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Collagen Type II enhances chondrogenic differentiation in agarose-based modular microtissues

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Abstract

Background aims. Cell-based therapies have made an impact on the treatment of osteoarthritis; however, the repair and regeneration of thick cartilage defects is an important and growing clinical problem. Next-generation therapies that combine cells with biomaterials may provide improved outcomes. We have developed modular microenvironments that mimic the composition of articular cartilage as a delivery system for consistently differentiated cells. *Methods.* Human bone marrow–derived mesenchymal stem cells (MSC) were embedded in modular microbeads consisting of agarose (AG) supplemented with 0%, 10% and 20% collagen Type II (COL-II) using a water-in-oil emulsion technique. AG and AG/COL-II microbeads were characterized in terms of their structural integrity, size distribution and protein content. The viability of embedded MSC and their ability to differentiate into osteogenic, adipogenic and chondrogenic lineages over 3 weeks in culture were also assessed. *Results.* Microbeads made with <20% COL-II were robust, generally spheroidal in shape and 80 ± 10 μm in diameter. MSC viability in microbeads was consistently high over a week in culture, whereas viability in corresponding bulk hydrogels decreased with increasing COL-II content. Osteogenic differentiation of MSC was modestly supported in both AG and AG/COL-II microbeads, whereas adipogenic differentiation was strongly inhibited in COL-II containing microbeads. Chondrogenic differentiation of MSC was clearly promoted in microbeads containing COL-II, compared with pure AG matrices. *Conclusions.* Inclusion of collagen Type II in agarose matrices in microbead format can potentiate chondrogenic differentiation of human MSC. Such compositionally tailored microtissues may find utility for cell delivery in next-generation cartilage repair therapies.

Key Words: *Agarose, Biomaterials, Cartilage tissue engineering, Chondrogenesis, Collagen, Mesenchymal stromal cells, Microencapsulation, Modular tissue engineering*

Introduction

Repair and regeneration of cartilage is a difficult orthopedic problem due to the low inherent healing capacity of the native tissue [1–3]. Articular cartilage is a well-organized tissue with remarkable durability; however, damage may result in debilitating joint pain and functional impairment. Clinical approaches such as osteotomy and osteochondral graft transplantation [4] have shown benefits in terms of relieving pain, delaying further deterioration and restoring partial function, but these therapies do not result in the regeneration of fully competent tissues, and their sustainability in a load-bearing environment remains uncertain. The lack of long-term clinical solutions for cartilage repair has motivated the search for improved regenerative therapies that achieve full recovery of intractable and large defects.

Cell-based approaches have emerged as a clinical therapy to regenerate damaged cartilage [5–10]. However, challenges associated with naked chondrocyte delivery [11,12] have led to the development of matrix-assisted strategies for cell implantation [13–16]. Such biomaterial-based approaches use a preformed scaffold or a hydrogel matrix, often supplemented with biochemical cues to provide mechanical stability while sustaining chondrogenic differentiation. It has also been shown that native extracellular matrix components in combination with autologous cells can to some degree recapitulate the native microenvironment and

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architecture, which can improve clinical outcomes [17]. Pre-clinical studies have also shown that cell-seeded scaffolds can be maintained in perfusion cultures with exogenous stimuli before implantation to further improve graft maturation and host–implant integration [18–20]. Despite these efforts, a repair tissue with functional properties and stability comparable to articular cartilage has yet to be engineered.

Bone marrow-derived mesenchymal stem cells (MSC) have been investigated widely for cartilage repair applications [21–24]. These progenitor cells are readily available, have demonstrated multilineage potential [25] and also exhibit valuable immunomodulatory and tissue homing properties [26]. MSC have been shown to differentiate into both cartilage [27–30] and bone [31–33], supporting their use in orthopaedic applications. In addition, MSC can exhibit both trophic [34] and chemotactic effects [35] to create a regenerative tissue environment. Notably, even MSC isolated from the marrow of patients with advanced osteoarthritis retain their chondrogenic potential and synthesize cartilage-specific matrix [27]. Taken together, the tissue-specific and pro-regenerative capabilities of MSC make them an excellent cell source for engineering of cartilage tissue. Combination of MSC with an appropriate biomaterial scaffold that mimics key aspects of the native extracellular matrix architecture and biochemistry may further enhance their regenerative potential.

The major material components of articular cartilage are large proteoglycans with interspersed fibrillar collagen, which constitute approximately 15–25% and 50–60% of dry weight, respectively [36–39]. Proteoglycans contribute to the compressive stiffness of the cartilage, while the tensile strength and resilience are dependent on collagen fibers [40]. In articular cartilage, chondrocytes are embedded in the matrix at a relatively low cell density (~10^4 cells/mm^3) and account for only approximately 1% of the tissue volume [41,42]. In their native differentiated state, chondrocytes have a generally spheroidal morphology and synthesize collagen Type II (COL-II) and large proteoglycans. However, when isolated and placed in conventional two-dimensional culture, chondrocytes will de-differentiate, spread on the culture surface and produce predominantly collagen Type I and small proteoglycans [43–45]. Although collagen Type I is commonly used as a scaffold in tissue engineering because of its wide availability [46,47], it has been shown that COL-II can preferentially promote cell proliferation, extracellular matrix deposition and wound healing by chondrocytes [48–50]. The polysaccharide agarose (AG) has been used as a mimic of the proteoglycan component of cartilage and has been shown to maintain the spherical morphology of chondrocytes [51,52], as well as support the deposition of an appropriate pericellular matrix [51–54]. *In vivo* studies have demonstrated that AG provides a microenvironment that supports the non-hypertrophic and non-proliferative chondrogenic phenotype [55]. The physiological response to AG resembles a wound-healing response similar to other biomaterials commonly used in the context of tissue repair [56]. Studies in both animals and humans have shown that AG can be completely biodegraded and cleared after implantation without adverse effects [55,57].

The goal of the present study was to fabricate and characterize modular microenvironments that mimic the proteoglycan-protein composition of cartilage tissue, with an emphasis on the ability to support lineage-specific differentiation of human bone marrow–derived MSC. The proteoglycan component was represented by AG, a polysaccharide that can easily be formed into a hydrogel and that has found utility as a matrix in cartilage tissue engineering [51,58]. The protein component was represented by reconstituted COL-II, which can form fibrillar structures as in the native tissue [37]. Cells were embedded directly into the AG/COL-II matrix using a water-in-oil emulsion technique, producing discrete "microbeads" (~80 μm in diameter) consisting of MSC embedded in a spheroidal hydrogel matrix. The modular format has several potential advantages over bulk gel methods in terms of reducing diffusion path lengths, allowing pre-culture of matrix-adhered cells, and delivering a differentiated cell population. Microbeads made with specific COL-II contents were characterized for their structural integrity, size distribution and protein content. MSC viability and their potential to differentiate into osteogenic, adipogenic and chondrogenic lineages were also assessed. The long-term goal of this work is to develop injectable, cell-based modular microenvironments that promote specific tissue regeneration, as shown schematically in Figure 1 in the case of articular cartilage. Such a therapy would provide a three-dimensional matrix environment and cells of specified function that could be delivered minimally invasively to sites of tissue damage.

Methods

Biopolymers and MSC culture

AG (Fisher Scientific) was low melting point grade with an average molecular weight of 120 kDa, gelling temperature between 34.5 and 37.5°C and gel-strength of ~500 g/cm^2 min. AG stock solution was made at 2.0 wt% by dissolving the appropriate amount of powdered AG in DI water heated to 60°C and sterilized using a 0.2-μm filter. COL-II (Elastin Products Company) was from mouse sternum with a molecular weight of 1000 kDa, <0.4% proteoglycan and was tested for the absence for collagen Type I. COL-II stock