Surface Modification and Characterization of Collagen-based Artificial Cornea for Enhanced Epithelialization

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Abstract

Argon plasma treatment of collagen-based artificial corneas led to an increase in the *in vitro* biocompatibility. Artificial corneas were prepared by stabilization of collagen matrices through glutaraldehyde and glutaraldehyde-polyethylene oxide (PEO) cross-linking. Characterization of polymer surface properties such as surface hydrophilicity and surface roughness were also investigated by contact angle measurement and atomic force microscopy (AFM), respectively. The epithelialization of untreated and treated samples was evaluated by the seeding and growth of human corneal epithelial cells. Enhanced attachment and proliferation of epithelial cells to artificial cornea surfaces were observed after argon plasma treatment. Surface hydrophilicity was also increased considerably by argon plasma treatment. AFM characterization also showed an increase in surface roughness through argon plasma treatment. Based on the biological and surface analysis, argon plasma treatment displays promising potential for biocompatibility enhancement of collagen-based artificial corneas.

Key words: argon plasma treatment, collagen-based artificial cornea, epithelialization, atomic force microscopy (AFM), contact angle measurement.

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INTRODUCTION

One major complication that often results in the rejection of artificial corneas is the lack of epithelial coverage on the surface of these devices [1]. The absence of a continuous sheet of epithelium allows for bacterial invasion, inhibits the formation of a tear film, permits epithelial downgrowth, and results in a rough, dry surface; therefore one of the criteria for a successful artificial corneas is that the anterior surface of the hydrogel be covered with a continuous sheet of epithelium [2]. Low-pressure plasma treatment is a promising tool for the surface modification of polymeric biomaterials in particular to improve their biocompatibility. A shallow surface layer of a few nanometers is modified while the bulk properties of the material remain unchanged. There are different approaches to obtain tailored surface properties using low-pressure plasma techniques [3]. In the course of this research argon plasma was used to surface modify a collagen-based artificial cornea. Results from the *in vitro* study indicated that the epithelial cell attachment was enhanced on the argon plasma modified surfaces.

MATERIALS AND METHODS

Artificial cornea samples were prepared from purified, acid soluble collagen extracted from rat-tail. All the samples (7 mm in diameter discs with a thickness of 0.3 mm) were initially treated with glutaraldehyde. Some of the glutaraldehyde-crosslinked samples were then treated with polyethylene oxide dialdehyde (PEO-2CHO) for improved elasticity.

A radio frequency (RF) plasma system (SP 100 Anatech Ltd, Springfield, VA, USA) was then used. Artificial cornea discs were dried by nitrogen gas in a sterile isolated chamber before being exposed to argon plasma. Discs were then placed in the middle of the plasma reactor. Air in the reactor was removed by three evacuations (<30 mtorr) and purged with argon gas to around 2000 mtorr. After three repeated evacuations, argon gas was introduced to the system at a constant flow rate. The reactor pressure was controlled by adjusting the gas flow rate. An argon plasma was excited in the reactor at the selected power. After the plasma was turned off, the samples were further quenched in argon. After a final evacuation the reactor was returned to atmospheric pressure by purging with argon. The treated samples were removed and placed in a solution of 1 v/v% chloroform in sterile $_{dd}H_2O$ for sterilization. They were then

removed, and rinsed in sterile $_{dd}H_2O$ prior to evaluating the cellular response.

The *in vitro* biocompatibility test involved two stages. The first stage involved the construction of a fixed volume of glutaraldehyde-stabilized human stromal equivalent. Aliquots of 1.5 ml of stromal mixture were placed into a 12 well culture dish with inserts and incubated at 37 ^oC until it was partially gelled (less than 1 hour).

In the second stage, the sterile samples were laid on top of the partially gelled human stromal equivalents, forcing it to sink into the matrix to ensure full contact between the two elements. Epithelial cells were seeded on top of the exposed portions of the human stromal equivalent and the samples at a concentration of 1000 cells per insert. Approximately 4 ml of $KSFM^{++}$ growth medium was added to the interior and exterior of the insert covering the entire surface. The system was then placed in an incubator at 37 ^oC, replacing the medium every 48 hours.

Epithelial cell number and morphology were monitored with digital pictures on a digital camera attached to a light microscope (10 to 40X magnification, Olympus IMT-2 Optical Company Ltd., Japan with Nikon coolpix 990 Nikon, Japan) on days 1, 2, and 3.

To measure the contact angle, artificial cornea samples were soaked in distilled water for at least a few hours prior to measurement. Pincers were used to procure the artificial cornea and then place on the glass plate sample holder of a Horizontal Beam Comparator (Scherr Tumico Model 20-4200 Series, St. James, Minnisota). Excess water on the surface was removed by covering the sample with a lightweight lint free cloth (kimwipe). A five microliter (5 µl) drop of distilled deionised water was deposited onto the artificial cornea surface by a micro syringe and equilibrium contact angle (θ_e) was measured.

AFM studies were conducted on a tapping mode using a MultiMode^{TM} scanning probe microscope (MM-SPM)(Nanoscope III equipped with 1553D scanner, Digital Instruments, CA). Artificial cornea samples were soaked in distilled water prior to AFM studies. Each sample was placed on a metal disc and the disc was magnetically attached to the top of the scanner tube. As the scanner moves back and forth, the

sample moves with it, allowing the probe to extract information from the sample surface much like a photograph needle plays a vinyl record. Images produced from scans were analyzed and/or modified using the microscope's functions. For example, mean surface roughness (R_a) was determined by AFM software.

RESULTS AND DISCUSSION

Enhanced attachment of epithelial cells to artificial cornea surfaces was observed after argon plasma treatment while attachment of the cells onto untreated surfaces was found to be negligible. The *in vitro* biocompatibility test results are shown in Figure 1.

Among the treated surfaces, those crosslinked with glutaraldehyde-PEO showed better cell attachment rather than those crosslinked with glutaraldehyde only. It is hypothesized that enhanced cell attachment may be the result of hydrogen bonding between cell membranes and hydrophilic functional groups like hydroxyl or carbonyl groups on the plasma treated surfaces. The plasma-treated surfaces exhibited definite changes in wettability compared to untreated ones, i.e. equilibrium contact angle decreased from 70 ° to 32 ° in average by argon plasma treatment. Changes in surface wettability due to argon plasma treatment were demonstrated to be permanent by contact angle measurement over a period of one month. AFM data also showed that mean surface roughness was increased from 24 nanometers (nm) to 40 nanometers after exposing the samples to argon plasma. This increase is believed to be due to the etching of the artificial cornea surfaces.

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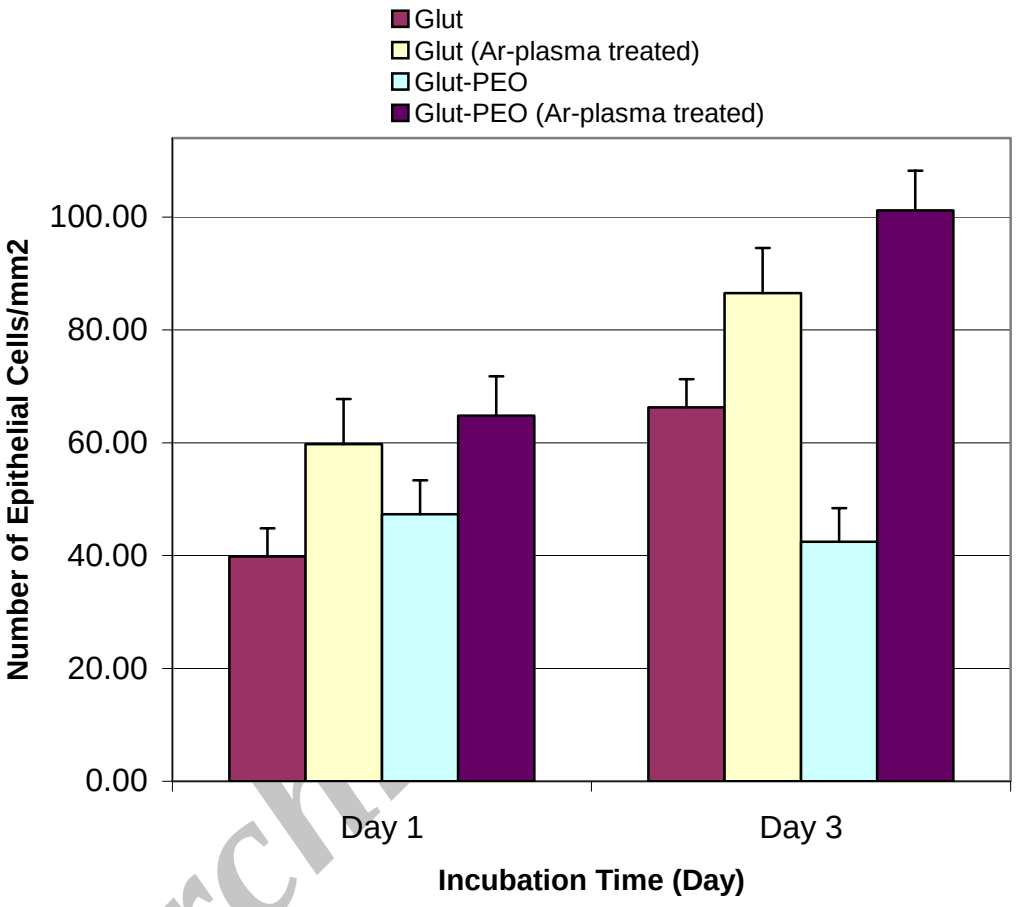

Figure 1 Effect of Argon Plasma Treatment on Epithelial Cells Attachment and Growth on Artificial Cornea Matrices.

*__Glut:__ Matrices Cross-linked by Glutaraldehyde
*__Glut-PEO:__ Matrices Cross-linked by Glutaraldehyde and Poly(ethylene oxide).
*__Ar:__ Argon