Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 27 (2006) 1670–1678
Biomaterials
www.elsevier.com/locate/biomaterials

Determination of human lens capsule permeability and its feasibility as a replacement for Bruch's membrane

Christina J. Lee[a], Jonathan A. Vroom[a], Harvey A. Fishman[b,1], Stacey F. Bent[a,*]

[a]Department of Chemical Engineering, Stanford University, Stanford, California 94305-5025, USA
[b]Department of Ophthalmology, Stanford University School of Medicine, Stanford, California 94305-5308, USA

Received 2 June 2005; accepted 1 September 2005
Available online 30 September 2005

Abstract

We have investigated human anterior lens capsule as a potential replacement for Bruch's membrane as a treatment for age-related macular degeneration. Any substrate to replace Bruch's membrane should possess certain characteristics to maintain proper function of the overlying retina. One of the important properties of Bruch's membrane is allowing the flow of nutrients and waste between the retinal pigment epithelium and the choriocapillaris. Here, we measured the permeability of the lens capsule by studying the diffusion of various molecular weight FITC–dextran molecules. Expressions for extraction of diffusion coefficients from concentration vs. time data from a blind-well chamber apparatus were derived for both a single and double membrane experiments. The diffusion coefficients in the lens capsule were found to be in the range of 10^{-6} to 10^{-10} cm^2/s. We demonstrated a power law relationship, with the diffusion coefficient possessing a -0.6 order dependence on molecular weight. The molecular weight exclusion limit was determined to be 150 ± 40 kDa. We have compared this value with reported values of Bruch's membrane molecular weight exclusion limit and find that the lens capsule has the potential to act as a substitute Bruch's membrane.

Keywords: Diffusion coefficient; Retina; Lens capsule; Dextran; Membrane

1. Introduction

Among individuals over 65 years of age, age-related macular degeneration (AMD) is the leading cause of blindness in industrialized nations [1]. It is often characterized by a loss of retinal pigment epithelial (RPE) cells and, in 10–20% of the cases, by the formation of new blood vessels (choroidal neovascularization (CNV)). Each RPE cell supports approximately 40 photoreceptors [2] by regulating the flow of nutrients and by metabolizing their wastes (renewing their outer segments); thus, the health of the RPE cells is vital to proper function of the retina. CNV is also a threat to vision, because the propensity of the new vessels to bleed can lead to scarring of the retina. Although there are treatments that destroy the new blood vessels [1], there is no available cure for AMD that restores RPE cells or the overlying retina.

The cause of AMD has yet to be proven, but evidence suggests that it is associated with RPE cell dysfunction and changes in its underlying basement membrane (Bruch's membrane) [3]. Specifically, one theory is that RPE cells stop degrading photoreceptor waste products properly, leading to accumulation of waste material in Bruch's membrane [3]. Since this material is high in lipid content, it causes changes in the membrane's composition and the membrane increases in thickness. As a consequence of these changes, the permeability of Bruch's membrane to nutrients, oxygen, and hormones is lowered. This results in a decreased availability of nutrients to the RPE and may contribute to the atrophy of the RPE cells and of the overlying photoreceptors, ultimately causing vision loss.

Replacement of the diseased RPE cells with healthy ones may provide a potential treatment for AMD. Limited success has been achieved in studies that inject a suspension of cells [4,5] and that transplant a sheet of cells [6,7].

*Corresponding author. Tel.: +1 650 723 0385; fax: +1 650 723 9780.
E-mail address: bent@stanford.edu (S.F. Bent).
[1]Current address: Plager Vision Center, Santa Cruz, CA 95065, USA.

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.09.008

A suspension of cells may result in multilayers which could lead to the improper orientation of the cells with respect to the photoreceptors. On the other hand, while sheets of RPE alone can possess the proper orientation, they may not adhere well to abnormal Bruch's membrane [8,9]. Therefore, it has been suggested that replacing the basement membrane in addition to RPE cells may be necessary for a successful transplant for AMD. Here we focus on evaluating a biocompatible substrate as a replacement basement membrane.

Bruch's membrane is a thin tissue, 2–4 μm thick, which lies between the retina and the choroid. It consists of five layers: basement membrane of the choriocapillaris, outer collagenous layer, elastin layer, inner collagenous layer, and basement membrane of the RPE. It is a complex, multilayered, gridlike structure composed mainly of glycoproteins: collagen types I and IV, laminin, fibronectin, and elastin [10]. It supports cell adhesion of both the RPE and the choriocapillaris. Additionally, Bruch's membrane allows for the transport of nutrients from the blood supply to the RPE and for the transport of waste products from the RPE to the choriocapillaris. Thus, any changes in Bruch's membrane that interfere with this transport pose a risk to the viability of the RPE and the photoreceptors.

Candidates to replace Bruch's membrane must possess similar properties as the native tissue. They should have comparable thickness, similar permeability to nutrients, and inherent ability to support cell adhesion in order to maintain the health of the retina. In addition, like in all implants, the candidates must be biocompatible. Many candidates to replace Bruch's membrane have been studied [11], such as anterior lens capsule [12,13], cadaveric Bruch's membrane [8], Descemet's membrane [14], synthetic biodegradable polymer films [15], and synthetic collagen type I [16]. Here we focus on lens capsule because it can act as a permanent barrier to prevent CNV and can be a potential autologous implant to circumvent the immune response. Additionally, it is easily obtained, as it is routinely removed in cataract surgery.

The lens capsule, which encapsulates the lens, is a basement membrane for lens epithelial cells. It is composed mainly of proteins, such as collagen IV, heparan sulphate proteoglycan, and fibronectin. The thickness of the human anterior lens capsule varies from 15 to 20 μm, with the higher thicknesses found as the eye ages [17]. Given its similar composition, the lens capsule may serve as a good substitute for Bruch's membrane. As evidence of its inherent biocompatibility, studies have shown that autologous lens capsule transplants into the subretinal space have been well-tolerated, with no signs of inflammation [12]. In addition, prior studies have reported that the human lens capsule is a good substrate for cell growth [18].

In the current study, we examine the permeability of lens capsule to determine if it is a viable replacement for Bruch's membrane. As previously mentioned, the nutrients to the RPE must be supplied from the choriocapillaris. Vitamin A, for example, is one such nutrient. Vitamin A is bound to a carrier to form a complex before being transported across Bruch's membrane, leading to a combined molecular weight of 75 kDa [19]. Therefore, Bruch's membrane is permeable to compounds of considerable mass and the replacement membrane must also possess these same diffusive properties to nutrients in order to support the overlying RPE.

While there have been numerous reports on permeability of ocular tissues such as cornea and sclera [20,21], there have been very few published reports on the permeability of lens capsule [22]. In addition, the existing literature on ocular tissues varies widely in the type of permeability values that they report. Commonly reported units are the diffusion coefficient (units of area/time), the permeability coefficient (length/time), and the flux (mass/time-area). The different values make comparisons between studies challenging because the values of flux and permeability coefficient are dependent on the experimental conditions, such as the thickness of the membrane and the initial concentration gradient. Therefore, we report here on the permeability of lens capsule in terms of diffusion coefficient (cm^2/s) because it is not dependent on experimental conditions.

2. Materials and methods

2.1. Tissue preparation

Human anterior lens capsules extracted during cataract surgery (IRB protocol ID # 74930) were stored in phosphate buffered saline (PBS) at 4 °C. For sterilization, the lens capsule was incubated for 30 min in a penicillin–streptomycin solution (6.25 mg/mL penicillin, 10 mg/mL streptomycin) and was exposed to UV light (254 nm) for 3 h. Native epithelial cells were removed with trypsin (0.05% trypsin–EDTA for 10 min at 37 °C) to provide a clean, initial substrate. With the aid of tweezers and a flexible, plastic spatula, the lens capsule was removed from solution and, under a stereoscope, was spread out in a single layer onto a piece of filter paper (mixed cellulose ester, 0.2 μm pore size, Millipore, Billerica, MA) to give it structural integrity. It was then allowed to dry in a laminar flow hood. (All reagents were obtained from Invitrogen, Carlsbad, CA.)

2.2. Diffusivity measurements

The diffusivity measurements were performed in blind-well chambers (Neuro Probe, Inc., Gaithersburg, MD). Fig. 1A is a schematic of the experimental apparatus. Fig. 1B shows the associated boundary conditions, which will be discussed in the next section. Two chambers, each with a capacity of 200 μL, were separated by a membrane with an exposed area of 18 mm^2. The bottom well (V_1) was filled with a fluorescein isothiocyanate–dextran (FITC–dextran) solution at an initial concentration C_1^0 between 1 and 10 mg/mL and the top well (V_2) was filled with PBS. The second chamber screwed down onto the membrane, sealing it such that the only transport of material was through the membrane. The dextran diffused across the membrane from the lower well to the upper well. No leakage around the membrane was observed with the use of a negative control. For the control, the membrane was replaced by a nonporous sheet of mylar plastic and no solute was detected in the second chamber.

The opening in the blind-well chambers was 4.88 mm in diameter. Since it was difficult to obtain a large, continuous piece of tissue without holes or tears, the system was modified to give the membrane a smaller exposed area. The lens capsule was spread onto a piece of filter paper (mixed