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Influence of macroporous protein scaffolds on bone tissue engineering from bone marrow stem cells

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Abstract

The aim of this study was to investigate the effect of three-dimensional silk fibroin scaffold preparation methods (aqueous and solvent) on osteogenic responses by human bone marrow stem cells (hMSCs). Macroporous 3D protein scaffolds with similar sized pores of $900 \pm 50\,\mu m$ were prepared either by an organic solvent process (hexafluoro-2-propanol, HFIP) or an aqueous process. hMSCs were expanded, seeded on the scaffolds, and cultured up to 28 days under static conditions in osteogenic media. hMSCs seeded onto the water-based silk scaffolds showed a significant increase in cell numbers ($p < 0.01$) vs. the HFIP-prepared silk scaffolds. Significantly higher ($p < 0.01$) alkaline phosphatase (ALPase) activity and calcium deposition were apparent after 28 days of culture in the water-based silk scaffolds when compared to the HFIP-derived silk scaffolds. Transcript levels for collagen type I (Col I), ALP, and osteopontin (OP) increased ($p < 0.05$) in the water-based silk scaffolds in comparison to the HFIP-derived materials. At early stages of culture, increased expression of OP and collagen type II (Col II) were also observed in both scaffolds. Expression of Col II, MMP 13, Col I, and OP proteins increased in the water-based silk scaffolds in comparison to the HFIP-derived scaffolds while bone sialoprotein (BSP) proteins increased in the HFIP-derived silk scaffolds in comparison to the water-based scaffolds after 28 days of culture. Histological analysis showed the development of bone-like trabeculae with cuboid cells in an extracellular matrix (ECM) in the water-based silk scaffolds with more organization than in the HFIP-derived material after 28 days of culture. Alcian blue staining demonstrated the presence of proteoglycan in the ECM formed in the water-based scaffolds but not in the HFIP-prepared silk scaffolds. The results suggest that macroporous 3D aqueous-derived silk fibroin scaffolds provide improved bone-related outcomes in comparison to the HFIP-derived systems. These data illustrate the importance of materials processing on biological outcomes, as the same protein, silk fibroin, was used in both preparations.

Keywords: Silk; Fibroin; Bone; Stem cells; Tissue engineering; Scaffolds

1. Introduction

Bone regeneration via tissue engineering has emerged as an alternative approach in the treatment of malfunctioning or depleted bone. In this approach, a biomaterial scaffold is needed to serve as an adhesive substrate for seeded cells as well as a physical support to guide the formation of the new bone-related ECM. Mesenchymal stem cells (MSCs) are multipotent cells that can replicate as undifferentiated cells with the potential to differentiate and produce mesenchymal tissue like bone, cartilage, tendon, muscle, and marrow stroma [1]. This cell source has received widespread attention because of their potential use in tissue engineering [2–4].

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The design criteria for polymeric scaffolds for bone tissue support include high-porosity structural integrity and degradability at a rate commensurate with the elucidation of new ECM by the seeded cells. A highly porous scaffold is desirable to allow uniform cell migration throughout the material as well as to optimize transport to and from the cells. Pore size plays a role in tissue ingrowth with an internal surface area available for cell attachment, spreading and expansion. The mechanical properties of the scaffold are important especially with respect to hard tissues such as bone in order to transmit mechanical force and manage mineralization requirements [5].

Aside from metals and ceramics, commonly used biodegradable synthetic polymers in 3D porous scaffold formats for bone tissue engineering include poly(glycolic acid) (PGA), poly(lactic acid) (PLA), the copolymers of poly(DL-lactic-glycolic acid) (PLGA), and biodegradable naturally derived polymers such collagen and fibrin [6–11]. The unique strength and resistance to mechanical compression of silk fibroin materials [12–14], the biocompatibility [14–18], the slow rate of degradation [19–21], the utility of this protein in various forms for tissue engineering soft [22,23] and hard [12,24,25] tissue, as well as the longstanding use silk fibroin in suture applications suggest this biomaterial as a suitable substrate for tissue engineering. In our recent studies we have also demonstrated improved bone-related outcomes when MSCs were grown on silk fibroin 3D scaffolds in comparison to collagen scaffolds [24].

We have previously reported that 3D porous silk scaffolds prepared from hexafluoro-2-propanol (HFIP) could be used at a substrate for bone tissue engineering [24]. However, we have also recently reported a new, all-aqueous process to prepare silk scaffolds with a similar morphology to the HFIP-derived materials, but with a significantly higher susceptibility to proteolytic hydrolysis [13]. The higher rates of enzymatic degradability, combined with the more biocompatible aqueous processing approach are hypothesized to enhance MSC responses and scaffold remodeling into bone-like tissue in a more expeditious fashion.

In the present study, we sought to compare these two scaffolds (aqueous vs. HFIP-derived) in terms of MSC responses toward osteogenic outcomes. The significance of this comparative approach is that the two different processing approaches employ the same protein, thus, comparisons in terms of outcomes can be made based on cellular response either due to differences in structure and/or morphology due to the different processing methods. The results suggest that the water-based samples provide enhanced benefits toward osteogenic-related outcomes when compared with the HFIP-derived scaffolds.

2. Materials and methods

2.1. Materials

Fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), Minimum essential medium (MEM) α medium, basic fibroblast growth factor (bFGF), Penicillin–streptomycin (Pen–Strep), Fungizone, nonessential amino acids, and trypsin were from Gibco (Carlsbad, CA). Ascorbic acid phosphate, Histopaque-1077, dexamethasone, β-glycerophosphate, Nonidet P-40 (NP-40), NaF, protease inhibitor cocktail, and phosphatase inhibitor cocktail were from Sigma (St. Louis, MO). All other substances were of analytical or pharmaceutical grade and obtained from Sigma. Silkworm cocoons were kindly supplied by M. Tsukada (Institute of Sericulture, Tsukuba, Japan) and Marion Goldsmith (University of Rhode Island, Cranston, RI).

2.2. Preparation of scaffolds

Aqueous-based silk fibroin scaffolds were prepared by adding 4 g of granular NaCl (particle size; 1000–1180 μm) into 2 ml of 8 wt% silk fibroin solution in disk-shaped Teflon containers (1.8 cm in diameter × 2 cm in height) [13]. The containers were covered and left at room temperature for 24 h, and then immersed in water and the NaCl extracted for 2 days. HFIP-derived silk fibroin scaffolds were prepared by adding 4 g of granular NaCl (particle size; 850–1000 μm) into 2 ml of 8 wt% silk fibroin in HFIP. The containers were covered overnight to reduce the evaporation of HFIP for more homogeneous structures. Subsequently, the solvent was evaporated at room temperature for 3 days. The silk/porogen matrix was then treated in methanol for 30 min to induce the formation of the β-sheet structure and insolubility in aqueous solution. The matrices were then immersed in water for 2 days to remove the NaCl, and then air-dried. The residual HFIP was assessed by X-ray photoelectron spectroscopy (XPS: Surface Science Inc. Model SSX-100).

2.3. Human bone marrow stem cell isolation and expansion

Total bone marrow (25 cm^3, Clonetics, Santa Rosa, CA) was diluted in 100 ml of medium (10% FBS) and prepared as we have previously reported [24]. Briefly, cells were separated by density gradient centrifugation with 20 ml aliquots of bone marrow suspension overlaid onto a poly-sucrose gradient (1077 g/cm^3, Histopaque, Sigma, St. Louis, MO) and centrifuged at 800*g* for 30 min at room temperature. Cells were pelleted and suspended in expansion medium (DMEM, 10% FBS, 1 ng/ml bFGF, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml fungizone, 0.1 mM nonessential amino