Acta Biomaterialia 10 (2014) 1824–1835

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Direct and indirect co-culture of chondrocytes and mesenchymal stem cells for the generation of polymer/extracellular matrix hybrid constructs

Erica J. Levorson [a], Marco Santoro [b], F. Kurtis Kasper [a], Antonios G. Mikos [a,b,*]

[a] Rice University, Department of Bioengineering, MS-142, 6100 Main Street, Houston, TX 77005-1892, USA
[b] Rice University, Department of Chemical and Biomolecular Engineering, MS-362, 6100 Main Street, Houston, TX 77005-1827, USA

ARTICLE INFO

Article history:
Received 27 July 2013
Received in revised form 27 November 2013
Accepted 13 December 2013
Available online 21 December 2013

Keywords:
Cartilage tissue engineering
Co-culture
Extracellular matrix
Chondrocyte
Mesenchymal stem cell

ABSTRACT

In this work, the influence of direct cell–cell contact in co-cultures of mesenchymal stem cells (MSCs) and chondrocytes for the improved deposition of cartilage-like extracellular matrix (ECM) within nonwoven fibrous poly(ϵ-caprolactone) (PCL) scaffolds was examined. To this end, chondrocytes and MSCs were either co-cultured in direct contact by mixing on a single PCL scaffold or produced via indirect co-culture, whereby the two cell types were seeded on separate scaffolds which were then cultured together in the same system either statically or under media perfusion in a bioreactor. In static cultures, the chondrocyte scaffold of an indirectly co-cultured group generated significantly greater amounts of glycosaminoglycan and collagen than the direct co-culture group initially seeded with the same number of chondrocytes. Furthermore, improved ECM production was linked to greater cellular proliferation and distribution throughout the scaffold in static culture. In perfusion cultures, flow had a significant effect on the proliferation of the chondrocytes. The ECM contents within the chondrocyte-containing scaffolds of the indirect co-culture groups either approximated or surpassed the amounts generated within the direct co-culture group. Additionally, within bioreactor culture there were indications that chondrocytes had an influence on the chondrogenesis of MSCs as evidenced by increases in cartilaginous ECM synthetic capacity. This work demonstrates that it is possible to generate PCL/ECM hybrid scaffolds for cartilage regeneration by utilizing the factors secreted by two different cell types, chondrocytes and MSCs, even in the absence of juxtacrine signaling.

1. Introduction

The tissue engineering paradigm focuses on the combination of cells, scaffolds and signals, with the ultimate goal of regenerating functional tissues. Currently, much effort is focused on the development of bioactive materials that serve as a scaffold, providing structural and mechanical support, and also deliver the bioactive signals to direct cellular differentiation and tissue growth [1–5]. One approach to developing such bioactive materials is to utilize components of the extracellular matrix (ECM) that comprises a particular tissue as a signaling component in an implantable scaffold for the purpose of directing the regeneration of the tissue of interest.

Cartilage, a tissue which comprises relatively few cells, is highly dependent on the ECM for form and function. Furthermore, repair of this tissue in the absence of bioactive signals often results in the repair of a defect with fibrocartilage, which is inferior in compressive strength compared to articular cartilage [6,7]. As such, previous efforts have focused on creating scaffolds composed of processed excised cartilage and those composed solely of isolated collagen and/or glycosaminoglycans (GAGs), as well as engineered three-dimensional (3-D) scaffolds coated with cartilaginous ECM components [5,8–13]. ECM coated polymeric scaffolds garner the benefits of both the natural and synthetic components. The polymeric scaffold provides the strength and durability to support tissue development while the ECM coating acts as a bioactive signal providing necessary cues to direct regeneration by the host's cells upon implantation. Previous work has shown that chondrocytes cultured on a scaffold under flow perfusion are capable of depositing cartilage-like ECM within the scaffold that is effective at inducing the chondrogenic differentiation of mesenchymal stem cells (MSCs) [14].

As potential for the isolation of chondrocytes from healthy cartilage is limited and because chondrocytes often dedifferentiate upon expansion, other culture methods have been explored to

* Corresponding author at: Rice University, Department of Bioengineering, MS-142, 6100 Main Street, Houston, TX 77005-1892, USA. Tel.: +1 713 348 4204; fax: +1 713 348 4244.
E-mail address: mikos@rice.edu (A.G. Mikos).

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.12.026

effectively generate cartilage-like ECM in vitro while reducing the number of chondrocytes needed. One such method is by utilizing co-cultures of chondrocytes and MSCs to generate similar quantities of cartilage-like ECM as cultures of chondrocytes alone [15,16]. Various studies have shown that co-culturing MSCs with chondrocytes leads to increased chondrogenic gene expression and ECM deposition when cultured in direct cell–cell contact, separated by a barrier such as a Transwell® membrane, or in conditioned media systems [17–21]. These phenotypic changes are considered to be the result of signaling via direct cell–cell contacts [17,18,21], as well as secreted factors generated by MSCs and chondrocytes [19,22]. Many studies confirm the secretion of cytokines and growth factors from MSCs exhibiting anti-inflammatory effects in addition to an increase in matrix production by chondrocytes [23–25], while some studies have elucidated positive chondroinduction of MSCs co-cultured with chondrocytes shown to produce growth factors, MMPs and parathyroid hormone related protein [20,26,27]. Previous work showed that a 1:1 ratio of chondrocytes to MSCs was capable of producing similar quantities of cartilage-like ECM as cultures of chondrocytes alone and that the ECM exhibited a similar chondroinductive effect on MSCs as polymer/ECM scaffolds generated using cultures of chondrocytes [15,28].

The objective of this study was to examine the necessity of direct cell–cell contact in co-cultures of MSCs and chondrocytes for the improved deposition of a cartilage-like ECM coating, as defined by an increase in GAG and collagen deposition, within nonwoven fibrous poly(ϵ-caprolactone) (PCL) scaffolds. The hypothesis was that matrix production by chondrocytes co-cultured in direct contact with MSCs would differ in the deposition of cartilage-like ECM from indirectly co-cultured groups due to a potential combined effect of juxtacrine and paracrine signaling. This hypothesis was tested by culturing chondrocytes and MSCs in direct contact by mixing on the same PCL scaffold as well as in indirect co-cultures by seeding the two cell types on two separate scaffolds which were then cultured together in the same system. Utilizing both static and perfused culture conditions separately, PCL/ECM construct generation was then characterized by quantifying GAG and collagen contents as well as through imaging the distribution of cells and matrix throughout the scaffold via histology and scanning electron microscopy.

2. Materials and methods

2.1. Scaffold formation

Non-woven fibrous poly(ϵ-caprolactone) (PCL) scaffolds were fabricated by electrospinning using previously described methods [15]. PCL (18 wt.%) was first dissolved in a 5:1 (v/v) solution of chloroform:methanol and expelled at a flow rate of 25 ml h^{-1} into an electric field formed by a voltage source with 30 kV applied voltage. The collector plate was placed at a distance of 36 cm from the 16 G needle. Following fabrication, scanning electron microscopy (SEM) (FEI Quanta 400 ESEM FEG, FEICo, Hillsboro, OR) was employed to examine fiber morphology as well as to measure the average fiber size for each mat generated. This was achieved by taking a total of 45 measurements from three different locations on the mat using the manufacturer-software from which the average and standard deviation were calculated. Electrospun mats 1 mm thick with an average fiber diameter of 8.5 μm and a standard deviation of 1.2 μm were die cut into 3 mm diameter disks and used for the following cellular studies. The average porosity of the scaffolds was 91% as determined by mercury porosimetry using previously described methods [29].

2.2. Cell isolation and expansion

For this study, a previously validated xenogeneic co-culture model using rabbit MSCs and bovine chondrocytes was utilized [16,30]. Bovine chondrocytes were isolated from the femoral condyles of 7- to 10-day-old male calves obtained from Research 87 (Boston, MA). Following a previously established protocol [14,15,31], the chondrocytes from four legs were isolated via a 16 h incubation with 0.2 wt.% collagenase type II (Worthington, Lakewood, NJ) in culture medium, then pooled and cryopreserved. Before seeding scaffolds for both static and perfusion studies, the chondrocytes were first expanded for 7 days in chondrocyte growth medium consisting of Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA), 10% fetal bovine serum (FBS), 10 mM HEPES buffer (Gibco, Grand Island, NY), 1% non-essential amino acids (Gibco, Grand Island, NY), 0.28 mM ascorbic acid (Sigma, St Louis, MO), 0.4 mM L-proline (Sigma, St Louis, MO) and 1% penicillin–streptomycin–fungizone (Gibco, Grand Island, NY).

All rabbit MSC isolations were approved by the Rice University Institutional Animal Care and Use Committee and were in compliance with the NIH Guide for Care and Use of Laboratory Animals. Rabbit MSCs were isolated from the bone marrow of male New Zealand White rabbits weighing 0.9-1.2 kg using an established procedure [14,15,28,32]. After ~2 weeks of expansion following isolation, the MSCs from six rabbits were pooled and cryopreserved. Prior to scaffold seeding the MSCs were removed from cryopreservation and expanded to passage 3 in general growth medium consisting of DMEM (Invitrogen, Carlsbad, CA), 10% FBS and 1% penicillin–streptomycin–fungizone (Gibco, Grand Island, NY).

2.3. Cell seeding and culture

PCL scaffolds (Ø = 3 mm) were pressed into polycarbonate seeding cassettes and ethylene oxide sterilized for 12 h. Following sterilization the scaffolds were pre-wet with a decreasing ethanol gradient (100-35%) and then rinsed with sterile phosphate-buffered saline (PBS). After pre-wetting, the scaffolds were incubated overnight in general growth medium to facilitate cellular attachment.

The expanded MSCs and chondrocytes were then trypsinized and five cell seeding solutions were prepared. The same cell solutions were used to seed scaffolds for groups AC 35 and iAC 35 as well as MSC 35 and iMSC 35 (Table 1). In this work, iAC and iMSC indicate indirect co-cultured scaffolds containing chondrocytes and MSCs, respectively. Additionally, dAC:MSC indicates the direct co-culture group with chondrocytes and MSCs seeded together on the same scaffold (Fig. 1a and Table 1). The number following the group description indicates the initial seeding density. For the iAC 35 and iMSC 35 groups, 35,000 total cells per scaffold were seeded to control for the total cell number seeded on dAC:MSC scaffolds. Similarly, the seeding density of the iAC 17.5 and iMSC 17.5 groups was intended to control for the number of chondrocytes or MSCs seeded initially on the dAC:MSC scaffolds.

Table 1
Description of experimental groups with cell types and densities seeded.

Experimental group	Chondrocytes per scaffold	MSCs per scaffold
AC 35	35,000	–
MSC 35	–	35,000
dAC:MSC	17,500	17,500
iAC 35	35,000	–
iMSC 35	–	35,000
iAC 17.5	17,500	–
iMSC 17.5	–	17,500