

Decreased Infection and Epithelial Cell Attachment to Lubricin Coated Intraocular Lenses

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Statement of Purpose:

There are over 6 million world wide surgeries involving intraocular lenses (IOL) [1]. However, the encroachment of epithelial cells onto the surface of IOLs can lead to a significant loss of visual acuity [2]. Additionally, bacterial infection after cataract surgery is a major post operative complication [3]. Lubricin is a glycoprotein found in the synovial fluid that plays a major role in providing in the synovial fluid's lubricating and anti-adhesive properties[4]. The purpose of this *in vitro* study was to investigate the role that lubricin can play to prevent the encroachment of epithelial cells onto the surface of intraocular lens materials and also serve as an effective non-immune opsonification agent for bacteria colonization.

Methods:

Lubricin preparation: Directly-isolated bovine synovial lubricin (LUB) was diluted to a desired concentration of 100 μ g/ml.

Lens preparation:

Poly (methyl methacrylate) (PMMA) and IOSOFT 26 IOL sample materials, obtained from Vista Optics, were rinsed 3 times in PBS. IOSOFT 26 is a copolymer based on HEMA (2-hydroxyethyl methacrylate) and MMA (methyl methacrylate).

Lens coatings:

Lenses were soaked in 1ml of 100 μ g/ml LUB for a minimum of 2hrs. After coating, each sample was placed in a fresh well in a 24 well plate.

Human lens epithelial cell trials:

Human lens epithelial cells (HLEC) were obtained from the American Type Culture Collection (ATCC) (CRL-11421; population numbers 4 - 7) and were cultured in tissue culture flasks with cell culture media containing 80% DMEM, 20% fetal bovine serum, and 1% penicillin/streptomycin. Cells were cultured at 37°C and 5% CO₂ in a humidified environment. Once cells reached a minimum of 80% confluence they were released from the flask using Trypsin-EDTA. Monodispersed cells were then pelleted at 1180 rpm for 5 minutes. The supernatant media was removed and the cells were re-suspended in fresh media and counted for use in experiments.

Plating cells on lenses:

After monodispersed cells were counted, 1x10⁶ cells were slowly plated directly on each lens. The cells were allowed to settle for approximately 15 min and then fresh media was slowly added to each well to bring the volume to 1ml total. Samples were cultured for 4 days and on the 4th day media was removed and the samples were stained with Calcein AM (10 μ g/(1mlPBS)) (green) and Propidium iodide (40 μ g) (red). Samples were then imaged.

Bacteria trials:

Bacteria cell lines used in this study were *Staphylococcus aureus* (*S. aureus*) obtained in freeze-dried form from the American Type Culture Collection (25923) as dry pellets.

Overnight cultures (18-24 hours in 3ml tryptic soy broth at 200 rpm and 37°C) were seeded on the substrates in DMEM. The bacteria were allowed to adhere under standard cell conditions (5% CO₂/95% humidified air at 37°C) for 4 hours. The samples were then stained with 125 μ l of 0.1% crystal violet, dried overnight, and analyzed with a spectrophotometer. All experiments were conducted in triplicate.

Results:

Preliminary results for HLEC adhesion on PMMA indicated that LUB coatings on the contact lens prevented endothelial cell attachment and proliferation (Figure 1).

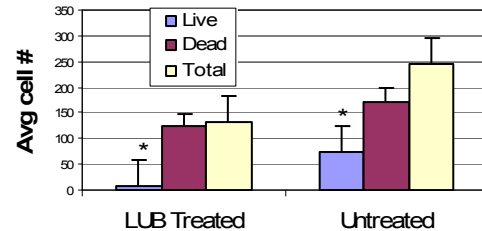


Figure 1: PMMA coated with lubricin decreased epithelial cell density after 4 days. Data = mean +/- SEM; N = 3. (*p<0.05)

Bacteria colonization was also reduced on lubricin coated compared to uncoated PMMA.

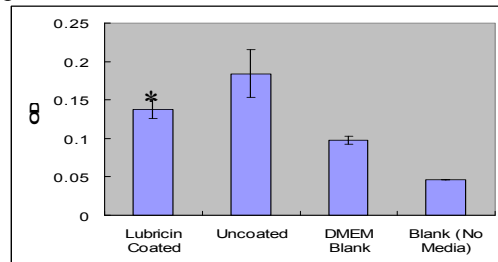


Figure 2. Bacterial adhesion onto lubricin coated (200 μ g/ml) or uncoated polystyrene after 4 hours determined by crystal violet. Lubricin coated substrates significantly decrease bacterial adhesion when compared to uncoated control. Data = mean +/- SEM; N = 3. (*p<0.05)

Conclusions:

Preliminary results showed that lubricin can be used to prevent cellular proliferation and bacterial adhesion polymer IOL surfaces.

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