Design and Validation of a Bioreactor for Scleral Tissue

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Statement of Purpose: The design of a bioreactor for study of the ocular sclera is described. The sclera, which is the outer connective tissue layer of the eye, is altered in myopia (near-sightedness), exhibiting thinning due to decreased collagen synthesis[1] and increased levels of active matrix metalloproteinase 2 (MMP-2).[2] We have previously reported the development of enzymatically-degradable semi-interpenetrating polymer networks (edsIPNs) for promotion of new scleral growth as a means of reinforcing the weakened walls of myopic eyes.[3] For *in vitro* testing of edsIPNs, it is important to simulate as closely as possible the natural environment of the sclera, which is under tension from intraocular pressure (IOP). This is likely to influence collagen synthesis and MMP levels, both of which are of interest in physiologically relevant evaluation of edsIPNs and tissue response to pharmacological therapies.

Methods: Our scleral bioreactor has two chambers, allowing independent treatment of the two surfaces of cultured sclera, which is secured and sealed by ring-shaped grips around its circumference. The grips can accommodate tissue punches from 8- to 14-mm in diameter, allowing samples from eyes of different animals to be studied. Media is supplied to each chamber from independent glass reservoirs that are loosely capped and adjustable in height to allow establishment of a pressure differential between the chambers, thereby simulating IOP.

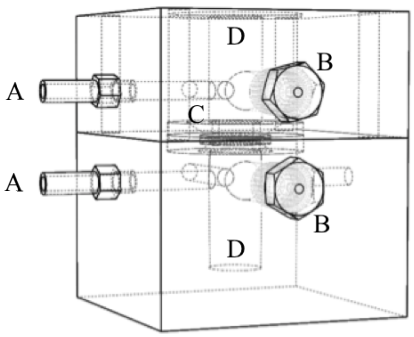

Figure 1. Bioreactor schematic.

Part	Description
A	Stop-cocks connect chambers with gas-permeable Silastic® tubing & 2 independent media reservoirs
B	Ports with an inner silicone septum lining for needle-based pressure monitoring
C	Disc of scleral tissue between two grips
D	Upper and lower chambers filled with media

Porcine sclera, chosen for its similarity to human sclera,[4] was cultured in the bioreactor for 48 hours, after which strips traversing the full diameter of the tissue were fluorescently labeled with 4μM of calcein AM and 4 μM of ethidium homodimer-1 for 30 minutes to evaluate cell viability and cytotoxity, respectively. Fresh scleral tissue, received on the day of imaging, and tissue simultaneously cultured in a cell culture plate, were used as controls. Tissue was imaged with a 2-photon microscope.

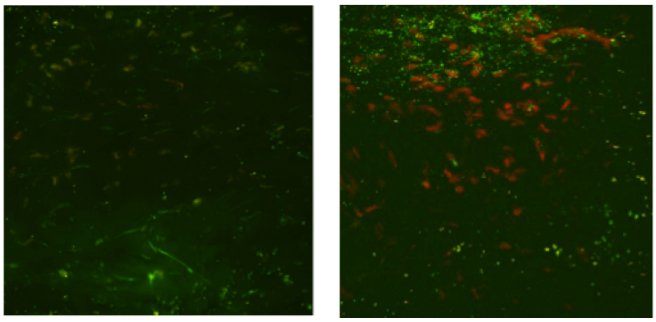

Figure 2. Live (green) and dead (red) scleral fibroblasts in the central (left) and clamped (right) parts of tissue cultured in the bioreactor for 48h.

In a separate experiment for assessing effectiveness of the seal between the two chambers, 148 kDa or 19.5 kDa FITC-labeled dextran (15 mg/mL) was added to the top chamber. The bottom chamber was monitored for fluorescence over a 30-minute period, for each of four bovine scleral samples.

To monitor collagen synthesis in sclera cultured in the bioreactor, an assay was developed using lamb scleral punches cultured in 24-well plates (n=5). Medium was labeled with 50 μCi of [3H]-proline for 6, 12, and 24 hours. Samples were then washed extensively, lyophilized, digested, and counted with a liquid scintillation counter. Values were normalized to tissue dry weight.

The large ratio of medium to tissue encountered in the bioreactor was simulated in a gelatin zymography experiment, which assessed its sensitivity to the levels of MMPs released from cultured sclera into media.

Results & Conclusions: The cell viability/toxicity assay showed cells to be alive through the full thickness of the sclera exposed to media; tissue between clamps showed few live cells (Figure 2). The short-term seal test revealed no leaks for any samples, as evidenced by the lack of fluorescence in the bottom chamber. Collagen synthesis rates were shown to be linear over time (R^2=0.99987). MMPs were detected in sampled media, with bands corresponding to proMMP-2, proMMP-9, and MMP-2 detected in the gel. Initial test results for our bioreactor prototype suggest its suitability for culturing sclera, with potential application in the evaluation of therapies for myopia.

References:

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