Optimizing Stem Cell Engineering for Orthopaedic Applications

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Articular cartilage repair remains a significant challenge in orthopaedic surgery and this has led to increased interest in cell-based therapies for cartilage regeneration. Although autologous chondrocyte implantation has been used with reasonable success, there are several disadvantages associated with this technique, such as donor site availability and morbidity, dedifferentiation of the isolated chondrocytes during monolayer expansion, and poor integration of repair tissue into the native cartilage. This has led to a growing interest in the use of mesenchymal stem cells (MSCs) as an alternative cell source. MSCs are easy to isolate and are self-renewing, have a high expansion potential, and are capable of differentiating into several cell lineages, including chondrocytes and osteoblasts. Here, we highlight recent advances in the field of tissue engineering that seek to combine MSCs, biomaterial scaffolds, and chondrogenic signals (the so-called tissue engineering triad) to create functional cartilage tissue replacements.

Mesenchymal Stem Cell Source

MSCs have been isolated from a variety of human tissues, including, among others, bone marrow, synovium, and adipose tissue. Bone marrow stem cells (BMSCs) have been most extensively studied. However, BMSCs have been shown to express markers for hypertrophic chondrogenesis (eg, collagen type X, matrix metalloproteinase–13) that mineralize when exposed to osteogenic stimuli.[1] Adipose-derived stem cells (ADSCs) have emerged as an attractive alternative because of the ease of the isolation procedure and relative abundance of cells compared with BMSCs. Whereas most early comparative studies found BMSCs to have increased chondrogenic potential under identical culture conditions, it has now become clear that these are two distinct cell types that require specifically tailored chondrogenic signals. Furthermore, recent studies have shown that ADSCs can be expanded and can proliferate more rapidly and possess a stable, undifferentiated status and pluripotency, which decreases less than that of BMSCs.[2,3] Human infrapatellar fat pad contains abundant MSCs that can be easily harvested during arthroscopic procedures. Infrapatellar fat pad–derived stem cells isolated from osteoarthritic patients have been shown to be highly clonogenic and have demonstrated chondrogenesis similar to that of cells isolated from adult articular cartilage.[4]

Environmental Factors

One of the key challenges in tissue engineering using MSCs is to identify the optimum environmental conditions needed to develop functional cartilaginous grafts. Both biochemical and biomechanical cues can be combined to play synergistic roles to enhance chondrogenesis, achieve phenotypic stability, and generate grafts that approach the complex design of native cartilage.

Multiple growth factors, either alone or in combination, have been explored in an attempt to improve

the isolation and expansion of progenitor cells isolated from different tissues. The transforming growth factor (TGF)-β superfamily contains many that promote chondrogenesis including TGF-β1 and TGF-β3. The addition of soluble bone morphogenetic protein (BMP)-6 increases the expression of TGF-β receptor I and has been shown to enhance chondrogenesis of both BMSCs and ADSCs, although it appears particularly important for inducing robust differentiation of the latter.[4] Fibroblast growth factor–2 has been demonstrated to significantly lower the cell-doubling time of MSCs and to delay the loss of chondrogenic potential.[4]

Articular cartilage exists naturally in a low oxygen environment, and hypoxia appears to be an important factor for cartilage engineering. Expansion of MSCs at a low oxygen tension has been shown to increase cell yield and to reduce doubling times as well as promote chondrogenic differentiation of MSCs.[1] The functional properties of cartilaginous tissues engineered using fat pad–derived stem cells are also improved in a hypoxic environment.

With mechanical forces playing a key role in the development and maintenance of normal articular cartilage, mechanical stimulation has emerged as another strategy to optimize chondrogenesis and improve the functional properties of MSC-based constructs. It has now been well established that the timing of load initiation is crucial in determining functional outcomes and that this is a TGF-β3–mediated process: loading initiated before establishment of a chondrogenic phenotype reduces the mechanical properties of constructs, whereas loading initiated after a brief period of chondrogenesis and matrix elaboration dramatically improves the mechanical properties, but only when TGF-β3 levels are maintained.[5]

Scaffolds

The design and manufacture of scaffolds is another key element of cartilage tissue regeneration. Whereas classic tissue engineering has focused on using natural and synthetic biomaterials, recent work has focused on the development of biomimetic scaffolds that recapitulate the spatially varying mechanical properties and complex three-dimensional zonal architecture of native cartilage. Recent examples of such an approach include the work of Nguyen and colleagues,[6,7] who layered specific combinations of synthetic and natural biopolymers to create unique niches in a polyethylene glycol–based hydrogel. These niches then "directed" a single BMSC population to differentiate into zone-specific chondrocytes and organize into a complex tissue structure, leading to an increasing gradient of compressive modulus from the superficial to the deep zone. Others have used electrospun fibers embedded in a hydrogel to form a composite scaffold that mimics native extracellular structure.[8] A related approach developed in our laboratory involves modulating the gradients in biomechanical and biochemical signals within the developing tissue. By varying both the oxygen tension and mechanical environment through the depth of BMSCs seeded in hydrogels, we were able to induce cartilage-like zonal variations in extracellular matrix content in one cohesive construct.[9]

Manipulating Endogenous Stem Cells to Augment Cartilage Repair

An emerging approach that is receiving considerable attention in the field is the concept of using acellular constructs that deliver biologic cues to manipulate endogenous stem cells. Using an acellular bioscaffold created from composite poly-ε-caprolactone and hydroxyapatite infused with TGF-β3, Lee et al[10] were able to guide the homing and differentiation of endogenous stem cells to regenerate the entire surface of a rabbit proximal humeral head. Other studies have used bioinstructive scaffolds to facilitate osteochondral integration by incorporating TGF-β1 in the top layer and BMP-4 in the bottom layer to provide targeted chondrogenic and osteogenic differentiation of endogenous stem cells in a spatially distinct manner.[11] An alternative approach involving the use of an acellular nanofiber scaffold infused with chondroitin sulfate[8] was able to mimic the physical and biologic cues of native extracellular matrix, leading to the synthesis of type II collagen by endogenous cells. As these approaches are further developed, manipulation of endogenous stem cells may act as an adjunct or alternative approach for cell delivery in cartilage tissue regeneration.

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