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Stem cell delivery in tissue-specific hydrogel enabled meniscal repair in an orthotopic rat model

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ABSTRACT

Interest in non-invasive injectable therapies has rapidly risen due to their excellent safety profile and ease of use in clinical settings. Injectable hydrogels can be derived from the extracellular matrix (ECM) of specific tissues to provide a biomimetic environment for cell delivery and enable seamless regeneration of tissue defects. We investigated the *in situ* delivery of human mesenchymal stem cells (hMSCs) in decellularized meniscus ECM hydrogel to a meniscal defect in a nude rat model. First, decellularized meniscus ECM hydrogel retained tissue-specific proteoglycans and collagens, and significantly upregulated expression of fibrochondrogenic markers by hMSCs versus collagen hydrogel alone *in vitro*. The meniscus ECM hydrogel in turn supported delivery of hMSCs for integrative repair of a full-thickness defect model in meniscal explants after *in vitro* culture and *in vivo* subcutaneous implantation. When applied to an orthotopic model of meniscal injury in nude rat, hMSCs in meniscus ECM hydrogel were retained out to eight weeks post-injection, contributing to tissue regeneration and protection from joint space narrowing, pathologic mineralization, and osteoarthritis development, as evidenced by macroscopic and microscopic image analysis. Based on these findings, we propose the use of tissue-specific meniscus ECM-derived hydrogel for the delivery of therapeutic hMSCs to treat meniscal injury.

1. Introduction

Promising new tissue engineering approaches have incorporated natural biomaterials made from the extracellular matrix (ECM) of decellularized tissues, such as heart [1], lung [2], and bone [3]. Decellularization preserves the molecular composition of the native ECM with tissue-specific molecules, including the structural and mechanical features of the original tissue, which guide the behavior of therapeutic cells and facilitate tissue development [4]. The applications of decellularized biomaterials range from whole organ replacements to tissue patches and hydrogels that can be therapeutic or serve as delivery vehicles for drugs, biologics, or cells [5]. Injectable hydrogels have enabled novel treatment options due to the modes of administration that can be tailored to specific tissues and diseases. One important focus is in sports medicine, where injectable orthobiologics such as hyaluronic acid and platelet-rich plasma [6] are broadly utilized for treating osteoarthritis (OA) and injuries to tendons, ligaments, and menisci. The latter are fibrocartilaginous tissues that specialize in load-bearing and stabilization of the knee joint [7,8].

Meniscal injury is of particular interest, as they are implicated in over half of all arthroscopic procedures performed by orthopaedic surgeons [9], the majority of which involves surgical resection (partial meniscectomy), despite knowledge that partial removal of meniscal tissue contributes to the development of OA [10].

Given the importance of the meniscus in the biomechanics of the knee, and the role of meniscus ECM in maintaining the functional properties of meniscal tissue, repair of the meniscus must regenerate the native ECM. In the subpopulation of patients with meniscal damage but otherwise healthy knee joints, meniscal replacement has been studied by implanting tissue allografts [11], synthetic biomaterials [12–14], and decellularized xenografts [15–18]. However, it has long been appreciated that the dense ECM of the meniscus obstructs cellular infiltration and integration. In a

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canine model, cells were shown to repopulate devitalized meniscal allografts, but the central core of the tissue remained acellular even at six months after transplantation [19]. Cell migration into the decellularized meniscus was limited to ~150 μm over seven days *in vitro*, precluding robust tissue formation [20].

To overcome these limitations, we developed a hydrogel from decellularized bovine meniscus ECM to deliver human mesenchymal stem cells (hMSCs) into full-thickness defects in explant models *ex vivo* and animal models *in vivo*, and promote growth of viable repair tissue. Mesenchymal stem cells (MSCs) were selected for our study as they have been widely studied in musculoskeletal diseases [21–23], with previous clinical reports on their use in meniscal repair and OA treatment [24,25]. To improve repair outcomes, hMSCs need to differentiate and grow new tissue, and to produce trophic factors for tissue remodeling and regeneration. Intra-articular delivery of cells has been previously applied in preclinical [26–31] and clinical [25,32–34] studies of various osteochondral conditions. However, without an appropriate carrier, large numbers of cells need to be injected to compensate for the massive cell loss by dispersion from the defect site [29]. We hypothesized that a hydrogel derived from decellularized meniscus ECM (mECM) can protect exogenously delivered cells, localize them to the site of injury, and provide signals for the formation of new repair tissue. To test this hypothesis, we investigated the development of meniscal tissue by hMSCs in mECM and collagen hydrogels *in vitro*, and the capacity of hMSC-mECM hydrogel constructs to integrate with native meniscus *in vitro* and *in vivo*, and to repair meniscal injury in an orthotopic nude rat model.

2. Materials and methods

2.1. Culture of human mesenchymal stem cells

hMSCs were isolated from fresh, unprocessed human bone marrow aspirates (Lonza, Basel, CH), and characterized as previously described [3]. Cells were expanded to passages 4–5 in basal medium consisting of high glucose (hg) DMEM, 10% fetal bovine serum (FBS), 1 × antibiotic-antimycotic, and 0.1 ng/mL bFGF (Thermo Fisher Scientific, Waltham, MA).

2.2. Decellularization and digestion of bovine meniscus extracellular matrix

Juvenile bovine menisci were dissected within 36 h of slaughter (Green Village Packing Company, Green Village, NJ), minced to 1–2 mm^3 pieces, and lyophilized for 24 h. Tissues were decellularized at 25 °C with agitation in 2% SDS and 10 mM Tris (3 cycles, 24 h each), followed by 0.1% peracetic acid (2 h), washed with sterile water and PBS (3 cycles), and lyophilized again (24 h). The resulting meniscus ECM (mECM) was digested at an initial concentration of 40 mg/mL at 25 °C with agitation in 0.1% pepsin +0.01 M HCl (12 h), resulting in a mECM digest solution. Biochemical analysis confirmed the retention of ECM constituents and removal of DNA (Fig. S1). The mECM digests, along with type I collagen controls (BD Biosciences, San Jose, CA) and Precision Plus Protein Dual Color Standards (Bio-Rad, Hercules, CA), were analyzed by SDS-PAGE on 4–20% gels (Bio-Rad), stained with Coomassie Brilliant Blue (Bio-Rad), and imaged by a Canon imageCLASS D480 (Melville, NY; Fig. S2).

2.3. Encapsulation of hMSCs in hydrogel

For hydrogel studies, mECM digests and type I collagen (rat tail; BD Biosciences) were gelated based on a previously established protocol [4]. Briefly, type I collagen hydrogels (3 mg/mL) were formed by mixing collagen solution (30% by volume), 0.1 N NaOH (3%), 10 × PBS (3.3%), hgDMEM (63.7%) at physiologic pH and 4 °C. Similarly, mECM hydrogels (5.61 mg/mL) were formed by mixing mECM digest (56% by volume), 0.1 N NaOH (5.6%), 10 × PBS (6.2%), hgDMEM (32%) at 4 °C. The concentration of mECM in hydrogel (5.61 mg/mL) was chosen to match the hydroxyproline content of the type I collagen hydrogel (3 mg/mL), as quantified by a modified acid hydrolysis assay [35].

hMSCs were encapsulated in type I collagen or mECM hydrogels at 30 × 10^6 cells/mL, then injected in 25 μL aliquots into 4-mm diameter poly(dimethylsiloxane) rings. Gelation occurred over 40 min at 37 °C, and subsequent hMSC-laden constructs in either mECM or type I collagen hydrogel were cultured for 28 days in chondrogenic media (CM), containing hgDMEM, 1 × antibiotic-antimycotic, 0.1 μM dexamethasone, 50 μg/mL ascorbate 2-phosphate, 40 μg/mL L-proline, 100 μg/mL sodium pyruvate, 1 × insulin/transferrin/selenium premix (BD Biosciences), with or without 10 ng/mL TGF-β3 (PeproTech, Rocky Hill, NJ). Samples were collected at days 0, 14, and 28 for biochemical, histological, and gene expression analyses.

2.4. Mechanical integration testing in an in vitro tissue explant model

Juvenile bovine menisci were dissected as previously described [36]. Explants were harvested from the central tissue region using sterile 4 mm diameter biopsy punches, and cut to 1.5 mm height using a custom microtome device. A 1.5-mm diameter central core was punched out, leaving a tissue ring. Explant rings were devitalized at 4 °C with agitation in sterile water (5 cycles, 24 h each, sterile water changes between cycles) [37]. hMSCs were encapsulated in mECM hydrogel as described above, and after 3 days of culture in basal medium, two hMSC-laden mECM gels were press-fitted into the devitalized rings. mECM-explant composites were cultured for 42 days in CM with 10 ng/mL TGF-β3. Samples were collected at timed intervals for mechanical, biochemical and histological analyses.

Integration of full-thickness defects in meniscus explants was tested using a custom device consisting of a 1.33-mm diameter indenter in series with a 50 g load cell, placed above a cup with a 2 mm diameter hole using our previously established protocol [38]. Prior to testing, the height of each sample was measured using a digital caliper. The indenter was displaced at a ramp rate of 0.3% of the sample height per second, until the central core was pushed fully through the outer ring. Integration strength was calculated as the ratio of the peak force to the surface area of contact between the central core and outer ring.

2.5. Phenotype stability in a subcutaneous implantation model in nude mouse

After 6 weeks of *in vitro* culture, devitalized meniscal explants containing mECM-encapsulated hMSCs were implanted subcutaneously into nude mice for 4 weeks *in vivo*, according to an approved protocol at Columbia University. Female NOD/SCID mice at 6 weeks of age (Harlan, Indianapolis, IN) were anesthesized by intraperitoneal injections of ketamine (80–100 mg/kg) and xylazine (5–10 mg/kg), with analgesia by subcutaneous administration of buprenorphine SR (1.2 mg/kg). Constructs were implanted into separate subcutaneous dorsal pockets (1 construct per pocket, 2 pockets per animal). Implants were collected after 4 weeks *in vivo* and evaluated for expression of chondrogenic, hypertrophic, and osteogenic markers.