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Engineered μ-bimodal poly(ε-caprolactone) porous scaffold for enhanced hMSC colonization and proliferation

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Abstract

The use of scaffold-based strategies in the regeneration of biological tissues requires that the design of the microarchitecture of the scaffold satisfy key microstructural and biological requirements. Here, we examined the ability of a porous poly(ε-caprolactone) (PCL) scaffold with novel bimodal-micron scale (μ-bimodal) porous architecture to promote and guide the in vitro adhesion, proliferation and three-dimensional (3-D) colonization of human mesenchymal stem cells (hMSCs). The μ-bimodal PCL scaffold was prepared by a combination of gas foaming (GF) and selective polymer extraction (PE) from co-continuous blends. The microarchitectural properties of the scaffold, in particular its morphology, porosity distribution and mechanical compression properties, were analyzed and correlated with the results of the in vitro cell–scaffold interaction study, carried out for 21 days under static conditions. Alamar Blue assay, scanning electron microscopy, confocal laser scanning microscopy and histological analyses were performed to assess hMSC adhesion, proliferation and 3-D colonization. The results showed that the combined GF–PE technique allowed the preparation of PCL scaffold with a unique multiscaled and highly interconnected microarchitecture that was characterized by mechanical properties suitable for load-bearing applications. Study of the cell–scaffold interaction also demonstrated the ability of the scaffold to support hMSC adhesion and proliferation, as well as the possibility to promote and guide 3-D cell colonization by appropriately designing the microarchitectural features of the scaffold.

Keywords: Gas foaming; Microstructure; Poly(ε-caprolactone); Scaffold; Stem cell

1. Introduction

Tissue engineering aims at the repair of damaged biological tissues circumventing the limitations of the traditional medical approaches, such as transplantation, by using a combination of material, cells and molecular cues [1,2]. One of the most important challenges in tissue engineering is the appropriate design of open-pore biocompatible and biodegradable porous scaffolds that are able to provide a temporary substitute for the extracellular matrix. The scaffold must promote cell adhesion, proliferation and biosynthesis, must allow fluid transport, and must sustain the external mechanical stress until the regeneration of the functional new tissue is completed [3–5].

Although not all the details of scaffold design requirements have been completely defined, a large number of studies have explored the effects of the topographical features of the scaffold on cellular responses [6–9]. These studies demonstrated that the microarchitecture of the scaffold

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may guide cell functions by regulating the interaction between cells, and the diffusion of nutrients and metabolic wastes throughout the three-dimensional (3-D) construct [6–9]. In particular, the scaffold-assisted regeneration of specific tissues has been shown to be strongly dependent on the scaffold's surface-to-volume ratio and pore size and interconnectivity. Indeed, these microarchitectural features significantly influence cell morphology, cell binding and phenotypic expression, but also control the extent and nature of nutrient diffusion and tissue ingrowth [6–9].

Furthermore, it has been suggested that the pore dimensions may directly affect some biological events and, as a result, different tissues require optimal pore sizes for their regeneration [6–8]. Therefore, scaffolds with bimodal-micron scale (μ-bimodal) porosities may be often necessary for the regeneration of highly structured biological tissues, such as bone and cartilage [7].

On the other hand, transport issues, 3-D cell colonization and tissue ingrowth would be inhibited if the pores are not well interconnected, even if the porosity of the scaffolds is high [9].

For the success of any tissue engineering scaffold-based strategy, the issue of the mechanical function is essential, and is one of the critical aspects in scaffold design. Indeed, there is often a conflict between maximizing surface-to-volume ratio to enhance cell colonization and fluid transport, and optimizing the mechanical response of the scaffold. This conflict often leads to a compromise in the optimal design solution [10].

To date, many different strategies have been developed to overcome the transport limitations and the lack of tissue infiltration into the interior of the scaffold, without affecting its mechanical function.

The use of dynamic cell seeding and cultivation devices, in particular perfusion bioreactors or spinner flasks, results in increased proliferation, differentiation and distribution of cells into the scaffold [11–14]. Nevertheless, without an appropriate scaffold microarchitecture, this solution may be unsuitable to ensure the ingrowth of new tissue and to prevent the development of a necrotic core caused by diffusion constraints into the interior of the construct.

In this study, a new approach in the design of μ-bimodal porous scaffold is presented, and the microstructural properties and biocompatibility of the obtained scaffold evaluated. Poly(ε-caprolactone) (PCL) was selected for the preparation of the scaffold because of its proved biocompatibility [15–17] and processability properties [18]. As previously reported [19], the PCL scaffold was prepared by the combination of gas foaming (GF) and selective polymer extraction (PE) from co-continuous blends. In particular, the μ-bimodal porous architecture of the scaffold was designed with a strait porosity of the order of hundreds of microns (macroporosity), for rapid cell colonization and tissue ingrowth, with a built-in one order of magnitude lower porosity (microporosity), that may serve as preferential route for fluid transport.

The microstructural features and the mechanical function of the scaffold were evaluated by scanning electron microscopy (SEM), porosity distribution analysis and static compression testing. Furthermore, the cell–scaffold interaction was studied using human mesenchymal stem cells (hMSCs) in vitro culture, for 21 days and under static conditions. Alamar Blue assay was performed to evaluate cell proliferation, while cell adhesion and 3-D colonization were assessed by SEM, confocal laser scanning microscopy (CLSM) and histological analyses. Finally, the results of the cell–scaffold interaction study were compared with those obtained by seeding hMSCs into PCL scaffolds with monomodal porosity distribution in order to address whether different scaffold microarchitectures may govern cell adhesion and spatial distribution in 3-D porous structures.

2. Materials and methods

2.1. Scaffold fabrication

As schematically illustrated in Fig. 1, the GF–PE technique is characterized by three different steps [19]: (1) 3/2 (w/w) PCL/thermoplastic gelatin (TG) co-continuous blend was prepared by melt mixing in an internal mixer (Rheomix® 600 Haake, Germany) at 60 °C and 80 rpm for 6 min (Fig. 1A); (2) the PCL/TG blend was subsequently gas foamed with a 4/1 (v/v) N_2/CO_2 blowing mixture, at a foaming temperature (T_F) of 44 °C and with a pressure drop rate (PDR) of 700 bar s^{-1} (Fig. 1B); (3) finally, the TG was selectively extracted from the foamed blend by soaking the sample in dH_2O (Fig. 1C).

2.2. Microstructural characterization

2.2.1. Morphology and porosity

The microstructure of the scaffold was analyzed by SEM (S440, Leica, Germany). The scaffold was cross-sectioned, gold sputtered and analyzed at an accelerating voltage of 20 kV.

The overall porosity was determined from the mass and the volume of the scaffold by using the following equation [20]:

$$\%\ \text{porosity} = [1 - (\rho S/\rho_{PCL})] \times 100, \quad (1)$$

where ρ_S is the apparent density of the scaffold calculated from mass and volume measurements. The mass was measured by using a high accuracy balance (10^{-3} g, AB104-S, Mettler Toledo, Italy), while the volume was determined by geometrical calculation. The overall porosity data represents the mean value of five different porosity measurements.

Image analysis (Image J®) was used to assess the volume fraction of the two different scaled porosities by means of area fraction measurements [21,22]. In particular, the area fraction of the TG foamed phase provided the macroporosity volume fraction, while the difference between the mean overall porosity and the macroporosity amount yielded the

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