Investigating Lubricin to Prevent Post-operative Infection of Intraocular Lenses Following Cataract Surgery

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Statement of Purpose: Introduction

The goal of this research was to investigate the ability of Lubricin to prevent bio-fouling of intraocular lenses after cataract surgery. Cataract is the leading cause of blindness in the world [1]. There are over 6 million cataract surgeries involving intraocular lenses (IOLs) worldwide every year [2]. There are three main post-operation complications of these surgeries. First, the encroachment of epithelial cells onto the surface of IOLs can lead to a significant loss of visual acuity [3]. Secondly, the accumulation of calcium deposits on IOLs may lead to total lens opacification [2, 4]. Thirdly, bacterial infection after cataract surgery is a major post operative complication known as endophthalmitis [4, 5].

Lubricin (LUB) is a glycoprotein found in the synovial fluid that plays a major role in providing lubricating and anti-adhesive properties to synovial fluid [6]. The current stage of this research focused on using LUB to prevent the colonization of bacteria on polystyrene and poly (methyl methacrylate) (PMMA) IOL materials.

Methods:

Planktonic Bacteria Growth in the Presence of LUB <u>4hr study</u> *Staphylococcus aureus* (*S. aureus*) obtained from the American Type Culture Collection (25923) was cultured in tryptic soy broth (TSB) (Sigma Aldrich, St. Louis, MO, USA). Polystyrene well plates were soaked in LUB 200µg/mL LUB solution for 2 hours. LUB solution was removed and *S. aureus* was seeded on the substrates at a density of 1×10^7 bacteria/mL (as estimated by the McFarland scale) by diluting the bacteria cultures to an optical density of 0.52 at 562 nm and then further diluted at a ratio of 1:90. *S. aureus* was seeded into wells with and without LUB coatings in Dulbecco's Modified Eagle Medium (Invitrogen). After 4 hours, crystal violet was used to determine the quantity of bacteria on the sample. Crystal violet staining was quantified with a spectrophotometer (Spertamax 340PC). **<u>Extended study</u>** *Staphylococcus epidermidis* (*S. epi*) obtained from the American Type Culture Collection (35984) was used in extended growth studies in addition to *S. aureus*. The bacteria were seeded, in 96 well plates in TSB with or without LUB, and optical density measurements were taken every 4 minutes for 24 hours, while the temperature was maintained at 37°C. No crystal violet was used over the course of the extended growth studies.

Adherent Bacteria Growth in the Presence of LUB

PMMA IOL (Vista Optics, Cheshire, UK) samples were cleaned and soaked in solutions of 200µg/mL LUB dissolved in phosphate buffer solution (PBS). *S. aureus* bacteria were allowed to adhere under standard cell conditions for 1hr. Samples were then stained with the Live/Dead fluorescent stain (Invitrogen) and analyzed with a fluorescence microscope (Leica DM5500 B).

Statistics

All proliferation assays were performed at least in duplicate. Error in these trials was calculated through use of standard error of means. Student t-test was used to determine statistical significance.

Results

Results of this study showed decreased *S. aureus* growth when cell culture wells were treated with LUB compared to the control without LUB (Figure 1).

Moreover, planktonic *S. aureus* growth decreased after 24 hours when cultured in the presence of LUB compared to without (Figure 2). Preliminary results for *S. epi* also indicated that LUB reduced bacterial proliferation (not shown here). Preliminary results for adherent *S. aureus* growth on PMMA also indicated that LUB prevented bacteria proliferation (not shown here).

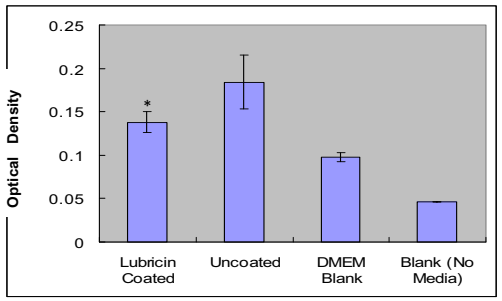

Figure 1. *S. aureus* growth on LUB (200µg/mL) or untreated polystyrene cell culture wells after 4 hours as determined by crystal violet. LUB coated substrates significantly decreased bacterial growth when compared to uncoated control. Data = mean +/- SEM; N = 3 (*p<0.05 compared to untreated).

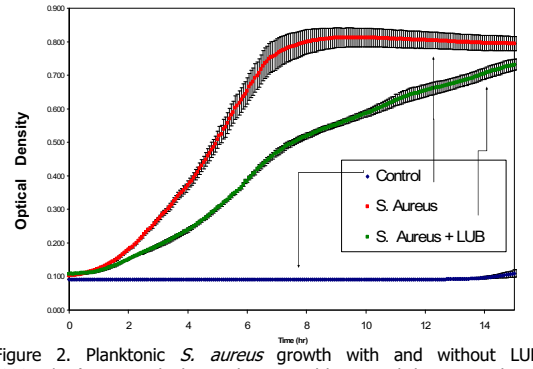

Figure 2. Planktonic *S. aureus* growth with and without LUB (200µg/mL) over 15 hr hours determined by optical density readings. LUB treatment significantly suppressed bacterial growth over the course of 15 hrs. Data = mean +/- SEM; N = 3

Conclusions:

At a concentration of 200µg/mL, LUB decreased S. aureus and *S. epi* growth, both when used as a coating and in solution. Thus, LUB should be further studied for anti-bacterial applications.

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