International Society for Cellular Therapy ISCT

Cytotherapy (2004) Vol. 6, No. 6, 596–601

Taylor & Francis healthsciences

Mesenchymal stem cell-based cartilage tissue engineering: cells, scaffold and biology

L Song*, D Baksh* and RS Tuan

Cartilage Biology and Orthopedics Branch, National Institute of Arthritis, and Musculoskeletal and Skin Diseases, Department and Health and Human Services, NIH, Bethesda, Maryland, USA

Cartilage repair and regeneration by stem cell-based tissue engineering could be of enormous therapeutic and economic potential benefit for an aging population. However, to use stem cells effectively, their natural environment must be understood in order to expand them in vitro *without compromising their multilineage potential and their specific differentiation program. Collaboration between diverse academic disciplines and between research and regulatory government agencies and industry is crucial before cell-based cartilage tissue engineering can achieve its full therapeutic potential.*

Introduction

Damage to cartilage is of great clinical consequence given the lack of the tissue's intrinsic ability to heal. Cartilage damage may occur in a number of different ways, including congenital diseases, pediatric growth plate disorders, trauma-induced injuries, age-related degenerative joint diseases, osteoarthritis and rheumatoid arthritis. Due to the lack of blood supply, the subsequent wound-healing response, and the lack of the intrinsic ability of native chondrocytes to participate in regeneration at the injury site, damage to cartilage results in incomplete repair. As the degeneration progresses through the cartilage into the underlying bone, partial healing may be attained; however, the fibrous tissue formed is functionally inferior to native hyaline cartilage. The current treatment options (e.g. surgical intervention) to repair damaged articular cartilage are less than satisfactory, and rarely restore full function or return the damaged tissue to its normal biologic state. Tissue engineering, therefore, may offer great promise for the regeneration of diseased and damaged tissue such as cartilage. One of our laboratory's specific research initiatives is to develop cell-based tissue engineering strategies for the repair of articular cartilage degeneration.

Tissue engineering combines the principles of engineering and life sciences to design and fabricate constructs to expedite the replacement and regeneration of damaged tissue. The underlying principle of tissue engineering (Figure 1) involves the utilization of biocompatible and mechanically suitable scaffolds (conductive cues), combined with an appropriate source of cells (productive cues) and bioactive molecules (inductive cues) to promote the differentiation and maturation of the cell type of interest. These components, when combined, form a tissue-engineered construct, which can function as the tissue replacement material and, in principle, facilitate a faster rate of tissue repair.

Cartilage tissue engineering

One of the great challenges facing cartilage tissue engineering is designing constructs that mimic the unique multiphasic cellular architecture and mechanical properties of native cartilage tissue. As such, successful cell-based cartilage tissue engineering will depend on not only the selection of an appropriate biocompatible scaffolding material, but also materials with suitable mechanical properties, that when combined with cells and bioactive molecules will result in a desirable clinical outcome.

In our laboratory, human marrow-derived mesenchymal stem cells (MSC) have been prepared as high-density pellet cultures and subsequently press-coated onto the surface of porous biodegradable polymer blocks consisting

* These authors contributed equally.

Correspondence to: **Rocky S Tuan**, Cartilage Biology and Orthopedics Branch, National Institute of Arthritis, and Musculoskeletal and Skin Diseases, 50 South Dr., Room 1503, MSC 8022, National Institutes of Health, Bethesda, MD 20892–8022, USA.

DOI: 10.1080/14653240410005276

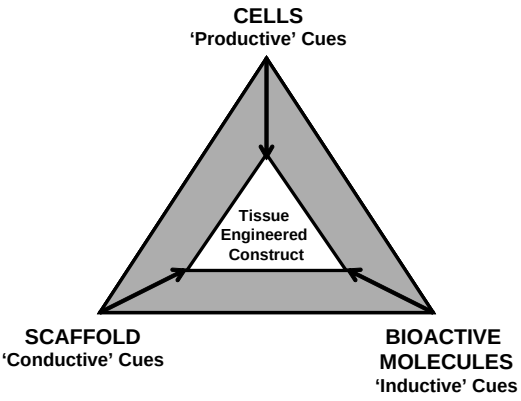

Figure 1. *Basic principles of tissue engineering.*

of poly-D,L-lactic acid (PLA) [1] to fabricate a cartilage tissue engineering construct. BM-derived MSC were used in these studies because of their capacity to differentiate down multiple connective tissue lineages, including both cartilage and bone [2]. Furthermore, these cells are ideal sources of cells for such a strategy because they possess a lower propensity to dedifferentiation in monolayer culture compared with isolated primary chondrocytes, a property also observed in trabecular bone-derived mesenchymal progenitor cells (MPC). PLA was chosen as a candidate scaffold for these studies because it is biodegradable and can be constructed into a three-dimensional (3-D) scaffold that mimics the structure of the native tissue, which provides a suitable template for cell colonization and tissue elaboration. The press-coating technique allows the design of cartilage constructs with layers of different sizes and shapes on biodegradable polymers to generate *in vitro*-engineered osteochondral grafts.

We have also recently demonstrated a suitable strategy for articular cartilage repair, which involves the design of an *in vitro*-engineered osteochondral plug [1,3]. This novel osteochondral plug is constructed by combining either human BM-derived MSC [1] or trabecular bone-derived MPC [3] with a biodegradable PLA polymer. As described earlier, the cartilage layer is fabricated by press-coating a chondrifying high-density cell pellet onto the scaffold, which is then seeded with cells that have been induced previously to differentiate into the osteogenic lineage. Maintenance of such a construct in the presence of suitable inductive cues necessary to maintain chondrogenesis (e.g. transforming growth factorβ, TGFβ) and osteogenesis (e.g. glucocorticoids, such as dexamethasone) results in an engineered tissue with a cartilage-like layer adherent to, and overlying, a dense bone-like constituent. This osteochondral plug resembles that of a core sample taken from a normal articular joint surface, in that this single-unit construct comprises both a cartilage-like and bone-like layer in direct apposition to each other. Analyses using reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry and histology, all revealed hyaline-like cartilage and bone with an interface mimicking the native osteochondral junction. Thus, by applying the principles of tissue engineering, a single-cell source construct applicable for articular cartilage repair was successfully designed.

MSC are a good source for cell-based cartilage tissue engineering

Selecting an ideal source of cells for cartilage tissue engineering requires achieving a number of criteria, including:

- easy access and availability of the source cells;
- demonstration of an extensive self-renewal or expansion capability of the source cells to generate sufficient quantity;
- a capacity of the source cells to differentiate readily into cell lineages of interest upon instructive differentiation cues;
- a lack of or minimal immunogenic or tumorigenic ability of the source cells.

Over the years, three major cell types have been examined for their potential application in cartilage repair and tissue engineering, namely committed chondrocytes, embryonic stem (ES) cells, and adult stem cells. Each of these cell types has demonstrated its limitations and advantages over the others due to their intrinsic biological properties, such as their proliferative and differentiation potentials, availability of large quantity of cells for transplantation, and ethical and legal concerns. For example, committed chondrocytes isolated from hyaline cartilage by enzymatic digestion have been tested extensively as a cell source to be injected into the damaged articular cartilage site for repair. However, their wide usage in clinical trials and tissue engineering applications has been hindered because of the loss of redifferentiation capability after relatively limited *in vitro* expansion. In contrast, ES cells derived from the inner cell mass of the embryonic blastocyst have infinite proliferation potential and are able to generate cells characteristic of all three germ layers under appropriate inductive conditions. However, the ethical and legal

concerns associated with ES cells and their tumorigenic potential make them a less ideal cell source in basic research and clinical applications. On the other hand, adult stem cells (e.g. MSC) derived from various tissues (e.g. BM, fat, trabecular bone) demonstrate a promising future in regenerative medicine because of their ability of self-renewal and differentiation along specific lineages upon stimulation.

MSC are pluripotent undifferentiated cells residing in various adult tissues, including BM, muscle, trabecular bone, dermis, adipose tissue, periosteum, blood and synovial membrane [3]. They can be distinguished from hematopoietic stem cells in terms of cell-surface antigens and adhesion capability. Upon culture under appropriate conditions, MSC show an extensive but finite proliferative capability, raising little risk of tumorigenicity when transplanted *in vivo*. Additionally, MSC can be induced to differentiate along numerous cell types, such as chondrocytes, osteoblasts, adipocytes, cardiomyocytes, hepatocytes and neurons, by specific growth factors [e.g. bone morphogenetic proteins (BMP), the mammary tumor proto-oncogene family (Wnts), fibroblast growth factors (FGF) and culture environment (such as monolayer or 3-D culture)]. Such multidifferentiation potential has elicited continued interest in MSC as one of the top candidate sources of cells for bone and cartilage tissue regeneration and repair.

Multipotential MSC were originally isolated and identified from BM aspirate based on their tendency to adhere to tissue culture plastic. Recently our laboratory has demonstrated that MSC can also be obtained from explant cultures of collagenase-treated trabecular bone fragments [4,5]. These trabecular-derived MSC are able to maintain an undifferentiated phenotype through long-term *in vitro* culture (up to 40 cumulative population doublings). They express putative MSC cell-surface markers, including Stro-1, CD105, CD73, and lack CD34 and CD45, typical markers for hematopoietic stem/progenitor cells. Similar to BM-derived MSC, trabecular bone-derived MSC can differentiate along the osteoblastic, adipocytic and chondrocytic lineages *in vitro* under controlled culture conditions, suggesting a useful alternative system for cell-based tissue engineering applications.

Scaffolds for tissue engineering

In addition to selecting an appropriate source of cells for cartilage tissue engineering, the design of an engineered cartilage construct, i.e. the scaffolding material in which the cells will reside, needs careful consideration. The physical properties (e.g. architecture, macro/microporosity, interconnecting porosity and topography) of an engineered tissue substitute will play an integral role in altering its biologic performance. Therefore, an ideal tissue engineering scaffold should possess a number of key properties, including being biocompatible, biodegradable, porous, mechanically stable, cell permissive and conducive to extracellular matrix production and deposition and biomolecular signal transmission. There are a number of candidate scaffolding materials that have been designed for cartilage tissue engineering strategies that include natural biopolymers, such as agarose, alginate and collagen, and synthetic polymers, such as polyglycolic acid (PGA), polylactic acid (PLA) and their co-polymers, poly (D,L-lactic-*co*-glycolide) (PLGA) [6].

One major criterion in designing tissue engineered constructs is designing a scaffold that mimics the architecture of the native tissue. Our laboratory has employed an electrospinning process to design novel polymeric-based scaffolds for tissue engineering applications [7]. Electrospinning is a process whereby ultra-fine fibers are formed in a high-voltage electrostatic filed. We have specifically employed this technique to generate 3-D, nanofibrous poly(ε-caprolactone) (PCL) scaffolds for cartilage tissue engineering applications [6]. These scaffolds are unique in that they feature morphologic similarity to the extracellular matrix of natural tissue, namely collagen fibers (Figure 2). Furthermore, the resultant nanofibrous scaffold possesses qualities ideal for cartilage tissue engineering. For example, nanofibrous spun scaffolds have a porosity of 90%, which thereby provides an open-pore template for cell invasion and exchange of nutrients and metabolic waste. Additionally, the majority of the pore diameters range from 25 to 100 μm. This size range is sufficient for cell invasion and subsequent colonization of cells on the scaffold substrate. Importantly, electrospun nanofibers (200–700 μm) possess better mechanical properties than scaffolds composed of larger diameter fibers. Our PCL scaffolds support a chondrocytic phenotype of fetal bovine chondrocytes and the condrogenic induction and maintenance of TGFβ-1 treated MSC. These homogeneous, 3-D, porous electrospun structures provide a suitable template for cell colonization and tissue elaboration and possess effective mechanical

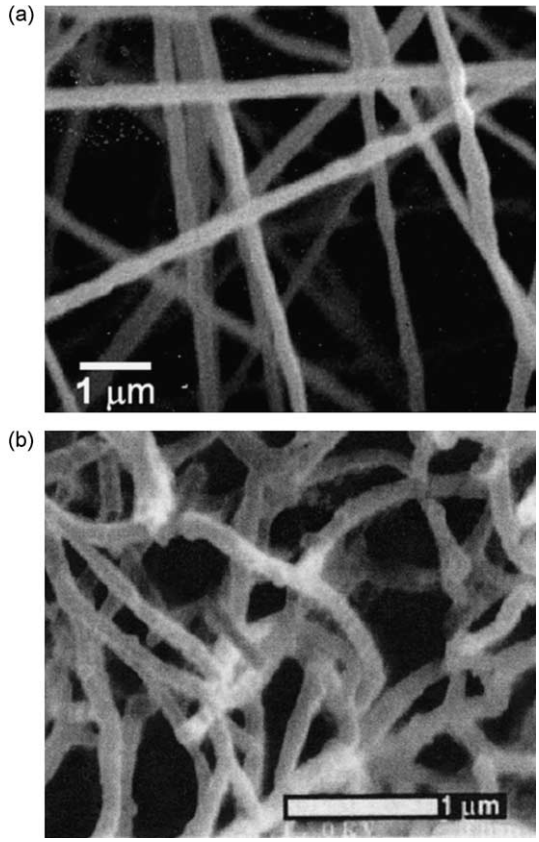

Figure 2. *Scanning electron micrographs of PCL nanofiber (a) and native collagen fibrils (b), reprinted with permission from the* Journal of Cellular Biochemistry.

properties for soft tissue, such as skin and cartilage, replacement and/or repair.

Developmental mesenchymal chondrogenesis as a model for regenerative chondrogenesis

Although MSC have shown great promise in cartilage repair and regeneration, several questions still need to be addressed before we can maximize the potential of these cells in cartilage tissue engineering. For example, how can MSC be induced effectively to differentiate into chondrocytes in an engineered scaffold *in vitro* and maintain this differentiated phenotype prior to implantation? What are the cellular and molecular signals involved in mesenchymal chondrogenesis? What is the requirement for growth factors (i.e. dose, timing of dose) and culture environment (i.e. static vs. dynamic) during chondrogenesis? How is the chondrogenic phenotype maintained after the MSC-seeded scaffold is transplanted into a damaged cartilage lesion *in vivo*? To answer these questions, it is necessary to examine how MSC differentiate into chondrocytes in response to specific stimuli *in vitro*.

Induction of chondrogenesis *in vitro* from adult MSC requires a high cell seeding density, similar to the condensation process observed in embryonic chondrogenesis, e.g. in the developing limb bud. Two cell adhesion molecules have been identified as important players in mediating cell–cell contact and communication during the condensation and early commitment of undifferentiated mesenchymal cells into chondrocytes, namely N-cadherin and neural cell adhesion molecule (N-CAM). Their expression profiles are tightly controlled in a spatiotemporal manner, up-regulated during initial cell adhesion and down-regulated once cells begin differentiation. TGFβ family members also play an important role in chondrogenic differentiation. In the absence of TGFβ, MSC undergo condensation without progressing into chondrogenic differentiation and maturation, as evidenced by the lack of expression of collagen type II, collagen type X, Sox9 and sulfated proteoglycans. Tuli *et al*. [8] have demonstrated that TGFβ-1 stimulated MSC chondrogenesis by activating intracellular mitogenic activated protein (MAP) kinases p38, ERK-1 and JNK. Addition of MAP kinase inhibitors in the TGFβ-1-treated MSC pellet cultures led to the downregulation or complete abolition of chondrogenic lineage-specific gene expression. Through N-cadherin-mediated cell–cell adhesion and communication, TGF-β1 activates collagen type II and Sox9 promoter activities. In addition, TGFβ-1-activated MAP kinases increased the cytoplasmic pool of β-catenin (an important signaling molecule in mediating canonical Wnt signaling) as well as decreased β-catenin-dependent TCF promoter activity, suggesting that canonical Wnt signaling is a negative regulator of MSC chondrogenesis.

Wnt is a family of secreted cysteine-rich glycoproteins that have been implicated in the regulation of stem cell maintenance, proliferation and differentiation during embryonic development. Canonical Wnt signaling increases the stability of cytoplasmic β-catenin by receptor-mediated inactivation of glycogen synthase kinase (GSK-3) kinase activity, and promotes β-catenin translocation into the nucleus. The active β-catenin–T-cell factor (TCF)/lymphoid enhancing factor (LEF) complex then regulates the transcription of genes involved in cell proliferation and differentiation. In humans, mutations in the Wnt co-receptor, LRP5, lead to defective bone formation. Gain of function mutation results in high

bone mass, while loss of function causes an overall loss of bone mass and strength, indicating that Wnt signaling is positively involved in embryonic osteogenesis. The function of Wnt signaling in MSC osteogenesis has been demonstrated by Boland *et al*. [9]. By incubating MSC with Wnt-3a-conditioned medium or overexpressing Wnt3a, Boland *et al*. [9] have shown that Wnt-3a, a prototypic canonical Wnt signaling factor, inhibits osteogenesis *in vitro* through β-catenin-mediated downregulation of TCF activity, while stimulating MSC proliferation. Thus, it is of particular interest to determine whether the inhibitory effect of Wnt-3a on osteogenesis is the direct result of suppression of osteogenic gene expression or the secondary effect of increasing cell proliferation. These findings also provide crucial insights on cartilage repair and regeneration, as the progressing of osteoarthritis is always accompanied by the damage of subchondral bone and formation of osteophytes.

To exploit fully the potential of MSC in cartilage tissue engineering, it is of importance to understand the characteristics of adult MSC, such as their proliferation, maintenance and differentiation. One emerging concept about adult stem cells is their transdifferentiation potential, i.e. cells differentiated along one developmental lineage can switch their gene expression program and become cells of a different lineage. Song and Tuan [10] have recently shown that human MSC that have been precommitted to one mesenchymal lineage were able to convert to other cell types in response to inductive extracellular cues (Figure 3). By labeling and selecting fully differentiated osteoblasts using a lineage-specific promoter reporter system, they demonstrated that fully committed, homogeneous osteoblasts can transdifferentiate into mature adipocytes and chondrocytes at both a population and single colony level, further confirming that transdifferentiation is an intrinsic property of adult MSC. Extensive cell proliferation was also observed to precede the phenotypic change during the transdifferentiation process, suggesting that genetic reprogramming is a crucial step for differentiated MSC to acquire a new phenotype. Understanding the cellular and molecular signals that regulate the transdifferentiation/dedifferentiation process will not only shed light on fundamental mechanisms controlling development, but provide tools to manipulate adult stem cells for cell-based approaches in regenerative medicine.

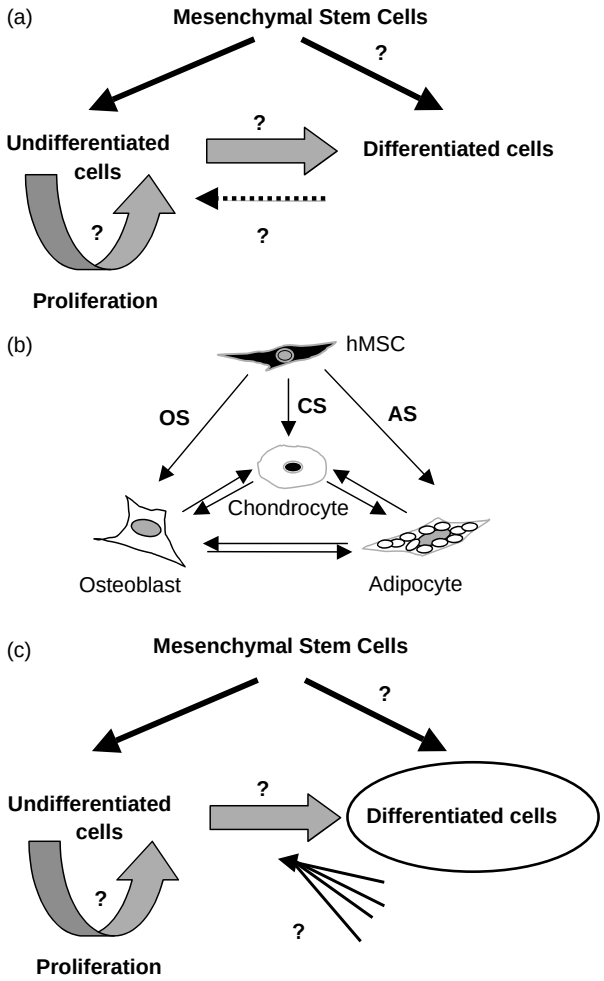

Figure 3. *Schematic diagrams depicting the biological properties of adult MSC. (a) MSC are traditionally viewed as cells that have limited self-renewal and differentiation potential. (b) Experimental evidence has demonstrated the ability of MSC to transdifferentiate and dedifferentiate as a function of specific culture conditions (OS, osteogenic conditions; CS, chondrogenic conditions; AS, adipogenic conditions. (c) An updated view of MSC. Differentiated cells from MSC are able to re-enter the proliferation stage and resume the characteristics of undifferentiated MSC through genomic reprogramming. Factors or signals involved in maintaining the MSC biological properties (question marks) require further investigation.*

Conclusion

In the USA, approximately 90% of the population over the age of 40 demonstrate some form of degeneration in their weight-bearing joints, resulting in pain and immobility [2], leading to a reduced workforce and increased medical expenses. Thus improvements in cartilage repair using a cell-based tissue engineering approach will be of great benefit to public health as well as to the economy.

However, we still face a number of challenges. In order to use adult stem cells effectively, it is important to understand their native environment so that we can manipulate their *in vitro* growth environment to recapitulate their native environment, with the goal in mind to expand them *in vitro* without compromising their multilineage potential and their specific differentiation program. Despite the significant progress that has been made in this field, significant questions remain. For example, how can we develop adequate delivery vehicles and stimulate their integration between the graft and host? How can we establish valid *in vitro* and animal model systems to test our regimen? Therefore, collaboration between biologists, physicians and engineers, and between academia, government (both research and regulatory agencies) and industries, is not only necessary but crucial before cell-based cartilage tissue engineering can reach its full potential in cartilage repair and regeneration.

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