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Aligned silk-based 3-D architectures for contact guidance in tissue engineering

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ABSTRACT

An important challenge in the biomaterials field is to mimic the structure of functional tissues via cell and extracellular matrix (ECM) alignment and anisotropy. Toward this goal, silk-based scaffolds resembling bone lamellar structure were developed using a freeze-drying technique. The structure could be controlled directly by solute concentration and freezing parameters, resulting in lamellar scaffolds with regular morphology. Different post-treatments, such as methanol, water annealing and steam sterilization, were investigated to induce water stability. The resulting structures exhibited significant differences in terms of morphological integrity, structure and mechanical properties. The lamellar thicknesses were ~2.6 μm for the methanol-treated scaffolds and ~5.8 μm for water-annealed. These values are in the range of those reported for human lamellar bone. Human bone marrow-derived mesenchymal stem cells (hMSC) were seeded on these silk fibroin lamellar scaffolds and grown under osteogenic conditions to assess the effect of the microstructure on cell behavior. Collagen in the newly deposited ECM was found aligned along the lamellar architectures. In the case of methanol-treated lamellar structures, the hMSC were able to migrate into the interior of the scaffolds, producing a multilamellar hybrid construct. The present morphology constitutes a useful pattern onto which hMSC cells attach and proliferate for guided formation of a highly oriented extracellular matrix.

1. Introduction

The potential of silk as a biomaterial has been widely recognized, owing to its processing versatility, impressive mechanical performance, biocompatibility, oxygen and water vapor permeability and tailorable degradability [1–3]. Silk fibroin (SF) is a structural protein which can be extracted from larvae cocoons of the species *Bombyx mori*. In the process of preparing new SF-based biomaterials, microstructure, porosity and surface chemistry are important features that modulate cell behavior, migration and proliferation towards the desired engineered tissue [4]. Based on this knowledge, SF has been generated in a wide variety of morphologies for engineering different tissues in terms of structure and function [5–13].

Most functional tissues, such as musculoskeletal, tendon and ligament, cardiac, nervous and vascular, present significant cell alignment and anisotropic morphologies. To mimic such intricate structures and develop materials with properties/direction-controlled functions constitutes an important challenge and has attracted considerable interest in recent years. A significant amount of research has been directed towards controlling the spatial organization of cells in well-defined microarchitectures [14–18]. Several techniques have been described to control two-dimensional cellular alignment [15,17,19]. However, cellular organization within three-dimensional (3-D) architectures remains challenging. Considerable progress has been achieved using strategies of mechanical [20,21], electrical [22] or magnetic [23] stimulation. Still, there is a need for simple 3-D systems for investigating cell alignment and guidance cues for various cell types without mechanical force or other external stimulation, for a wide range of applications, from tissue engineering to the control of cellular behaviors such as differentiation and function.

The freeze-drying process has been widely exploited for producing viable porous architectures for tissue engineering, mostly in the case of polymer-based systems [24–29]. This technique does not require additional chemicals, relying instead on the water already present in solution/hydrogel to form ice crystals, which can be sublimated from the polymer, creating a particular microarchitecture. The direction of growth and size of the ice crystals are a function of the temperature gradient and concentration of

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