Acta Biomaterialia 10 (2014) 4798–4810

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Enhanced depth-independent chondrocyte proliferation and phenotype maintenance in an ultrasound bioreactor and an assessment of ultrasound dampening in the scaffold

Sanjukta Guha Thakurta, Mikail Kraft, Hendrik J. Viljoen, Anuradha Subramanian *

Department of Chemical Engineering, 207L Othmer Hall, University of Nebraska-Lincoln, Lincoln, NE 68588-0643, USA

ARTICLE INFO

Article history:
Received 8 January 2014
Received in revised form 11 July 2014
Accepted 14 July 2014
Available online 25 July 2014

Keywords:
Tissue engineering
Chondrocyte
Bioreactor
Low-intensity continuous ultrasound
Ultrasound dampening

ABSTRACT

Chondrocyte-seeded scaffolds were cultured in an ultrasound (US)-assisted bioreactor, which supplied the cells with acoustic energy around resonance frequencies (~5.0 MHz). Polyurethane-polycarbonate (BM), chitosan (CS) and chitosan–n-butanol (CSB) based scaffolds with varying porosities were chosen and the following US regimen was employed: 15 kPa and 60 kPa, 5 min per application and 6 applications per day for 21 days. Non-stimulated scaffolds served as control. For BM scaffolds, US stimulation significantly impacted cell proliferation and depth-independent cell population density compared to controls. The highest COL2A1/COL1A1 ratios and ACAN mRNA were noted on US-treated BM scaffolds compared to controls. A similar trend was noted on US-treated cell-seeded CS and CSB scaffolds, though COL2A1/COL1A1 ratios were significantly lower compared to BM scaffolds. Expression of Sox-9 was also elevated under US and paralleled the COL2A1/COL1A1 ratio. As an original contribution, a simplified mathematical model based on Biot theory was developed to understand the propagation of the incident US wave through the scaffolds and the model analysis was connected to cellular responses. Scaffold architecture influenced the distribution of US field, with the US field being the least attenuated in BM scaffolds, thus coupling more mechanical energy into cells, and leading to increased cellular activity.

Published by Elsevier Ltd. on behalf of Acta Materialia Inc.

1. Introduction

The field of tissue engineering promises to yield substitutes that could potentially overcome the limited availability of native explants [1–3]. For example, tissue engineered neo-cartilage with appropriate biomechanical properties holds promise both for graft applications and as a model system for controlled studies of chondrogenesis [4,5]. Research into the "engineering aspects" of cartilage-tissue equivalents typically involves the fabrication of scaffold, design and evaluation of appropriate bioreactors, and controlling stem-cell fate to produce an alternative source of cells [6,7]. Currently, all aspects of the tissue engineering process are being intensively researched, starting with the choice of cell source, cell selection, in vitro cell expansion, scaffold design, cell seeding and bioreactor cultivation and conditioning [8–11]. Typically, many of these aspects are interrelated. For example, while bioreactors are mainly designed to alleviate mass-transfer limitations, they also provide mechanical conditioning to the developing tissue and impact cell colonization depending upon the scaffold microstructure [12–16]. The long-term research objective is to achieve uniform cell distribution and cell differentiation throughout the scaffold volume so that a robust tissue, both biochemically and biomechanically, may be generated.

To obtain uniform cell colonization and cellular ingrowth into the thickness of the scaffold over the duration of culture, scaffold designs offering highly interconnected and accessible pore networks are often fabricated. Most of the scaffolds used in current tissue engineering applications possess pore diameters ranging from 50 to 500 μm, with a total porosity of 48–95% [17]. Other features indicative of successful cell infiltration include pore interconnectivity/tortuosity and scaffold permeability. We note that reduced pore connectivity may indicate closed pores, thus limiting the route for colonization with duration of culture.

Factors that impact cell colonization other than the structural features of scaffold are: (i) the cell seeding method employed which controls the initial spatial distribution of cells; and (ii) mechanical conditioning of the cell–scaffold construct during culture [11,18,19]. In the static surface seeding method, where the cells are first evenly layered on top of the scaffold and cultured, variable results were obtained and many studies report non-uniform cellular distributions [20]. To better exploit the principle

* Corresponding author. Tel.: +1 (402) 472 3463; fax: +1 (402) 472 6989.
E-mail address: asubramanian2@unl.edu (A. Subramanian).

http://dx.doi.org/10.1016/j.actbio.2014.07.013
1742-7061/Published by Elsevier Ltd. on behalf of Acta Materialia Inc.

of convective transport of cells in scaffold seeding, perfusion of cell suspensions through porous polymeric foams in flow bioreactor or under orbital shaking and centrifugation was investigated [18,21–23]. Variable results have been attained with dynamic seeding; orbital shaking has been noted to yield the highest spatial distribution of cells in the construct at 7 days in culture [21]. In general, static or dynamic cell-seeding methods used in conjunction with perfusion bioreactors yield a uniform initial cell distribution.

Conditioning of cell-seeded constructs during culture offers several important advantages compared to static culture systems, such as enhanced mass transfer of O_2 and nutrients by convective fluid flow, the ability to provide mechanical forces influencing tissue development, and better control over culture conditions [24]. The flow of medium through the scaffold porosity benefits cell differentiation by enhancing nutrient transport to the scaffold interior and by providing mechanical stimulation to cells in the form of fluid shear [25,26].

Our previous work has shown that the stimulation of in vitro chondrocyte cultures by low-intensity continuous ultrasound (US) can modulate the signal-transduction pathways leading to chondrocyte-specific gene regulation or RNA translation of a protein product, or both [27,28]. Thus, to capitalize on the positive bioeffects of low-intensity continuous US and apply them to the field of cartilage tissue engineering, our laboratory has designed and developed an ultrasonic bioreactor configuration that uses US to stimulate chondrocytes maintained in an in vitro culture [29]. Aspects of US that would negatively affect cells, including temperature and cavitation, were shown to be insignificant for the US protocols used covering a wide range of frequencies and pressure amplitudes, including the ones used in the present study.

This paper has two research focuses. First, we assess whether culturing chondrocyte-seeded scaffold under low-intensity continuous US stimulation in an US-assisted bioreactor that supplies the cells with acoustic energy around resonance frequencies can yield uniform cell proliferation and cell population density throughout the porous scaffold. Second, we investigate whether the spatial architecture of scaffold and US stimulation can regulate post-expansion redifferentiation and maintenance of chondrocyte phenotype. We posit that the use of the US-assisted bioreactor will result in a higher cell population density throughout the scaffold volume by preventing peripheral encapsulation, and coupled with mechanical stimulation of the cells, will result in an improved chondrogenic response by the bovine articular chondrocytes (BAC) cells cultured on scaffolds.

For the current study, we have used (i) chitosan (CS) scaffolds fabricated via the conventional freeze–drying–lyophilization (FDL) process [30,31]; (ii) chitosan-10% n-Butanol scaffolds with improved porosity prepared via the emulsion FDL [32]; and (iii) polycarbonate–polyurethane-based elastomeric scaffold, a generous gift from Biomerix Corporation, CA. We have employed a static surface-seeding method to minimize the orthogonal effects of flow-assisted cell seeding. We assessed cell proliferation with respect to US stimulation and culture duration. Next, we have evaluated cell population density (i.e. an indirect measure of cell proliferation) at a given depth in the axial direction of the scaffold and their distribution on a particular scaffold via image analysis obtained with confocal microscoscopy. We observed cell morphology with scanning electron microscopy (SEM). Our studies are supported with gene expression analyses for Collagen 1A1, Collagen 2A1, Sox-9, Aggrecan, Collagen 10A1, TGFβ1 and TGFβ3 via real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR), and protein expression analyses for Collagen 1A1, Collagen 2A1, Sox-9 and Aggrecan protein expression by Western blotting. To better explain the experimentally observed cellular distributions, we developed a simplified mathematical model based on Biot theory that (i) captures the essential interactions to predict the propagation of the incident US wave through the scaffolds with different geometries, and (ii) assesses the dampening of the US in the scaffold and, finally, connects the analysis to cellular responses.

2. Materials and methods

2.1. Reagents

Unless otherwise specified, all reagents were of analytical grade or better and were purchased from Sigma Aldrich. CS with a degree of deacetylation of 83% was purchased from Vanson (Redmond, WA) and used without further purification. A polycarbonate polyurethane-based scaffold (Biomerix 3D Scaffold™) was a generous gift from Biomerix, Inc. (Freemont, CA) and is denoted as BM.

2.2. Scaffold preparation

CS scaffolds were prepared by the FDL method detailed elsewhere [30,31]. In parallel, CS was also mixed with 10 vol.% n-butanol and the resultant scaffolds (denoted as CSB) were prepared by emulsion FDL [32]. The CS, CSB and BM scaffolds were cut with a biopsy punch into specimens of $5\,mm \times 2.5\,mm$ (diameter × thickness). CS and CSB scaffolds were neutralized with 0.25 M NaOH followed by thorough rinsing with deionized water. BM, and neutralized CS and CSB scaffolds were either directed to the scaffold sterilization step or dried in the lyophilizer for material characterization.

2.3. Characterization of scaffolds

2.3.1. Variable-pressure scanning electron microscopy (VPSEM)

The morphologies of the scaffolds were characterized by VPSEM (Hitachi S-3000N) at the Center of Biotechnology, University of Nebraska-Lincoln (Lincoln, NE) following the standard procedure detailed elsewhere [33]. Pore diameters were measured using image analysis software (ImageJ™, National Institutes of Health, USA).

2.3.2. Mercury intrusion porosimetry (MIP)

MIP measurements were performed at the Materials Science and Engineering Research Facility at the University of Washington (Seattle, WA). A Micromeritics Autopore IV 9500 porosimeter was used to analyze the samples and Autopore IV software was used to generate pore-related data.

2.4. Cell culture

2.4.1. Bovine chondrocyte isolation and culture

Bovine articular chondrocytes (BACs) were isolated using the standard procedure detailed elsewhere [33]. Frozen cell stocks were thawed and expanded in RPMI 1640 medium supplemented with 10% FBS, 2 g $NaHCO_3$, 1 mM sodium pyruvate, 1 mM antibiotic–antimycotic and 25 $\mu g\,ml^{-1}$ L-ascorbic acid. The same medium was used in the culture of cell-seeded constructs. Cultures were maintained at 37 °C under a 5% CO_2 humidified atmospheric chamber. Passage 2 cells were serum deprived for 24 h by replacing 10% FBS with 0.1% FBS in the culture medium, trypsinized and used in all cell-seeding experiments.

2.4.2. Scaffold sterilization and cell seeding

CS, CSB and BM scaffolds were sterilized with sequential treatments of 70% and 90% ethanol solution for 1 h followed by sterile 1× PBS rinse and incubation in cell culture medium (RPMI with 10% FBS) for 12 h. Prewetted scaffold disks were seeded with

Download English Version:

https://daneshyari.com/en/article/10159061

Download Persian Version:

https://daneshyari.com/article/10159061

Daneshyari.com