IOL materials activate macrophages and induce an inflammatory phenotype in lens epithelial cells

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Introduction:

Compromise of the crystalline lens often causes partial or complete blindness. One potential complication is the development of cataracts, or a clouding of the lens. At present, the only effective treatment for cataracts is the surgical removal of the lens and its substitution with an artificial implant known as an intraocular lens (IOL). However, in some cases the process of surgically removing cataracts and implanting an IOL can cause posterior capsule opacification (PCO), or secondary cataracts. PCO is the most frequent complication arising from cataract surgery, occurring in 12% of patients after one year, 21% of patients after 3 years, and 28% of patients after 5 years [1]. With an ever-aging population, it is anticipated that by 2020 almost 25 million cataract surgeries will be performed in the United States, with over 2.5 million of those patients requiring additional surgery within one year to restore their vision [2]. Despite efforts to improve IOL biomaterials and surgical techniques, PCO remains an important issue in ophthalmology [3].

The direct cause of PCO is a proliferation and posterior migration of lens epithelial cells into the visual axis of the lens, where their light scattering properties cause loss of vision [4]. Concurrent with this growth and movement are the classic signs of inflammatory fibrosis: an increase in extracellular matrix deposition, and epithelial cell transdifferentiation into myofibroblasts by way of the epithelial-mesenchymal transition (EMT) [5]. Both of these phenomena contribute to the clouding of vision. Previous work has focused on developing IOLs that prevent lens cell growth and migration. However, macrophages have been observed on the surface of excised IOLs [6] and yet little is known about their possible role in PCO. The purpose of this study is to characterize the activation of macrophages by various IOL materials, and to investigate what effect this interaction has on lens cell phenotype. Macrophage activation will be characterized by increased expression of the cell-surface activation markers CD54 (ICAM-1), CD14, CD36 and CD45. The change of lens cell phenotype will be measured by the increased expression of the fibroblast proteins alpha smooth muscle actin (αSMA) and fibronectin, and the subsequent reduced production of the epithelial protein E-cadherin [7].

Materials and Methods:

A co-culture *in vitro* model of the lens epithelium was developed to investigate the interaction between IOLs and their implanted environment. The human acute monocytic leukemia cell line (THP-1) was cultured in RPMI 1640 cell culture medium supplemented with 10% fetal bovine serum (FBS). Monocytes were differentiated with phorbol 12-myristate 13-acetate into macrophage-like cells in 6-well tissue culture polystyrene plates. After 72 hours the media was replenished and cells were allowed to rest in fresh media for two further periods of 48 hours. Human lens epithelial cells (cell line: HLE B-3) were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 20% FBS and were seeded into 12-well polyethylene terephthalate cell culture inserts. After 12 hours, macrophages were transferred into the bottom of the 12-well polystyrene plates along with one of four IOLs (PMMA or hydrophilic acrylic, square or round edged) or no lens. This co-culture was incubated for either 48, 96 or 144 hours with media replenished every 48 hours. Lens cells were examined for changes in phenotype via immunostaining and flow cytometry. The macrophages were analyzed via flow cytometry for changes in expression of CD54, CD36, CD14 and CD45. Data was

analyzed via ANOVA using a GLM in SAS v9.3. Pair wise comparisons between treatments were performed using Tukey's HSD Test. The correction for multiple comparisons was performed post-hoc via Bonferroni's correction.

Results and Discussion:

The macrophage activation marker CD54 showed a 61% increase in fluorescence with acrylic hydrophilic versus PMMA lenses ($n=3$, $p<0.0001$) and a 91% increase with acrylic hydrophilic versus control ($n=3$, $p<0.0001$). No statistically-significant difference in macrophage activation was observed between PMMA lenses and the control groups, although square-edged lenses showed an increase versus round-edged lenses ($n=3$, $p<0.0663$). The co-culture of macrophages on IOLs and lens cells did not result in significant changes in the expression of CD36, CD14 or CD45.

For the lens cell observations, one replicate each of 96 and 144 hours were discarded due to deviations in the experimental protocol, resulting in only 2 replicates at those time points. Due to the reduced sample size, no statistical analysis has been performed on the expression of lens cell proteins. However, a 16% increase in fibronectin expression has been observed thus far between the acrylic hydrophilic IOLs and the control.

Our results show that foldable, hydrophilic acrylic IOLs induce an inflammatory response in macrophages when compared to PMMA lenses. Further, the co-culture model indicates a relationship between macrophage activation and fibronectin expression by lens epithelial cells. Future experimentation will involve the use of better established FHL-124 lens epithelial cell line, kindly donated by Dr. Judith West-Mays of McMaster University. Additionally, the presence of various inflammatory mediators in the co-culture model will be investigated to determine which exact inflammatory pathway is responsible for this interaction.

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