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# Utilization of in vitro methods to determine the biocompatibility of intraocular lens materials

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ABSTRACT

In vitro methods for measuring the adhesion and viability of lens epithelial cells on implant devices are needed to assess material biocompatibility. We investigated whether the use of confocal microscopy and spectrophotometric methods could determine the viability and adhesion of cells on a silicone biomaterial. Human lens epithelial cells adhered to silicone were treated with 0.01% benzalkonium chloride (cationic surfactant), 0.1% sodium dodecyl sulfate (anionic surfactant), and 10% Tween 20 (nonionic surfactant). Cell viability was then assessed using two fluorescent dyes (calcein and ethidium homodimer-1). Adhesion was determined directly by measuring the number of attached cells after surfactant treatment and by an indirect method that utilized the colorimetric agent crystal violet. The number of viable cells remaining on the biomaterial was determined both immediately after exposure and after the cells were allowed to grow for 1 day following surfactant exposure. The measurements for adhesion showed that the anionic surfactant weakened cell surface binding more than the cationic or nonionic surfactant. This study demonstrated that confocal microscopy in conjunction with crystal violet as an indirect colorimetric indicator can quantify the viability and adhesion of human lens epithelial cells attached to a material surface.



## 1. Introduction

Posterior capsule opacification (PCO, secondary cataract) is a severe long-term complication of cataract surgery (Schmidbauer et al., 2001). The pathogenesis of PCO begins with the adhesion and subsequent proliferation and posterior migration of residual lens epithelial cells (LEC) across the visual axis (Schmidbauer et al., 2001). While emphasis is placed on the careful removal of all visible LEC during surgery, often there remains a number of residual cells that escape notice, which can induce PCO even long after a successful surgery (Nishi, 1999; Trivedi et al., 2002). Many changes to intraocular lens (IOL) designs have been made to alleviate the problem of PCO (Schmidbauer et al., 2001). However, despite this, PCO remains a serious complication associated with IOL surgery. In addition to design changes, material changes may also improve IOL biocompatibility and it has been proposed that it is critical that new IOL materials prevent LEC adhesion (Werner, 2008). Furthermore, the lens material needs to inhibit LEC proliferation without excessive toxicity to the cells, which could cause severe necrosis and subsequent inflammation of surrounding tissues.

The cell-adhesive properties of IOL materials need to be better understood. Thus, there has been a growing demand and need for appropriate in vitro cellular adhesion assays. Cell adhesion has traditionally been evaluated by measuring the ability of adherent cells to remain attached to a surface after exposure to a detachment force (Jarrell et al., 2007; Katayama et al., 2007; Klenkler et al., 2009; Reyes and Garcia, 2003; Yan et al., 2005). The most rudimentary detachment force is the mechanical force from the washing off of the loosely adhered or non-adhered cells with a physiological buffer. Micromanipulation, hydrodynamic shear, and centrifugation are among the currently used forms of applying detachment forces (Reyes and Garcia, 2003). These methods quantify cell adhesion either by directly counting the number of cells or indirectly by colorimetric detection (Kueng et al., 1989).

Benzalkonium chloride (BAK), Tween 20 (polysorbate 20), and sodium dodecyl sulfate (SDS) are common surfactants found in ophthalmic solutions and pharmaceutical eye drops. BAK is a cationic surfactant and is the most commonly used preservative in ophthalmic solutions (Pisella et al., 2000). Tween 20 is a nonionic surfactant that efficiently solubilizes anti-inflammatory agents to allow for better penetration into the eye (Ayaki et al., 2007). SDS is an anionic surfactant that is found in commercial products such as shampoos and in contact lens cleaners (Bennett and Weissman, 2005).

This study was undertaken to determine the effects of BAK, Tween 20, and SDS on the adhesion and growth of human lens epithelial cells (HLEC) adhered to silicone surfaces. HLEC on silicone surfaces were chosen because silicone IOL are one of the

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more popular soft IOL (Yao et al., 2006). Also, silicone-based materials have widespread clinical applications due to its favorable qualities, including its inertness, chemically stability, autoclavability, flexibility, and minimal immunologic reactivity (Habal, 1984). In addition, silicone can be made transparent and has a suitable refractive index, which makes it an excellent IOL material (Yao et al., 2006). Thus, this study was undertaken to determine whether surfactants could be used to quantify the adhesion and viability of human lens epithelial cells adhered to a material surface by using the calcein and ethidium homodimer-1 with confocal microscopy in conjunction with crystal violet as an indirect colorimetric indicator.

## 2. Materials and methods

### 2.1. Cell culture

Human lens epithelial cells (HLEC) (CRL-11421, American Type Culture Collection, Manassas, VA, USA) were cultured in MEM (minimum essential medium with Earle's salts and L-glutamine; Invitrogen, Carlsbad, CA, USA) supplemented with 20% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen), and were incubated at 37 °C in a humidified atmosphere with 5% $CO_2$. Cells were grown to confluence. At confluence, HLEC were washed with PBS (phosphate buffered solution; Lonza, Basel, Switzerland), dissociated with TriplExpress (Invitrogen), and seeded onto silicone discs.

### 2.2. Test vessel preparation

Silicone disks were cut from silicone sheeting (Specialty Manufacturing, Inc., Saginaw, MI, USA) and soaked in 70% ethanol to sterilize the surfaces (at least 1 h) prior to use. The silicone disks were then placed on the bottom of a well in the 24-well tissue culture plate (BD Biosciences, Mississauga, ON, Canada). Tygon silicone tubing (Saint-Gobain Performance Plastics, Akron, OH, USA) were cut into rings of approximately 1 cm in length and were inserted on top of the silicone disks in the well to prevent cell attachment to the sides of the culture wells. The wells were then seeded with $5 \times 10^5$ cells per well and were incubated at 37 °C with 5% $CO_2$ in 1 mL MEM with serum for 1 day before exposure to test substances.

### 2.3. Test substance preparation

The monolayer cultures on silicone surfaces were exposed to PBS solutions of benzalkonium chloride (0.01% BAK, vol/vol), sodium dodecyl sulfate (0.1% SDS, vol/vol), and Tween 20 (polysorbate 20) (10%, vol/vol). The BAK and Tween 20 were obtained from Sigma–Aldrich, Oakville, ON, Canada, while the SDS was obtained from Rockland Immunochemicals, Gilbertsville, PA, USA.

Control monolayer cultures on silicone surfaces were treated in the same manner as the test cultures with regards to handling and medium change, except they were exposed to PBS instead of the test surfactants. All samples were run in triplicate in at least three independent experiments.

### 2.4. Confocal analysis of cell adhesion and proliferation

Cell adhesion on control and treated monocultures on silicone surfaces was determined quantitatively using a confocal laser scanning microscope (510 Meta CLSM, Carl Zeiss Inc., Germany). At the given time point, the silicone disks were removed and treated with the test surfactant for 2 min. After the treatment, the disks were submerged 1 cm into 25 mL of PBS for 1 s three times. One set of disks was evaluated immediately after exposure and a second set was assessed after one additional day of incubation in growth media.

Immediately after exposure and after 1 day recovery, the disks were then stained with calcein and ethidium homodimer-1 (Invitrogen) for 30 min at 37 °C in a humidified atmosphere of 5% $CO_2$. The silicone disks were then imaged on petri dishes with glass cover slips (MatTek Corporations, Ashland, MA, USA) with the confocal laser microscope.

Each silicone disk was divided into five sectors (Fig. 1) and a random microscopic field in each quadrant was selected and counted as representative samples of each disk. The number of live and dead cells in each field were counted and converted into a live-to-dead ratio.

### 2.5. Crystal violet analysis of cell adhesion

Cell adhesion was also quantified using the traditional crystal violet analysis (Humphries, 2009). Immediately after exposure to surfactants as described above, the HLEC on the disks were fixed with 5% glutaraldehyde for 30 min, and then washed with PBS. The HLEC on the disks were then stained with 0.1% crystal violet for 60 min, and destained with washes of deionized water. The dye was then solubilized with 2% sodium dodecyl sulfate in PBS. Adhesion was quantified by reading absorbance at 560 nm in Multiskan Spectrum (Thermo Labsystems, Waltham, MA, USA). All samples were run in triplicate in at least three independent experiments. The absorbance readings of the samples were averaged and compared.

### 2.6. Statistics

Statistical significance was determined by analysis of variance using the Kruskal–Wallis one-way analysis of variance test for the comparison between the groups exposed to surfactant versus the controls. The Wilcoxon signed-ranked test was used to determine the statistical significance between the immediate and one-day effects of the surfactants. Statistical significance was taken at $p < 0.05$.

## 3. Results

Following the initial seeding of $5 \times 10^5$ cells per surface, human lens epithelial cells (HLEC) adhered readily onto the silicone surface to form a fairly confluent layer within 1 day. The cells grew on the silicone controls and most of the cells were viable as observed and imaged with the Live/Dead assay using the confocal microscope. Following the exposure to different surfactants, the monolayer would loosen and fold over itself or detach all together. The images of the cells after exposure to the surfactants are shown

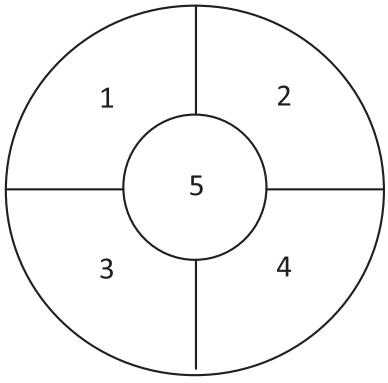


**Fig. 1.** Sector divisions of silicone disks.