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Articular chondrocytes and mesenchymal stem cells seeded on biodegradable scaffolds for the repair of cartilage in a rat osteochondral defect model

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ABSTRACT

This work investigated the ability of co-cultures of articular chondrocytes and mesenchymal stem cells (MSCs) to repair articular cartilage in osteochondral defects. Bovine articular chondrocytes and rat MSCs were seeded in isolation or in co-culture onto electrospun poly(ε-caprolactone) (PCL) scaffolds and implanted into an osteochondral defect in the trochlear groove of 12-week old Lewis rats. Additionally, a blank PCL scaffold and untreated defect were investigated. After 12 weeks, the extent of cartilage repair was analyzed through histological analysis, and the extent of bone healing was assessed by quantifying the total volume of mineralized bone in the defect through microcomputed tomography. Histological analysis revealed that the articular chondrocytes and co-cultures led to repair tissue that consisted of more hyaline-like cartilage tissue that was thicker and possessed more intense Safranin O staining. The MSC, blank PCL scaffold, and empty treatment groups generally led to the formation of fibrocartilage repair tissue. Microcomputed tomography revealed that while there was an equivalent amount of mineralized bone formation in the MSC, blank PCL, and empty treatment groups, the defects treated with chondrocytes or co-cultures had negligible mineralized bone formation. Overall, even with a reduced number of chondrocytes, co-cultures led to an equal level of cartilage repair compared to the chondrocyte samples, thus demonstrating the potential for the use of co-cultures of articular chondrocytes and MSCs for the *in vivo* repair of cartilage defects.

1. Introduction

While a number of treatment options currently exist for the repair of articular cartilage defects, these options primarily lead to short-term functional repair, but are not capable of achieving stable, long-term repair of the tissue [1,2]. Autologous chondrocyte implantation (ACI) is generally one of the most often-used procedures for the treatment of cartilage defects, and has been shown to have some success in repairing the damaged tissue [1,3]. However, the isolation of appropriate numbers of autologous chondrocytes is not without challenges. Chondrocytes are present in relatively low densities in native articular cartilage [4], and the isolation of sufficient numbers would lead to large donor site morbidity [5]. Furthermore, the *in vitro* expansion of chondrocytes is associated with a rapid dedifferentiation of the cells into a more fibroblastic phenotype, which ultimately leads to the production inferior tissue [6]. Thus, numerous approaches have been investigated in order to enhance the chondrogenic phenotype of expanded cells or to reduce the demand for chondrocytes in the treatment of articular cartilage defects [7].

Co-cultures of articular chondrocytes and mesenchymal stem cells (MSCs) are one approach that has been proposed to reduce the demand for articular chondrocytes and thus improve articular cartilage treatments [8–11]. When co-cultured with MSCs, articular chondrocytes have been observed to undergo enhanced proliferation and matrix production [9,12–14]. This effect, which has been shown to be independent of MSC source or culture condition [15], would allow for the use of reduced numbers of chondrocytes to achieve an equal chondrogenic outcome [11]. Furthermore, the co-cultured cell population has been demonstrated to be more sensitive to chondrogenic stimuli, such as transforming growth factor-β3 (TGF-β3), and to produce a phenotype that is more stable after the

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removal of the stimuli, compared to monocultures of either cell type [8]. While the beneficial effects of MSCs on chondrocytes are crucial to the performance of these co-cultures, chondrocytes have similarly been demonstrated to have beneficial effects on MSCs, which mitigates some disadvantages associated with MSC chondrogenesis. The chondrogenesis of MSCs is challenged by the eventual hypertrophy and mineralization of these cells after extended culture in chondrogenic conditions [16]. However, co-culture with articular chondrocytes has been demonstrated to reduce the hypertrophy of MSCs in culture [10,17,18]. Thus, the advantages of co-cultures of articular chondrocytes and MSCs for the *in vitro* generation of articular cartilage is well-documented; however the use of this cell population for *in vivo* repair of articular cartilage defects has not been investigated.

The objective of the present study was to investigate the use of co-cultures of articular chondrocytes and bone marrow-derived MSCs for the *in vivo* repair of articular cartilage in a rat osteochondral defect. We hypothesized that the use of co-cultures of chondrocytes and MSCs would lead to equal or greater cartilage repair compared to chondrocytes alone, thus allowing for the use of reduced numbers of chondrocytes. Therefore, we implanted electrospun poly(ε-caprolactone) (PCL) scaffolds, seeded with MSCs, chondrocytes, or co-cultures of chondrocytes and MSCs into the trochlear groove of rats and evaluated the tissue repair via histology and microcomputed tomography.

2. Methods

2.1. Study design

The groups investigated in this study are outlined in Table 1. Briefly, bovine articular chondrocytes and rat bone marrow-derived MSCs were seeded onto electrospun PCL scaffolds to create three separate experimental groups. The AC group consisted of articular chondrocytes seeded in monoculture at a density of 40,000 cells per scaffold; the MSC group consisted of MSCs seeded in monoculture at a density of 40,000 cells per scaffold. The CC group consisted of articular chondrocytes and MSCs seeded in a 1:3 ratio at a density of 40,000 cells per scaffold (i.e. 10,000 chondrocytes and 30,000 MSCs). Additionally, an empty control (empty) and a material control (PCL) were also investigated. All samples ($n = 8$ per group) were implanted into defects created in the trochlear groove of Lewis rats for 12 weeks. Samples were analyzed for cartilage tissue formation through histological scoring and for the formation of mineralized bone through microcomputed tomography.

2.2. Scaffold fabrication

Non-woven mats were electrospun using PCL (Sigma–Aldrich, St. Louis, MO) with a number-average molecular weight (Mn) of 114,000 ± 4000 Da and a polydispersity index (Mw/Mn) of 2.02 ± 0.04, as determined by gel permeation chromatography (Phenogel Linear Column with 5-μm particles, Phenomenex, Torrance, CA; Differential Refractometer 410, Waters, Milford, MA, $n = 3$) and a calibration curve generated from polystyrene standards (Fluka, Switzerland). Briefly, a 14 wt% solution of PCL was prepared by dissolving the polymer in a 5:1 volume ratio of chloroform to methanol. The polymer solution was extruded at 25 ml/h through a 16 G needle, charged to 30 kV, towards a grounded collecting plate 40 cm away. Fiber morphology was inspected using scanning electron microscopy and determined to be 9.51 ± 0.75 μm ($n = 32$ fibers). Scaffolds were punched from mats using a 1.5 mm dermal biopsy punch. Scaffolds approximately 1.6 mm in thickness were used for this study.

After preparation, scaffolds were loaded into custom-designed polycarbonate blocks designed to confine the cell suspension during seeding and sterilized by exposure to ethylene oxide (Anderson Sterilizers, Haw River, NC) for 14 h. Scaffolds were then prewet by soaking in a graded ethanol series, rinsed in phosphate buffered saline (PBS) three times, and soaked in general medium (DMEM, 10% FBS, 1% PSF) for 72 h.

Table 1
Outline of experimental groups.

Group	Abbreviation	Scaffold	Rat MSCs (cells)	Bovine ACs (cells)
Empty control	Empty	No scaffold	0	0
Material control	PCL	Electrospun PCL	0	0
MSCs	MSC	Electrospun PCL	40,000	0
Co-cultures	CC	Electrospun PCL	30,000	10,000
Articular chondrocytes	AC	Electrospun PCL	0	40,000

2.3. Cell isolation and culture

Bovine articular chondrocytes were isolated from the femoral chondyles of 7–10-day old calves (Research 87, Boylston, MA) within 24 h of slaughter using previously described methods [19]. Briefly, cartilage was isolated, minced to 1 × 1 × 1 mm pieces, washed with PBS, and incubated in chondrocyte growth medium (DMEM, 10% FBS, 1% non-essential amino acids, 50 μg/ml ascorbic acid, 46 μg/ml L-proline, 20 mM HEPES, 1% PSF) supplemented with 2 mg/ml collagenase type II (Worthington biochemical corporation, Lakewood, NJ) on a shaker table at 37 °C for 16 h. Cells were isolated from 4 legs, pooled, aliquoted and cryopreserved in freezing medium (DMEM containing 20% FBS and 10% dimethyl sulfoxide).

MSCs were isolated from the femora and tibiae of five 6-week old, male Lewis rats (150–174 g; Harlan Laboratories, Indianapolis, IN) [20]. Care of the animals was provided in accordance with the Rice University Institutional Animal Care and Use Committee. Isolation was performed using previously described methods [20]. Briefly, after euthanasia, the tibiae and femora were aseptically removed and the marrow was flushed from each bone using 5 ml of general media. Marrow pellets were collected, broken up, and plated in 75-cm^2 tissue culture flasks. Medium was replaced after one day in order to remove the non-adherent cell population. Cells were cultured for 5 days, after which they were lifted using 0.05% trypsin-EDTA, pooled, and cryopreserved in freezing medium for storage.

MSCs and chondrocytes were then thawed, plated, and expanded in chondrocyte growth medium for 5 days. Cells were then lifted using 0.05% trypsin-EDTA, suspended in chondrocyte growth medium. 30 μl of cell suspension, containing 40,000 cells, was pipetted on top of each scaffold. Scaffolds were seeded with MSCs, chondrocytes, or a 1:3 mixture chondroctyes and MSCs. Scaffolds were then incubated overnight to allow for cell attachment. Empty scaffolds were also incubated for an additional night after prewetting in chondrocyte growth medium. Prior to implantation scaffolds were removed from loading blocks and rinsed in sterile PBS.

2.4. Animal surgeries

Animal surgeries were performed according to protocols approved by the Rice University Institutional Animal Care and Use Committee, and NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) were observed. Forty healthy male Lewis rats (12-weeks old and weighing 300–350 g) were purchased from Harlan Labs (Indianapolis, IN). Animals were