Acta Biomaterialia 9 (2013) 6563–6575

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

The influence of scaffold material on chondrocytes under inflammatory conditions

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ARTICLE INFO

Article history:
Received 17 July 2012
Received in revised form 2 January 2013
Accepted 7 January 2013
Available online 16 January 2013

Keywords:
Cartilage matrix production
Scaffold
Pro-inflammatory cytokines
Cytokine release
Water uptake

ABSTRACT

Cartilage tissue engineering aims to repair damaged cartilage tissue in arthritic joints. As arthritic joints have significantly higher levels of pro-inflammatory cytokines (such as IL-1β and TNFα that cause cartilage destruction, it is critical to engineer stable cartilage in an inflammatory environment. Biomaterial scaffolds constitute an important component of the microenvironment for chondrocytes in engineered cartilage. However, it remains unclear how the scaffold material influences the response of chondrocytes seeded in these scaffolds under inflammatory stimuli. Here we have compared the responses of articular chondrocytes seeded within three different polymeric scaffolding materials (silk, collagen and polylactic acid (PLA)) to IL-1β and TNFα. These scaffolds have different physical characteristics and yielded significant differences in the expression of genes associated with cartilage matrix production and degradation, cell adhesion and cell death. The silk and collagen scaffolds released pro-inflammatory cytokines faster and had higher uptake water abilities than PLA scaffolds. Correspondingly, chondrocytes cultured in silk and collagen scaffolds maintained higher levels of cartilage matrix than those in PLA, suggesting that these biophysical properties of scaffolds may regulate gene expression and the response to inflammatory stimuli in chondrocytes. Based on this study we conclude that selecting the proper scaffold material will aid in the engineering of more stable cartilage tissues for cartilage repair, and that silk and collagen are better scaffolds in terms of supporting the stability of three-dimensional cartilage under inflammatory conditions.

1. Introduction

Arthritis is the leading debilitating joint disease caused by the destruction of joint cartilage and is accompanied by inflammation and pain [1–3]. However, articular cartilage has a limited regenerative capacity in vivo, and optimal treatments for arthritis are still lacking. Cartilage tissue engineering has emerged as a potential therapeutic option for cartilage repair [4–7], which generally involves the reconstruction of three-dimensional (3-D) tissues by seeding chondrocytes into natural or synthetic scaffolds, although scaffold-free cultures have also been explored for tissue engineering applications [5,8,9]. In vitro grown cartilage constructs can then be transplanted into the host joint to resume function. In addition to cell-loaded scaffolds, cell-free materials may be placed in the cartilage defects to harbor migrating cells and provide mechanical support to enhance cartilage repair [10–12]. An appropriate scaffold architecture has the advantages of providing further mechanical support and open conduits for the mass transfer of oxygen and nutrients, thus constituting an important part of the chondrocyte microenvironment [5].

In selecting suitable scaffolding materials for cartilage constructs it is critical to consider the biocompatibility and mechanical strength of the material, as well as its ability to support maximum cartilage matrix production [13–15]. As bioengineered cartilage constructs will eventually be transplanted into arthritic joints that have elevated levels of pro-inflammatory cytokines that destroy cartilage, it is especially important to select scaffolds that support the stability of bioengineered cartilage in an inflammatory environment [4,16–18]. Multiple studies have shown that scaffolds

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http://dx.doi.org/10.1016/j.actbio.2013.01.004

made from various biomaterials have different surface features and physical characteristics that affect cell growth, cell attachment and matrix production [19–21]. However, little is known about how the scaffold material may influence homeostasis of the chondrocytes seeded within the scaffolds under inflammatory stimuli.

We hypothesized that the biophysical and chemical properties of the scaffold regulate the expression of cartilage matrix and degradation-related genes in chondrocytes in the presence of pro-inflammatory cytokines. To test this hypothesis we conducted a thorough investigation of cell morphology and gene expression in chondrocytes cultured in scaffolds derived from different biomaterials under treatment with the pro-inflammatory cytokines IL-1β and TNFα. We selected porous scaffolds derived from three different degradable and polymeric materials, silk, collagen (COL) and polylactic acid (PLA), as they are all widely used for tissue engineering [22–25]. As a natural material COL has adequate biocompatibility, biodegradability and a low immunogenic profile, but does not provide strong mechanical support [5,26–28]. Silk, a natural material prepared from silk fibroin of the silkworm, also has adequate biocompatibility. In addition, silk has impressive biomechanical properties [22,25,29–32]. PLA, on the other hand, is a synthetic polymeric material whose properties, such as mechanical strength, degradation rate and dimensions, can all be easily controlled [5]. However, PLA materials have been reported to provoke a higher inflammatory response in the host than silk [28].

In this study we found that primary bovine articular chondrocytes (BACs) when cultured within silk, COL and PLA scaffolds exhibited different cellular morphologies and expressed significantly different levels of cartilage matrix components and destruction genes. Furthermore, we characterized the biophysical properties of the scaffolds in terms of their abilities to release pro-inflammatory cytokines and take up water, which may influence the biochemical responses of chondrocytes under inflammatory conditions. Together, the study results strongly suggest that the scaffolding material plays an important role in the microenvironment of engineered cartilage and regulates the response of chondrocytes under inflammatory conditions.

2. Materials and methods

2.1. Scaffold preparation

3-D scaffolds derived from COL (bovine derived type I and III collagens), and PLA were purchased from BD Biosciences (San Jose, CA). The average pore sizes of the COL and PLA scaffolds were 100–200 μm and the dimensions were 5 × 3 mm (diameter × height), according to the manufacturer's specifications.

Silk scaffolds were prepared as previously described [27,33]. Briefly, cocoons of *Bombyx mori* were boiled for 30 min in an aqueous solution of 0.02 M Na_2CO_3 and rinsed with distilled water to eliminate sericin. Purified silk fibroin was solubilized in 9.3 M LiBr solution and dialyzed against distilled water. The resulting silk fibroin solution was lyophilized and dissolved in hexafluoroisopropanol (HFIP) to obtain a 10% (w/v) silk solution. To create the desired pore size 1 ml of the 10% silk–HFIP solution was added to 3.4 g of NaCl with a particle size of 106–212 μm in disk-shaped containers. The containers were tightly covered and left in a fume hood for 1–2 days for the silk–HFIP solution to evenly mix with the salt particles. The solvent was then evaporated for 3 days at room temperature. The scaffolds were treated with methanol for 1–2 days, and then the methanol was evaporated and the scaffolds were immersed in distilled water for an additional 2 days to extract the salt particles. Porous silk scaffolds were then cut into disks with the same dimensions as the COL and PLA scaffolds (5 × 3 mm (diameter × height)) and autoclaved for cell seeding. The pore sizes of these scaffolds were confirmed by ImageJ analysis of scanning electron microscopy (SEM) images (silk, 170 ± 34 μm; COL, 165 ± 31 μm; PLA, 184 ± 57 μm) [34,35]. The porosities measured by ImageJ analysis on SEM images using an established protocol [36–39] were: silk, 51.6 ± 8.5%; COL, 57.1 ± 4.3%; PLA, 53.4 ± 10.4%.

2.2. Isolation of bovine articular chondrocytes

BACs were isolated as previously described [40,41]. Articular cartilage from all surfaces of a bovine cadaver knee joint (Research 87 Inc., Boylston, MA) was dissected out and transferred to a tube containing phosphate-buffered saline and 10% penicillin/streptomycin. To dissociate the articular chondrocytes from the cartilage matrix minced cartilage pieces (12–15 cm^3 in volume) were treated with 20 ml of 1 mg ml^{-1} hyaluronidase solution (Sigma, St. Louis, MO) for 15 min, followed by treatment with 20 ml of 0.25% trypsin solution (Sigma) for 30 min, and 20 ml of 2 mg ml^{-1} collagenase solution (Sigma) for approximately 15 h at 37 °C. To remove any undigested cartilage to obtain a single cell suspension isolated chondrocytes were twice passed through a 40 μm strainer. The single cell population was resuspended in cell freezing medium (90% fetal bovine serum (FBS) (ThermoScientific HyClone, New Zealand), 10% DMSO (Sigma)), and stored in liquid nitrogen until experimentation. Cell viability was determined using the standard trypan blue staining protocol, where positive staining indicated cell death when isolated cells were mixed with trypan blue solution (Sigma). At isolation cell viability was 97.2 ± 2.4%. After freezing and thawing cell viability was 73.7 ± 4.3%. The viability of thawed cells after 3 days culture was 99.1 ± 4.5%. The purity of the chondrocytes was confirmed by immunocytochemistry for the cartilage marker Sox9, which showed that 99% of the cells were Sox9-positive (Supplementary Fig. S1). Only passage 0 cells (P0) were used for all experiments.

2.3. Cell seeding and 3-D culture

In preparation for cell seeding scaffolds derived from silk, COL and PLA were pre-wetted with sterile Dulbecco's modified Eagle's medium (DMEM) (Gibco, Carlsbad, CA) overnight. Scaffolds were then removed from the medium and chondrocytes were seeded in the scaffolds at a seeding density of 5×10^4 cells per scaffold. This cell density would allow easy access of all chondrocytes to both the scaffolds and pro-inflammatory cytokines. We have found that RNA yield from harvested chondrocytes at this seeding density rose consistently over the 16 day culture period (see Supplementary information), suggesting that cells were viable and proliferating. Based on the dimension of the scaffolds we calculated our initial seeding density to be 3.1×10^3 cells mm^{-3}. Taking into consideration cell proliferation over the culture period the cell density in the scaffolds should be comparable with the cellularity of adult native cartilage tissue (15×10^3 cells mm^{-3}) [42]. After seeding the cell-loaded scaffolds were placed in a humidified tissue culture incubator at 37 °C with 5% CO_2 for 2 h to allow cell attachment. Cell-loaded scaffolds were then cultured in fresh DMEM containing 10% FBS and 1% antibiotic–antimycotic (Gibco) for 8 or 16 days on a shaker (5–6 r.p.m., 6 h day^{-1}) in a tissue culture incubator. Three experimental groups were included in each independent experiment: control, 10 ng ml^{-1} IL-1β, and 10 ng ml^{-1} TNFα (Peprotech, Rocky Hill, NJ). The medium was changed every 2–3 days.

2.4. Scanning electron microscopy

Cell-loaded scaffolds were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at 4 °C overnight. Samples (two scaffolds from two independent experiments from each condition) were then treated with 1% osmium tetroxide for 1 h, dehydrated in