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Fabrication of degradable polymer scaffolds to direct the integration and differentiation of retinal progenitors

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Abstract

Retinal progenitor cells (RPCs) are self-renewing cells capable of differentiating into the different retinal cell types including photoreceptors, and they have shown promise as a source of replacement cells in experimental models of retinal degeneration. We hypothesized that a biodegradable polymer scaffold could deliver these cells to the subretinal space in a more organized manner than bolus injections, while also providing the graft with laminar organization and structural guidance channels. We fabricated highly porous scaffolds from blends of poly(L-lactic acid) and poly(lactic-*co*-glycolic acid) using a variety of techniques to produce pores oriented normal to the plane of the scaffold. RPCs were seeded on the polymer scaffolds and cultured for 14 days. Seeded scaffolds were then either fixed for characterization or used in an explant or in vivo rat model. The scaffolds were fully covered by RPCs in 3 days. Attachment of RPCs to the polymer scaffold was associated with down-regulation of immature markers and up-regulation of markers of differentiation. This suggests that the scaffold may promote differentiation of RPCs. The seeded cells elaborated cellular processes and aligned in the scaffold in conjunction with degenerating retinal explants. The cells also exhibited morphologies consistent with photoreceptors including a high degree of polarization of the cells. This data suggests that the scaffold may be a means to assist in the promotion of photoreceptor phenotypes. Implantation of the seeded scaffold into the rat eye is associated with increased RPC survival. Taken together, these data suggest that these polymer scaffolds provide a useful means for delivering RPCs to the subretinal space and may assist in the formation of retinal cell phenotypes, although it is clear that more cues are needed to direct the differentiation of RPCs into functional photoreceptors.

Keywords: Polymer; Scaffold; PLGA; Retina; Retinal progenitor cells

1. Introduction

Conditions of retinal degeneration including retinitis pigmentosa (RP) and age-related macular degeneration (AMD) affect over a million people in the US alone, where they comprise the leading cause of irreversible visual disability [1]. Outer retinal degeneration involves the loss of photoreceptor cells and may also involve the cells of the inner nuclear layer which connect to and support the photoreceptors. Neural progenitor cells (NPCs) have been shown to integrate morphologically into the inner nuclear layer in injury models but have not been seen to replace photoreceptors [2–4]. Recently, retinal progenitor cells (RPCs) have been isolated from the mature eye [5,6] and developing retina [7,8] and these cells may have promise for replacing photoreceptors [9].

The retina has a complex, multilayered architecture that is polarized with respect to the photoreceptors which are located at the back of the retina and

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intimately associated with the retinal pigment epithelium (RPE). Light must travel through the retinal processing layers before reaching the photoreceptor outer segments where phototransduction occurs. The integrity of the RPE layer is critical to the survival and function of photoreceptors and hence patterned vision. RPC grafts alone may be insufficient to recreate this complex cytoarchitecture on a large scale, particularly when multiple retinal layers have been lost or disrupted [10,11]. A second challenge for the use of RPCs is the delivery and survival of the cells. Studies indicate that the typical bolus injection of RPCs leads to a large degree of cell death. Another concern is reflux of cells from the subretinal space into the vitreous cavity at the time of injection. Vitreal cells can obscure the light path or in some instances even result in retinal detachment [12]. In addition, the widespread intraretinal migration frequently observed with these cells may not be desirable in all settings. For instance, the organized reconstruction of specific cellular layers may require that precise constraints be placed upon grafted cells.

We hypothesized that a tissue engineering approach, namely, using a degradable polymer scaffold with the appropriate architecture, might improve the survival and promote the organized differentiation of grafted RPCs in models of retinal degeneration and injury. In the intact retina, the orientation of photoreceptors and retinal bipolar cells is radial with respect to the curvature of the globe. To approximate the physical characteristics of this microenvironment, we experimented with a variety of techniques, including phase-inversion casting [13] and solid–liquid phase separation [14], to create porous scaffolds with pores oriented normal to the plane of the scaffolds. These scaffolds were then seeded with cells, the cells were characterized via RT-PCR and immunohistochemistry, and seeded scaffolds were co-cultured with degenerating retinal explants or implanted in vivo in models of retinal degeneration. The results of this work indicate that degradable polymer scaffolds improve the survival of RPCs in retinal degeneration models, promote differentiation of RPCs, and provide physical guidance to the RPCs resulting in a more normal anatomical organization.

2. Experimental

2.1. Polymer fabrication

2.1.1. Materials

Poly(lactic-*co*-glycolic acid) (PLGA) with a lactic to glycolic acid ratio of 50:50 and a number average molecular weight of Mn ~35,000 g/mol, noted here as PLGA 504, PLGA with a lactic to glycolic acid ratio of 50:50, a carboxylic acid end group, and a number average molecular weight of Mn ~25,000 g/mol, noted as PLGA 503H here, and PLGA with a lactic to glycolic acid ratio of 75:25 and a number average molecular weight of Mn ~45,000 g/mol, referred to as PLGA 755, were obtained from Boehringer Ingelheim GmbH (Germany). Poly(L-lactic acid) (PLLA) with a number average molecular weight of Mn ~100,000 g/mol, referred to here as PLLA 100k, and PLLA with a number average molecular weight of Mn ~50,000 g/mol, referred to here at PLLA 50k, were obtained from Polysciences (Warrington, PA). All solvents were from Aldrich (St. Louis, MO) (ACS Grade).

2.1.2. Phase-inversion membrane formation

Solutions were prepared with concentrations of 10, 20, 25 and 30% (w/v) polymer (either PLGA 504 or PLGA 503H) in dimethylsulfoxide (DMSO). 5–10% (v/v) of glycerol was added to the solutions to promote the formation of larger and less asymmetric pore structures. 0.4 ml of the complete solution was added to a glass slide (dimensions) and allowed to spread evenly over the entire surface of the slide. The glass slide was then immersed in 18 M-Ω water (MilliQ system, Millipore, Billerica, MA) at room temperature and the solvent transfer process initiated. Slides were removed from the water once the transfer process was complete, approximately 10 min after immersion. The completion of the transfer was indicated by the absence of solution at the glass-slide interface. Samples that completed the transfer were easily removed from the glass slides without any apparent sticky residue. Membranes were then dried with blotting paper and lyophilized overnight to remove residual water and solvent.

2.1.3. Solid-liquid phase separation

Five percent (w/v) solutions of PLGA, PLLA, or blends of PLGA/PLLA in dioxane were prepared. 0.3–0.5 ml of solution was added to a glass slide and allowed to spread uniformly across the slide. The slide was then placed on an ice bath so that the slide was in good contact with the ice, but residual water was not allowed to interact with the solution. After 1 min, a 20-gauge piece of copper wire, which had been sitting in dry ice, was touched to the surface of the slide. This initiated nucleation of dioxane crystals. The growth front spread along the surface of the slide and then moved normal to the slide, growing towards the solution/air interface. Once the dioxane had completely solidified, the slide was transferred to a freezer at −20 °C for at least 1 h. Slides were then placed on a lyophilizer to sublimate the dioxane, leaving behind the polymer scaffolds whose pore structure was a direct artifact of the freezing process.

During the above procedure, slides would occasionally become immersed in the ice water solution before the dioxane crystallization process was nucleated. These samples were placed in the freezer and lyophilized