Experimental Eye Research 121 (2014) 194–199

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Mechanical properties of murine and porcine ocular tissues in compression

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ARTICLE INFO

Article history:
Received 4 November 2013
Accepted in revised form 18 February 2014
Available online 5 March 2014

Keywords:
retinal degeneration
transplantation
implant
retinal modulus

ABSTRACT

Sub-retinal implantation of foreign materials is becoming an increasingly common feature of novel therapies for retinal dysfunction. The ultimate compatibility of implants depends not only on their *in vitro* chemical compatibility, but also on how well the mechanical properties of the material match those of the native tissue. In order to optimize the mechanical properties of retinal implants, the mechanical properties of the mammalian retina itself must be carefully characterized. In this study, the compressive moduli of eye tissues, especially the retina, were probed using a dynamic mechanical analysis instrument in static mode. The retinal compressive modulus was lower than that of the sclera or cornea, but higher than that of the RPE and choroid. Compressive modulus remained relatively stable with age. Conversely, apparent retinal softening occurred at an early age in mice with inherited retinal degeneration. Compressive modulus is an important consideration for the design of retinal implants. Polymer scaffolds with moduli that are substantially different than that of the native tissue in which they will ultimately reside will be less likely to aid in the differentiation and development of the appropriate cell types *in vitro* and will have reduced biocompatibility *in vivo*.

1. Introduction

Inherited retinal degenerative diseases such as retinitis pigmentosa, Leber congenital amaurosis, and Stargardt disease are characterized by death of the light sensing photoreceptor cells of the outer neural retina and irreversible blindness. For neurodegenerative diseases such as these, drug and/or gene therapy alone may not suffice, especially in patients who have suffered extensive photoreceptor cell loss prior to molecular diagnosis of their gene defect. Under these circumstances, strategies focused on cellular or tissue replacement will be beneficial. Many studies suggest that the use of stem cells to achieve such a goal is now feasible (Barber et al., 2013; Gonzalez-Cordero et al., 2013; Gust and Reh, 2011; Klassen et al., 2004; La Torre et al., 2012; Lakowski et al., 2011; Lamba et al., 2009, 2010; Ma et al., 2011; MacLaren et al., 2006; Tucker et al., 2010, 2011; 2013; West et al., 2012; Yao et al., 2011); however, a major remaining hurdle is the development of an optimal cell transplantation system. Current delivery methods typically result in massive cell loss and limited cellular integration following transplantation. For instance, several studies have shown that following bolus photoreceptor cell injection, less than 0.01% of transplanted cells survive and even fewer actually integrate within the host retina (Klassen et al., 2004; MacLaren et al., 2006). In large part, poor integration can be attributed to the lack of donor cell support following the bolus injection. These results are particularly common when attempting to perform subretinal transplants in late stage retinal degenerative hosts that have lost the majority of their outer retina due to photoreceptor cell death.

In an attempt to increase cellular survival and subsequent integration following retinal progenitor/stem cell (RPC) transplantation, several researchers have designed polymer scaffolds as

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http://dx.doi.org/10.1016/j.exer.2014.02.020

vehicles for delivery of drugs or cells to the subretinal space (da Silva et al., 2010; Diniz et al., 2013; Hu et al., 2012; Janoria et al., 2007; Pritchard et al., 2010; Tucker et al., 2010; Winter et al., 2008). Most implanted materials are tested for material or chemical biocompatibility (which is clearly an essential property of an implanted polymer) prior to implantation using *in vitro* culture techniques. However, the mechanical properties of material also play an important role in the ultimate compatibility, efficacy and outcome of the implant *in vivo*. To overcome potential rejection issues and for stimulation of optimal cellular differentiation and transplant integration, it is generally believed that the mechanical properties of an implanted material should match those of the recipient tissue as closely as possible. Thus, directing stem cell differentiation and cellular proliferation with mechanical cues (Zoldan et al., 2011; Lee et al., 2013) has long been utilized for generating cartilage (Little et al., 2011) and bone (Parekh et al., 2011). More recently this concept has also been applied to materials meant to regenerate softer tissues such as tendons (Kinneberg et al., 2011), cardiac valves (Wang et al., 2013), cardiac muscles (Guillemette et al., 2010; Chen et al., 2008), and neurons (Banerjee et al., 2009; Yao et al., 2013). In order to obtain an optimal match between implant and native tissue, therefore, the mechanical properties of the native tissue need to be thoroughly characterized in normal and diseased states.

Although some mechanical properties of the retina were described as early as 1987 (Wu et al., 1987), the extremely delicate nature of this neural tissue has limited the extent of its characterization. Attaching retinal samples to displacement probes, for example, is a commonly reported challenge, originally overcome by using synthetic adhesive to bind the retina to the probes. More recent work has addressed this challenge by using mechanical pressure to tightly clamp samples to analysis probes (Chen et al., 2010; Wu et al., 1987), leading to examination of retinal mechanical properties of the retina using uniaxial tension. Some insight into the pathophysiology of retinal tearing was gained following these analyses. For instance, the retina was found to have a relatively short (compared to the choroid) reversible elastic phase followed by a large irreversible plastic deformation (Wollensak and Spoerl, 2004). From these stress and strain studies, the tensile modulus of the retina was estimated to be about 100 kiloPascals (kPa) (Basinger et al., 2009; Chen and Weiland, 2012).

As described, the mechanical properties of the retina have been characterized thus far using tension applied parallel to the isotropic retinal plane. Given the layered nature of the retina, however, mechanical properties are not likely to be the same if characterized using compression perpendicular to the isotropic plane of the retina. For the purpose of developing cell delivery scaffolds, the transverse compressive mechanical properties are a better representation of actual mechanical pressures encountered by cells and tissues during chronic implantation. In this study, the compressive modulus of porcine and murine ocular tissues was examined. The effects of aging and disease on the murine retinal compressive modulus were also characterized. In an effort to begin to identify mechanically appropriate materials for retinal differentiation and transplantation, the transverse compressive modulus values for retinal tissue were compared to relevant synthetic materials.

2. Materials and methods

2.1. Animals and dissection

For testing of mouse retinal modulus, C57Bl/6J were used as a *wild type* control line (stock number 000664; Jackson Laboratories, Bar Harbor, ME). To test the effect of retinal degeneration on mouse retinal modulus we used the mutant line C3H/HeJ^{Pde6rd1} (stock number 000659; Jackson Labs), which develops rapid retinal degeneration due to rod photoreceptor cell death. Mice were euthanized using CO_2 inhalation, followed by cervical dislocation. Eyes were enucleated and the anterior segment was removed by carefully cutting around the circumference of the eye along the limbus. Once exposed, the lens and vitreous were removed, leaving the posterior cup consisting of the retina, choroid and sclera. Retinas were then carefully separated and maintained in their natural cup-like form with the cupped portion open, facing up in the sample basin. The media was removed to allow surface tension to flatten the tissue and the edges of the retinal cup were gently teased out to their most extreme limit, causing flattening of the retina, without damaging the sample (Fig. 2A and B). Each mouse retina measured was treated identically. Pig eyes (Iowa outbred swine, 5 months of age) were obtained from a local slaughterhouse, transported on ice and dissected within 2 h of harvest. Whole pig globes were processed in the same manner as mouse eyes above, except the retina was not removed from the choroid. Instead, a 5 mm biopsy punch was used to cut out retinal samples for modulus testing, which were then separated from the choroid and other layers. A biopsy punch was also used to isolate specimens of cornea, choroid and sclera for testing. Prior to modulus measurements, all tissues were maintained in 1× Hank's buffered salt solution (HBSS, 340 mOsm/L, Sigma–Aldrich, St. Louis, MO).

2.2. Polymers

Polydimethylsiloxane (PDMS, SYLGARD® 184 silicone elastomer kit, Dow Corning, Midland, MI) samples were formed using a 10:1 ratio of base to crosslinker. The blend was mixed by vortexing, poured into a petri dish, and cured in an oven for two days at 50 °C. The soft crosslinked poly(ethylene glycol) (PEG) samples were formed using 22.5 weight percent (wt%) PEG dimethacrylate (PEGDMA, MW 875, Sigma–Aldrich, St. Louis, MO), 22.5 wt% PEG methacrylate (PEGMA, MW 500, Sigma–Aldrich) and 0.1 wt% photoinitiator (Irgacure-651, Ciba Specialty Chemicals, Tarrytown, NY), in HBSS. The stiff crosslinked PEG samples were formed using 55 wt% PEGDMA, 22.5 wt% PEGMA, and 0.1 wt% photoinitiator in HBSS. These mixtures were vortexed and then photopolymerized with 365 nm light for 10 min in a laminate glass mold. Many other crosslinked PEG formulations with varying composition were prepared in the same manner to further demonstrate the flexibility of using polymeric materials. To prepare the gelatin samples, 4 grams (g) of gelatin crystals (Knox original unflavored gelatin) were mixed with 29 mL of cold HBSS. Boiling HBSS (88 mL) was added to this mixture, and the resulting solution was stirred to homogeneity, poured into a petri dish, and refrigerated for 24 h. All polymers were stored in HBSS for two days at room temperature, after which disks from each polymer species were punched with a 5 mm biopsy punch.

2.3. Stress and strain measurement

The mechanical properties of all samples were measured using a dynamic mechanical analysis instrument (DMA Q800 V7.0 Build 113, TA Instruments, New Castle, DE) equipped with a submersion compression clamp in static mode. Prior to each group of measurements, the drive shaft position, clamp mass, clamp offset, and clamp compliance were calibrated according to suggested protocols. Each sample was carefully transferred to the basin of the clamp either in HBSS using a wide-tipped Pasteur pipette (for soft tissue samples, Fig. 1A and B) or carefully with forceps (for polymer, cornea, and sclera samples). Any excess water or buffer surrounding the sample was removed using a syringe. Once the sample was installed, the top portion of the clamp was gently lowered onto the