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Osteogenic differentiation of dura mater stem cells cultured in vitro on three-dimensional porous scaffolds of poly(ε-caprolactone) fabricated via co-extrusion and gas foaming

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Abstract

A novel scaffold fabrication method utilizing both polymer blend extrusion and gas foaming techniques to control pore size distribution is presented. Seventy-five per cent of all pores produced using polymer blend extrusion alone were less than 50 μm. Introducing a gas technique provided better control of pore size distribution, expanding the range from 0–50 to 0–350 μm. Varying sintering time, annealing temperature and foaming pressure also helped to reduce the percentage of pore sizes below 50 μm. Scaffolds chosen for in vitro cellular studies had a pore size distribution of 0–300 μm, average pore size 66 ± 17 μm, 0.54 ± 0.02% porosity and 98% interconnectivity, measured by micro-computed tomography (microCT) analysis. The ability of the scaffolds to support osteogenic differentiation for subsequent cranial defect repair was evaluated by static and dynamic (0.035 ± 0.006 m s^{-1} terminal velocity) cultivation with dura mater stem cells (DSCs). In vitro studies showed minimal increases in proliferation over 28 days in culture in osteogenic media. Alkaline phosphatase expression remained constant throughout the study. Moderate increases in matrix deposition, as assessed by histochemical staining and microCT analysis, occurred at later time points, days 21 and 28. Although constructs cultured dynamically showed greater mineralization than static conditions, these trends were not significant. It remains unclear whether bioreactor culture of DSCs is advantageous for bone tissue engineering applications. However, these studies show that polycaprolactone (PCL) scaffolds alone, without the addition of other co-polymers or ceramics, support long-term attachment and mineralization of DSCs throughout the entire porous scaffold.

Keywords: Polycaprolactone; Polymer blend; Gas foaming; Bioreactor culture; Dura

1. Introduction

Restoration of form and function to the craniofacial skeleton following trauma, congenital malformations or resection remains a considerable clinical challenge for the plastic surgeon. Materials available to treat these conditions include autografts (tissue from the patient), allografts (tissue from a donor) and synthetic materials such as metals, ceramics and polymers. Although autografts provide the best clinical outcome when compared with other material options, complications, including post-operative pain [1], donor site morbidity [2] and infection [3], affect approximately 10–30% of patients [4]. Furthermore, autologous

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tissue may not be an option for large defects given the limitation in supply. In contrast, bone allografts are not limited in supply but often result in poor functional outcome due to limited revascularization, low cellularity and higher resorption rates [2]. Alternatively, metal synthetics are permanent fixtures which suffer from fibrous encapsulation [5], device translocation from bone apposition/resorptive patterns [6], hypersensitivity [7] and long-term stress shielding, ultimately resulting in bone failure [8]. A variety of biodegradable polymers in combination with novel fabrication techniques have been explored as alternative solutions to repair large cranial defects.

In cranial bone repair, dural and periosteal tissue layers directly apposed to cranial bone play an important role in the repair process. Recent findings have suggested that the secretion of cytokines and/or migration of osteoprogenitor cells from these layers contribute to the repair process and functional outcome of cranial regeneration [9–12]. Scaffold parameters such as pore size and pore structure may directly influence the diffusion of factors and migration of progenitor populations from these adjacent tissues into the defect area. Therefore, tailoring such scaffold characteristics to support these activities may significantly improve new bone growth.

Several different techniques have been established to create open pore structures using degradable polymers. The most common three-dimensional (3D) biodegradable polymer scaffolding fabrication techniques include solvent extraction [13], particulate leaching [14], microsphere sintering [15], gel casting [16], phase separation [17] and 3D printing [18,19]. Alternatively, polymer leaching is a particularly attractive technique. This cost-effective method yields fully interconnected porous networks where interconnections are characterized by cylinders and not small pores connecting larger ones. However, maximum porosity is usually limited to 50–60%. Alternatively, combination methods using both particulate and polymer leaching techniques have shown dramatic improvement in pore size control and have enabled the fabrication of scaffolds with multimodal pore size distributions [20].

More recently, Washburn et al. published a co-polymer extrusion technique using two immiscible polymers, where one is water-soluble [21]. In this study, a new scaffold fabrication technique is developed where this polymer blend extrusion method is combined with gas foaming to create scaffolds with optimal pore size distribution. Compared with polymer leaching techniques used to create porous structures, this method uses a water-soluble polymer, poly(ethylene oxide) (PEO), which does not require potentially toxic organic solvents for dissolution. Additionally, PEO itself is non-toxic to cells; therefore residual polymer should not affect cell viability. This technique allows for scaffolds to be fabricated into thin disks (<1 μm) using a microtome, an important feature for cranial defect implants where both rat and mouse skull anatomy dictates thicknesses <1 μm.

The blending process developed by Washburn et al. is accomplished using a twin-screw extrusion compounder. The polymers are added simultaneously to the compounder, heated to the operating temperature (above the melting temperature of both polymers) and the blend is mechanically mixed. Factors that alter the resulting blended morphology include polymer ratios, polymer viscosities and mixing conditions (temperature and shear) [19]. Here, poly(ε-caprolactone) (PCL) a Food and Drug Administration (FDA)-approved biodegradable polyester, is blended with PEO, which is also biocompatible and water-soluble. Following blending of PCL with PEO using a twin-screw extruder, a gas foaming technique is applied to create an open pore structure. Subsequently, the water-soluble PEO is dissolved to enhance the overall pore size.

Utilizing PCL to heal large cranial defects may be particularly advantageous given what is already known about this synthetic polymer. PCL degrades by hydrolytic scission with resistance to rapid hydrolysis via its hydrolytic aliphatic-ester linkage [22]. Degradation times can extend for up to 24 months. PCL scaffolds alone, without co-blending of other polymers, yield mechanical properties adequate for craniofacial bone repair [23]. Additionally, PCL scaffolds support mesenchymal stem cell attachment, proliferation, osteogenic differentiation, and aid in bone repair of critical sized rabbit cranial defects [24,25]. Its degradation, mechanical strength and biocompatibility properties make PCL an excellent polymer for long-term cranial bone applications.

Cells proposed for bone regeneration have been isolated from a range of different sources including bone, bone marrow, skin, adipose tissue and dental pulp [26–30]. Within the craniofacial community, it is widely accepted that progenitor cells located in underlying dural tissue play a crucial role in cranial skull morphogenesis and repair. Strategies to recruit these populations into damaged cranial bone areas have been a focus within our laboratory. Initial studies characterizing dura mater stem cells (DSCs), isolated from local stem cell niches underlying host cranial bone, indicate that these cells show promise as cellular agents for defect repair. Specifically, our group was the first to show cells from the dura mater tissue can be isolated, cultured and expanded in vitro up to passage 60 without changes in morphology or doubling time (2.5 days) [31]. Furthermore, this multipotent population demonstrates robust mineralized matrix deposition following 28 days induction using an osteogenic media [32]. Cells cultured long term on both 2D films and 3D microsphere-based scaffolds of 85:15 PLGA also demonstrate robust matrix deposition [33]. More research is needed to optimize the intended in vivo delivery construct. Others have previously shown that PCL is an excellent synthetic dural substitute showing no infection or significant adhesion to the underlying cortex [34]. Therefore, delivery of transplanted DSCs on a dural substitute material may encourage host DSCs to migrate to the wound bed to participate in tissue repair. Furthermore, this polymer has been shown to exhibit