mechanical integrity of the construct in the injured joint, which often presents an inflammatory environment that promotes degeneration, particularly in the case of rheumatoid arthritis. Immediate differentiation capability of adult MSCs, their easy isolation and expansion, coupled with our increasing knowledge of scaffolds and the environmental cues necessary for cartilage differentiation and maturation, strongly indicate that despite many challenges, these cells hold great potential for the development of functional cartilage replacements and amelioration of the deleterious effects of degenerative joint diseases [44].

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