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ScienceDirect
Biomaterials 28 (2007) 2192–2201
Biomaterials
www.elsevier.com/locate/biomaterials

Spatial cues for the enhancement of retinal pigment epithelial cell function in potential transplants

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Received 8 November 2006; accepted 4 January 2007
Available online 11 January 2007

Abstract

Retinal pigment epithelial (RPE) cellular morphology and function are vital to the health of the retina. In age-related macular degeneration, RPE dysfunction and changes in Bruch's membrane occur. Thus, a potential cure is a dual-layer biomimetic transplant consisting of a layer of healthy RPE cells cultured on a support membrane. In this study, we investigated human anterior lens capsule as a replacement for Bruch's membrane and also explored different seeding methods as ways of inducing the desired cellular morphology and function. Using *in vitro* assays, we demonstrated that RPE cells cultured on lens capsule exhibited epithelial characteristics, such as the presence of actin belts and the formation of tight junctions in the monolayer. Bovine photoreceptor outer segments were also incubated with the RPE cells in order to quantify the binding and ingestion activity of the RPE cells. With these assays, we determined that cells seeded by centrifugation appeared to possess the most epithelial-like morphology, with the shortest overall length and the smallest elongation. They also exhibited enhanced metabolic activity, with a 1.5-fold increase over conventional gravity seeding. Thus, the spatial cues provided by centrifugation may assist cells in assuming native RPE function. Therefore, a dual-layer transplant, with RPE cells organized by centrifugation onto lens capsule, appears promising in achieving native retinal function.

Keywords: Cell morphology; Epithelial cell; Membrane; Micropatterning; Ophthalmology; Retina

1. Introduction

The retinal pigment epithelium (RPE) is critical to vision because of its supporting role in maintaining the health of the overlying retina. However, RPE dysfunction can occur, such as in age-related macular degeneration (AMD), a disease which results in a loss of central vision. Over 10 million Americans are affected by AMD, with the loss of RPE cells occurring in the macula [1]. Unfortunately, the RPE has low regenerative capacity in the eye and only limited migration from the periphery. Thus, there are insufficient means of repopulating the macula, and transplantation of functional cells may be necessary to treat the dystrophy. In addition, accumulation of waste material in the underlying Bruch's membrane in AMD may also necessitate its replacement. Thus, a dual-layer transplant to replace both the RPE and Bruch's membrane has been under consideration [2–4]. However, there are various challenges that must be overcome in order for the transplant to be successful. Among the most important requirements to consider are avoiding immune rejection and mimicking *in vivo* function.

In this paper, we focus on the *in vivo* function that a retinal transplant must possess. In order to achieve the proper function, mimicking the morphology of the cells should be strived for since structure has been linked to function [5–8]. The RPE is a monolayer of columnar cells that are of compact size in the central retina (~10–14 μm wide) but are more spread in the periphery (60 μm wide). Hexagonal in shape, the cells also express apical/basal polarization. On the apical side, they are characterized by bundles of actin that are organized as belts around the cell

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doi:10.1016/j.biomaterials.2007.01.018

and by the presence of tight junctions that act as a blood-retinal barrier. Since the RPE is situated between the photoreceptors and its blood supply, the choriocapallaris, they form a semi-permeable membrane that regulates the flow of fluid, ions, and nutrients that travel between the choriocapallaris and the photoreceptors.

Also characteristic of the RPE is the presence of microvilli on the apical surface which are interdigitated with the photoreceptors. Hence, the RPE performs the crucial process of phagocytizing photoreceptor waste. In fact, the RPE is one of the most metabolically active cells in the body, with each cell renewing approximately 40 photoreceptors [9]. In the process of converting the light signal into an electrochemical response, the photoreceptor outer segments, which are composed of stacks of disks, become spent and require renewal. On a cyclic basis, the RPE ingests the tips of outer segments, degrades them, and transports the soluble waste material across the basal side into the choriocapillaris. As a result of diminished phagocytic capacity in diseased retinal pigment epithelial cells, insoluble material accumulates in Bruch's membrane and in the RPE, which are symptoms of AMD.

In attempts to culture cells that will duplicate the essential RPE functions, different potential substrates have been considered. For replacement membranes, candidates include cadaver Bruch's membrane, Descemet's membrane, anterior lens capsule, amniotic membrane, and synthetic polymers [2,3,10–14]. The best candidate, however, may be autologous tissue from the patient who requires the transplant. In our studies, we have investigated human anterior lens capsule because of easy availability through removal during cataract surgery and its potential to be an autologous transplant.

The lens capsule is an acellular tissue that encapsulates the lens. It is a basement membrane for lens epithelial cells, and it is composed of mainly proteins, such as collagen IV, heparan sulfate proteoglycan, and fibronectin. Given its similar composition to Bruch's membrane, the lens capsule may serve as a good substitute. As evidence of its inherent biocompatibility, studies have shown that autologous lens capsule transplants into the subretinal space were well tolerated, with no signs of inflammation [15]. In addition, prior studies have reported that the human lens capsule is a good substrate for cell growth [4,16,17]. In the present study, we investigate whether cells grown on the lens capsule also exhibit native function, such as the formation of tight junctions and the phagocytosis of photoreceptor outer segments.

To assess the RPE cell function, we use *in vitro* methods, such as immunofluorescence and phagocytosis assays. Specifically, the assays include an antibody to zonula occluden to show the presence of a major component of tight junctions, the stain phalloidin to illustrate the location and organization of filamentous actin, and an antibody to rhodopsin to quantify the binding and ingestion of bovine photoreceptor outer segments. We compare two different substrates for cell culture, human anterior lens capsule and polyester filters. The transwell polyester filter has been used extensively in cell culture studies because the porous substrate provides the cells with fluid access from both sides and has been shown to promote apical/basal polarization in RPE cells [17–19]. Thus, we use it as a control substrate and compare the morphology of cells cultured on the filters to those grown on lens capsule.

Finally, we examine whether the morphology of the cells can be improved and their activity enhanced by applying different methods to control cellular adhesion and spreading. Standard procedure for seeding cells onto substrates is to allow the cells to settle and attach simply under the force of gravity. Typically, the cells attach and spread over a wide range of times, leading to a monolayer of mixed morphology. In recent years, soft lithography has been utilized to provide control over cellular adhesion [20,21]. In this work, we include soft lithographic methods in our studies on cell function. In particular, cells were directed to adhere in patterns in attempts to mimic the *in vivo* morphology. The cells were restricted from spreading for a few days, providing some organization in the created RPE layer. We also investigate a third method of seeding cells. We examine the effectiveness of centrifuging the cells onto the surface in order to decrease the time necessary for cellular attachment to occur and to obtain some degree of homogeneity in cellular morphology. In sum, we compare the morphology and activity of the cells seeded by these three methods to determine if enhancement is achieved by the spatial cues that patterning and centrifugation provide.

2. Materials and methods

2.1. Lens capsule preparation

Human lens capsules extracted during cataract surgery (IRB protocol ID # 74930) were prepared as described in a previous study [16]. Lens capsules were sterilized under UV (254 nm) irradiation for 3 h and were treated with 0.05% trypsin–EDTA (Invitrogen, Carlsbad, CA) to remove native epithelial cells from its surface. Under a stereoscope, the lens capsule was spread out carefully in a single layer onto a polyester transwell filter (1 cm^2, Corning, Acton, MA) to give it structural integrity.

2.2. Soft lithography

Soft lithographic techniques were modified to allow for the printing of poly(vinyl alcohol) (PVA) as described previously [16]. Poly(dimethylsiloxane) (PDMS) (Sylgard 184, Dow Corning Corporation, Midland, MI) stamps, which were released from a patterned silicon wafer, possessed features that included an array of recessed hexagons 20 μm in diameter, separated by protruding lines 2 μm in width. To pattern lens capsule and polyester filter, the PDMS stamp was used to transfer the solution of 2% PVA (MW 70,000–100,000, Sigma, St. Louis, MO) and 0.1 mg/mL fluorescein (Sigma, St. Louis, MO) in a spatially resolved pattern. After removal of the elastomer stamp, the micropatterned lens capsules or polyester filters were sterilized under a UV lamp (254 nm) for 3 h.

2.3. Cell seeding

Human retinal pigment epithelial cells (ARPE-19, ATCC, Manassas, VA) were maintained in D-MEM/F-12 supplemented with 10% fetal