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

Mehrdad Rafat,[1,2] May Griffith,[2,3] Malik Hakim,[1,2] Lea Muzakare,[2] Frank Li,[2] K.C. Khulbe,[1] Takeshi Matsuura[1]

[1]*Department of Chemical Engineering, University of Ottawa, Ottawa, Ontario, K1N 6N5, Canada*
[2]*University of Ottawa Eye Institute, Ottawa, Ontario, K1H 8L6, Canada*
[3]*Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, K1H 8M5, Canada*

Received 20 January 2006; accepted 27 July 2006
DOI 10.1002/app.25305
Published online 23 July 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Argon plasma treatment enhanced the attachment of epithelial cells to a collagen-based artificial cornea crosslinked using glutaraldehyde (GA) and glutaraldehyde-polyethylene oxide dialdehyde (GA-PEODA) systems. The epithelialization of untreated and treated surfaces was evaluated by the seeding and growth of human corneal epithelial cells. Characterization of polymer surface properties such as surface hydrophilicity and roughness was also made by contact angle measurement and atomic force microscopy, respectively. Contact angle analysis revealed that the surface hydrophilicity significantly increased after the treatment. In addition, AFM characterization 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. It was also found that the cell attachment to artificial cornea surfaces was influenced by the combined effects of surface chemistry (i.e., surface energy), polymer surface morphology (i.e., surface roughness), and polar interactions between functional groups at the polymer surface and cell membrane proteins. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 2056–2064, 2007

Key words: atomic force microscopy; biocompatibility; crosslinking; modification; plasma

INTRODUCTION

The cornea is an avascular transparent tissue that act as the window to the eye. It forms part of the outer tunic of the eyeball that protects the inner contents of the eye, and functions as the main refractive component. When irreversibly diseased or damaged, corneas lose their transparency, leading to vision loss and/or blindness. Corneal blindness may be treatable by donor cadaver corneas.[1] However, at present, supply of healthy donated tissue is short, and an estimated 10 million patients worldwide are in need of corneas.[2] As a consequence, there have been considerable efforts put into the development of artificial replacements as keratoprostheses (KPros).[3]

One major complication in the development of KPros is the lack of adequate biocompatibility. It is generally agreed that the growth of corneal epithelial cells over the external surface of KPros is desirable for smooth integration of the devices into the host tissue.[4] The absence of a continuous sheet of epithelium exposes the eye to bacterial invasion, inhibits the formation of a tear film, permits epithelial downgrowth, and may result in a rough, dry surface. Therefore, an important criterion for a successful KPro is that the anterior surface rapidly becomes covered with a continuous sheet of epithelium.[5]

The past decade has witnessed a tremendous surge in interest regarding various techniques for surface modification of biomaterials. Typically, surface modification strategies such as grafting, immobilization, coating, plasma deposition, and plasma surface treatments are applied to impart desirable chemistry and morphology to facilitate interfacial interactions between biomaterials and biological species.[6] The critical principle behind these technologies is that they make it possible to change surface properties of a biomaterial without altering the bulk properties.[7] Plasma treatment is a promising tool for the surface modification of polymeric biomaterials, in particular, to improve their biocompatibility.[8] A surface layer of a few nanometers is modified, while the bulk properties of the material remain unchanged.[9] There are various approaches to obtain tailored surface properties using low-pressure plasma techniques.[10]

Correspondence to: T. Matsuura (matsuura@eng.uottawa.ca).

Contract grant sponsor: The Government of Ontario, University of Ottawa, and NSERC.

Several studies have been reported on the enhancement of corneal epithelial cell attachment and growth on synthetic biomaterials. For example, an ammonia plasma treatment was applied to artificial corneas fabricated from poly(2-hydroxyethyl methacrylate) (pHEMA) and intraocular lenses fabricated from poly(methyl methacrylate) (PMMA) to promote the migration of corneal endothelial cells during wound healing.[11] The attachment of the cells onto control untreated PMMA and pHEMA surfaces was found to be negligible, whereas there was an enhanced attachment of endothelial cells to the ammonia plasma treated surfaces. In another study, various plasma modification techniques were used to modify a synthetic cornea containing poly(vinyl alcohol) (PVA) hydrogel. Results from both the *in vitro* study and *in vivo* implantation in a rabbit model indicated that an enhanced epithelial layer was obtained on the plasma modified PVA surface when compared with the untreated surfaces.[5] Despite the great deal of research performed previously on plasma modification techniques, the effect of surface properties of collagen-based polymers, such as surface morphology and surface energetic, on cell attachment have not been addressed adequately.

Various *in vitro* experiments have shown that the cell behavior is influenced by the physicochemical properties of synthetic polymer surfaces. However, the relationship between substratum surface properties and *in vitro* cell response is not well understood.[12] Surface energy of the substratum was shown to influence the adhesion of various cells.[13–16] Materials with high surface energy were generally reported to promote cell adhesion, as opposed to low-energy surfaces, which deterred cell adhesion. The influence of surface chemical composition may also be related to such a macroscopic property or to the presence of specific chemical groups.[17–20]

In this article, the relationship between surface properties of crosslinked collagen and adhesion of epithelial cells has been studied. Argon plasma was used to modify the surface of collagen-based artificial cornea. Artificial corneas were biologically characterized *in vitro* by epithelial cell assay. Surfaces of artificial corneas were also characterized by contact angle measurement and atomic force microscopy (AFM). Results from the *in vitro* study indicated that epithelial cells attachment was enhanced on the argon plasma modified surfaces. Surface roughness and hydrophilicity were also increased by plasma treatment.

METHODS

Fabrication of artificial cornea hydrogels

Artificial cornea hydrogels were prepared from purified, acid-soluble rat-tail collagen films (Telo collagen Type I from BD) crosslinked with glutaraldehyde (GA) and poly ethylene oxide dialdehyde (PEODA). Three stages involved in the fabrication process of artificial cornea hydrogels are summarized here.

Production of collagen films

An evaporation technique was developed to produce solid collagen films prior to crosslinking. Purified rat-tail Type I (telo-) collagen (0.45% (w/v) in 0.05*N* acetic acid from Becton-Dickinson, Canada) was used for this study. Collagen solution was poured into a 35-mm diameter sterile culture dish. The culture dish was placed in a cold chamber at 4°C, and nitrogen gas was purged parallel to the surface of the solution in the evaporation dish using a gas-flow distributor. Additional collagen solution was added to the dish and then dried until sufficient collagen film thickness was obtained (i.e., 300 μm). The final collagen film that was transparent and flat was cut into 7-mm diameter disks, weighed, and prepared for the crosslinking reaction.

Fabrication of artificial cornea hydrogels using GA crosslinker

Selected collagen films were placed in flat Teflon reaction molds. A certain amount of GA solution was added to the top and bottom surfaces of the film at a GA to collagen molar ratio of 1.3 (1.3 aldehyde group in GA to 1 amine in collagen). The flat Teflon reaction mold was then closed and held in place using two aluminum plates and two clips. The reaction molds were placed in an isolated dust-free chamber at room temperature for 72 h. The top portion of the mold was then removed, and the samples were dried at room temperature for 24 h and rehydrated in sterile distilled-deionized water ($_{dd}H_2O$).

Further crosslinking of GA-crosslinked hydrogels with poly(ethylene oxide dialdehyde)

PEODA crosslinking was performed to enhance elastic properties of GA-crosslinked hydrogels. Some of the hydrogels that were initially crosslinked with GA were post crosslinked with PEODA at a molar ratio (%) of 7 (7 aldehyde groups in PEODA to 100 amine group in collagen). PEODA was used at 0.07-fold molar aldehyde to collagen amine based on our previous unpublished work. We found that the optimal PEODA-to-collagen molar ratio (aldehyde to amine groups) was about 0.07 that enhanced mechanical properties, while biological activity of the protein was well retained. A fixed amount of PEODA (poly ethylene oxide dialdehyde, ALD-3400 Shearwater Polymers, USA) was mixed with $_{dd}H_2O$ to form the solution. The desired PEODA to collagen

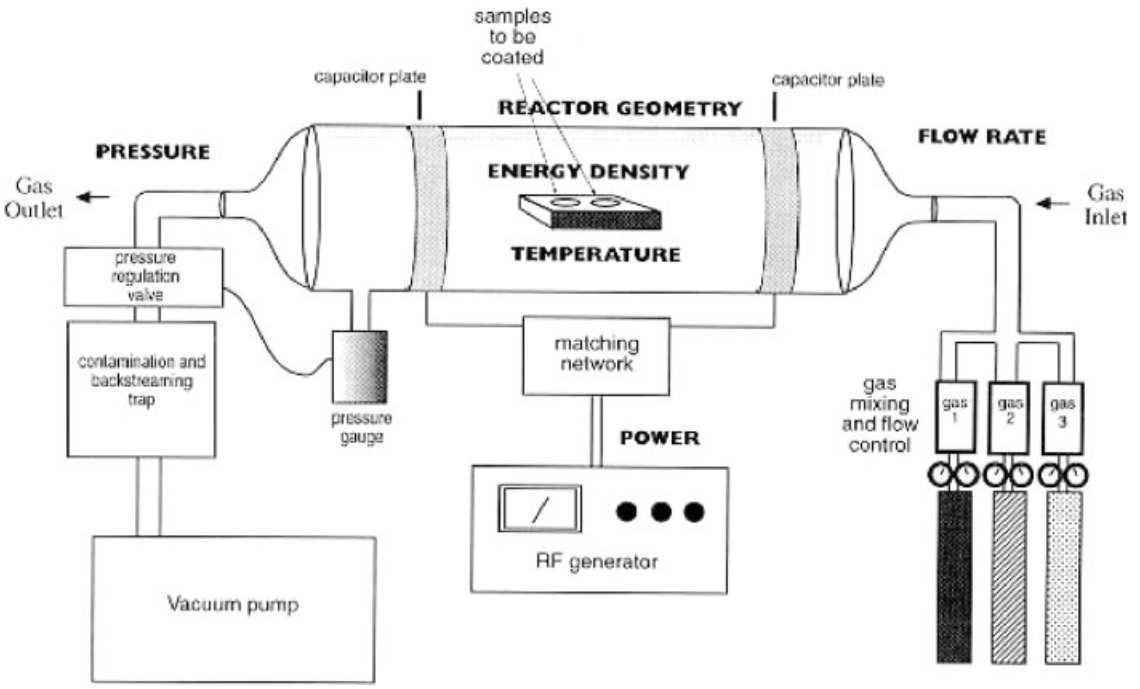

Figure 1 A diagram of a typical RF plasma reactor.[9]

molar ratio was utilized to calculate the concentration of PEODA solution. The GA-crosslinked samples were dried and subjected to PEODA crosslinking. The same technique used for GA-crosslinking was used for PEODA-crosslinking of hydrogels.

Modification of artificial cornea hydrogels

Artificial cornea hydrogels were subsequently dried using nitrogen gas in an isolated clean chamber (refrigerated at 4°C) before being exposed to argon plasma. Samples were then placed in the middle of a radio frequency (RF) plasma system (SP 100 Anatech, Springfield, VA). This is a small laboratory-scaled plasma system that generally comprises five main components: the reactor chamber in which the polymer samples are treated, a vacuum system, a gas-introduction and gas-control system, a high-frequency generator, and a microprocessor-based system controller as shown in Figure 1. Air in the plasma reactor chamber was removed through three evacuation-purge cycles with argon gas to around 2000 mTorr. After three evacuation/purge cycles, argon gas (Ultra High Purity from PRAXAIR) was again introduced to the system at a constant flow rate of 250 mL/min. The plasma reactor pressure was controlled and kept at 80 mTorr by adjusting the gas flow rate. Argon plasma was excited in the plasma reactor at 100 W for 30 min. After the plasma was turned off, the reactor was returned to atmospheric pressure purging with argon and samples were further quenched in argon for 10 min. The treated samples were rehydrated and treated with PBS solution and with 1% (v/v) chloroform for sterilization. Prior to evaluating the cellular response, samples were extraneously rinsed in sterile PBS/chloroform solution.

In vitro biocompatibility assessment

The *in vitro* biocompatibility test involved two stages. The first stage involved the construction of a fixed volume of collagen lightly crosslinked by GA that was also used as an *in vitro* control for epithelial cells growth. Aliquots of 1.5 mL of this viscous collagen were placed into a 12-well culture dish with

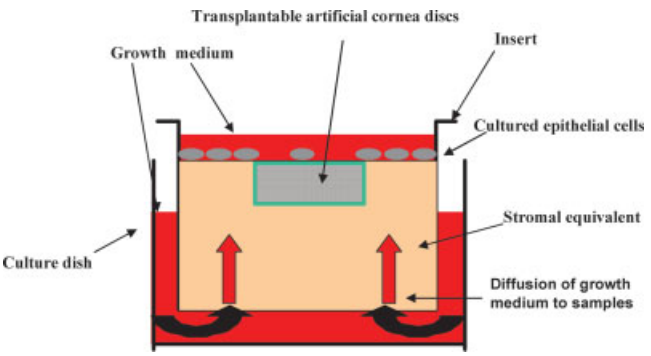

Figure 2 Schematic representation of an insert in a culture dish in which artificial cornea samples were placed and *in vitro* biocompatibility test was performed. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

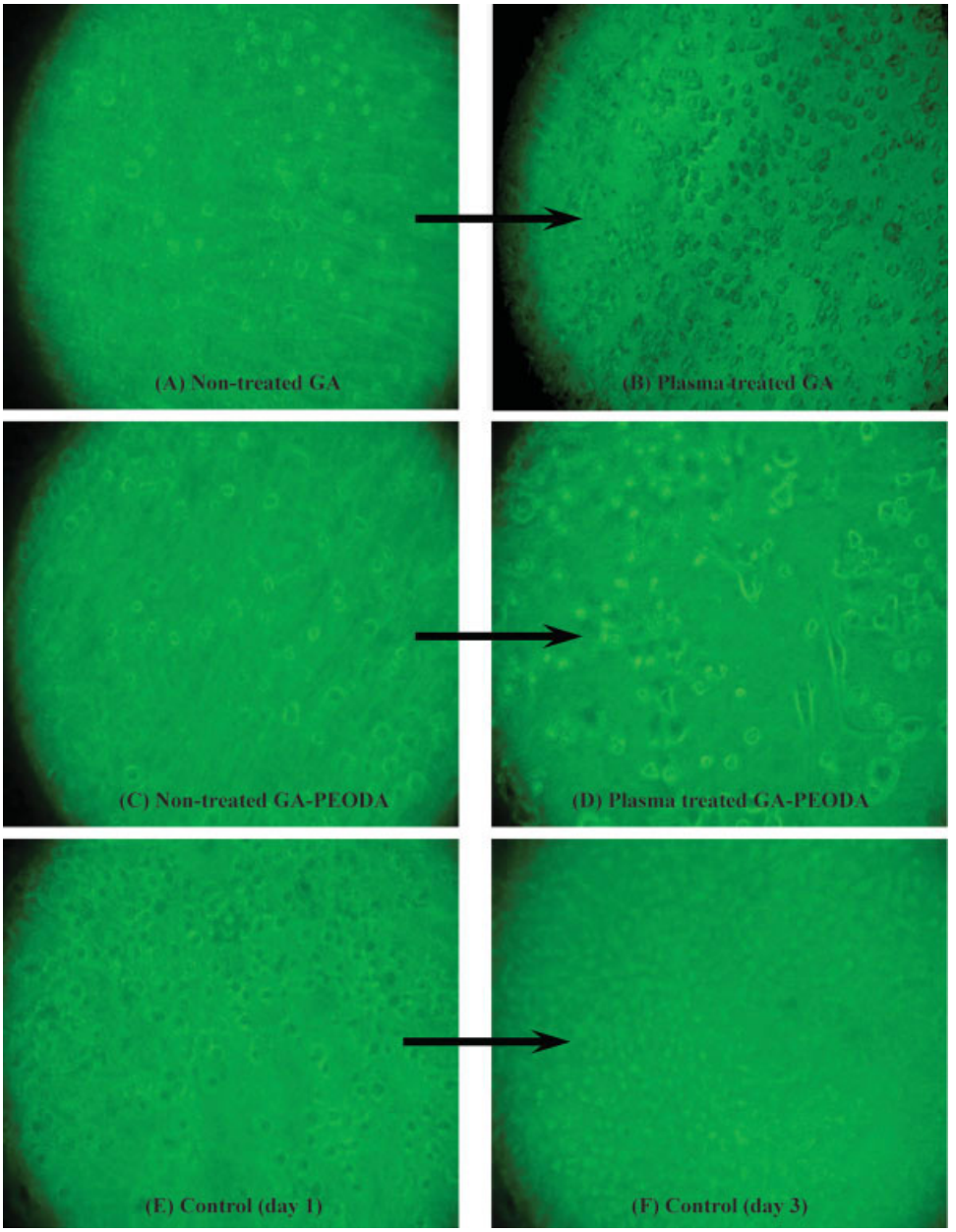

Figure 3 Microscopic images of epithelial cells cultured on various crosslinked matrices: (A) GA [Day 3]; (B) Argon-plasma-treated GA [Day 3]; (C) GA-PEODA [Day 3]; (D) Argon-plasma-treated GA-PEODA [Day 3]; (E) Stromal equivalent (control) [Day 1]; and (F) Stromal equivalent (control) [Day 3]. GA, artificial corneas fabricated by glutaraldehyde crosslinking of collagen matrices; GA-PEODA, artificial corneas fabricated by glutaraldehyde crosslinking of collagen matrices followed by crosslinking with poly(ethylene oxide dialdehyde). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

inserts and incubated at 37°C for less than 1 h until it was partially gelled to give a stromal equivalent.

In the second stage, each sterile sample was laid on top of a lightly gelled stromal equivalent and pressed into the matrix to give good contact between the two components (see Fig. 2). Immortalized human epithelial cells were seeded on top of the exposed portions of the stromal equivalent and the samples at a concentration of 1000 cells per insert. Approximately 4 mL of $KSFM^{2+}$ growth medium was added to the inside and outside of the insert. The system was then incubated at 37°C, and the medium was replaced every 48 h.

Epithelial cell density and morphology were monitored using a digital camera attached to a light microscope (10–40× magnification, Olympus IMT-2 Optical Company, Japan with Nikon Coolpix 990 Nikon, Japan) on Days 1, 2, and 3 after seeding.

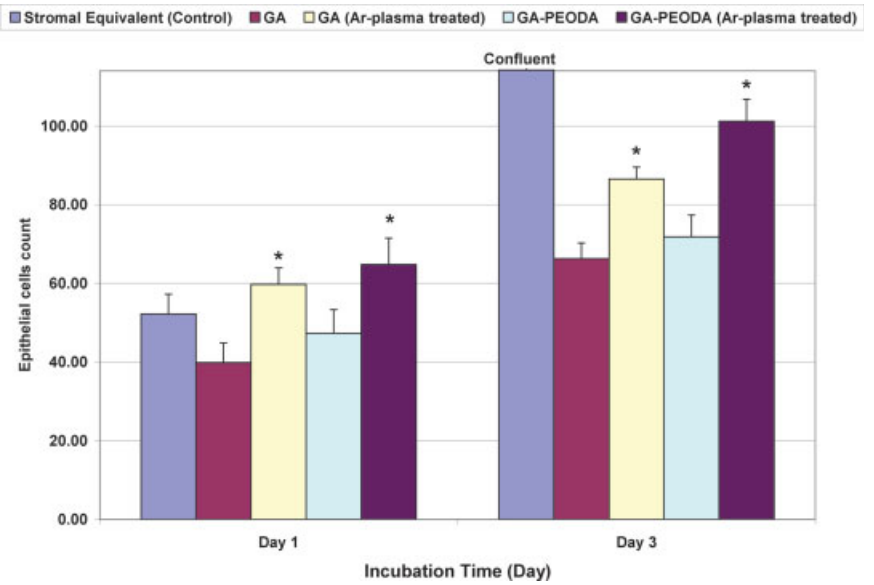

Figure 4 Effect of argon plasma treatment on epithelial cells attachment on GA and GA-PEODA crosslinked matrices. Five replicate samples were used in determining the mean value and standard deviation for each data set. The statistical difference between data sets was analyzed using one-way ANOVA. *$P = 0.0025$ and $P = 0.0022$ for the differences between GA and Plasma-treated-GA on Day 1 and Day 3, respectively. *$P = 0.0264$ and $P = 0.0032$ for the differences between GA-PEODA and plasma-treated-GA-PEODA on Day 1 and Day 3, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Contact angle measurements

To measure the contact angle, artificial cornea samples were soaked in sterile distilled water for 1–2 h prior to measurement. Each artificial cornea sample was placed on a glass plate and then on the sample holder of a Horizontal Beam Comparator (Scherr Tumico Model 20-4200 Series, St. James, MN). Excess water on the sample surface was removed by a lightweight lint free cloth (Kimwipe). A 5-μL drop of distilled, deionized water was deposited onto the artificial cornea surface by a microsyringe, and the equilibrium contact angle (θ_e) was measured.

Atomic force microscopy characterization

AFM studies were conducted by tapping mode using a MultiMode™ 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. Excess water on the surface was removed by a lightweight lint free cloth (Kimwipe). Each sample was then placed on a metal disc, and the disc was magnetically attached to the top of the scanner tube. Images produced from scans were analyzed using the microscope's software. The mean surface roughness (R_a) is the mean value of the surface relative to the center plane, the plane for which the volumes enclosed by the image above and below this plane are equal, and is calculated as

$$R_a = \frac{1}{L_x L_y} \int_0^{L_y} \int_0^{L_x} |f(x,y)| dx\, dy \qquad (1)$$

where $f(x, y)$ is the surface relative to the center plane, and L_x and L_y are the surface dimensions in x and y directions, respectively.[21]

RESULTS AND DISCUSSION

Enhanced attachment of epithelial cells to artificial cornea surfaces was observed after argon plasma treatment when compared with the low degree of cell attachment onto untreated surfaces (Fig. 3). The number of epithelial cells attached to the polymer surfaces on Day 1 and Day 3 were quantified in Figure 4. As shown in Figures 3 and 4, attachment of the cells onto untreated surfaces was found to be significantly lower than the plasma treated surfaces on Days 1 and 3 after seeding ($P < 0.05$). A confluent epithelium was observed on stromal equivalents (controls) on Day 3. In addition, comparing the plasma treated surfaces, those crosslinked using GA-PEODA system showed better cell attachment than those crosslinked using GA system.

The plasma-treated surfaces had significant increase in wettability compared with the untreated ones, with the equilibrium contact angle decreasing from 70° to 32° on average by argon plasma treat-

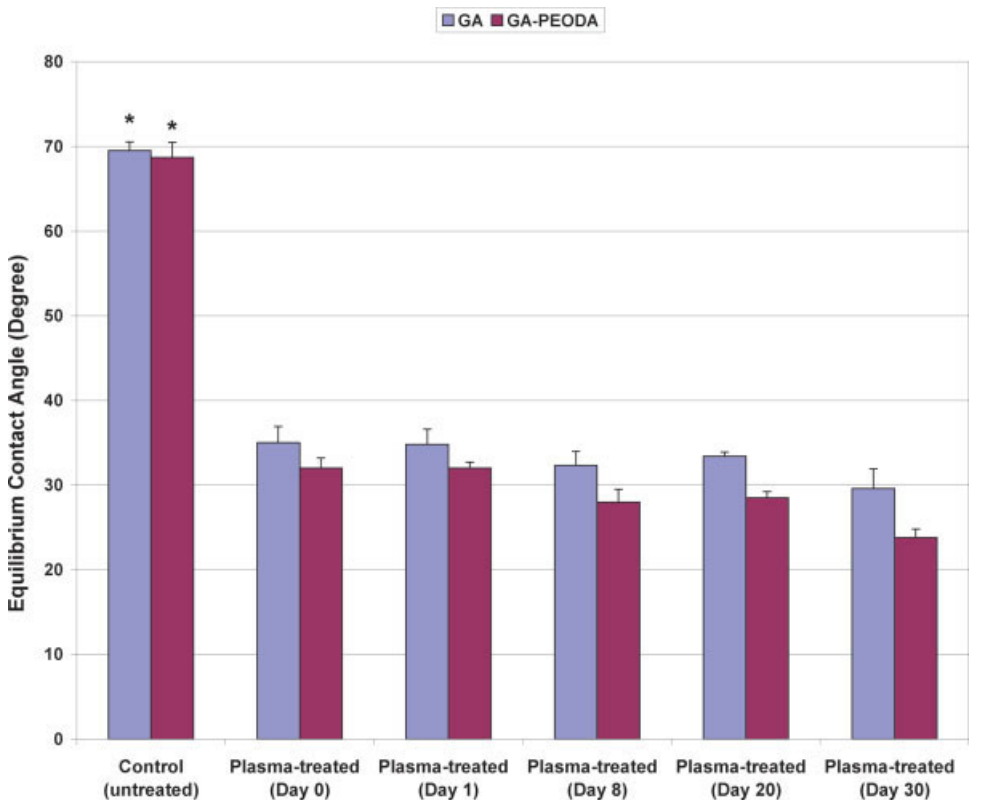

Figure 5 Effect of plasma treatment on surface hydrophilicity of the artificial cornea matrices fabricated by GA and GA-PEODA crosslinking of collagen films. Five replicate samples were used in determining the mean value and standard deviation for each data set. The statistical difference between untreated and plasma treated data sets was analyzed using one-way ANOVA. *$P = 2.2 \times 10^{-14}$ for GA and $P = 1.2 \times 10^{-5}$ for GA-PEODA. *x*-Axis represents the number of days after plasma treatment. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ment as shown in Figure 5 ($P = 2.2 \times 10^{-14} < 5 \times 10^{-2}$ for GA and $P = 1.2 \times 10^{-5} < 5 \times 10^{-2}$ for GA-PEODA). The contact angle measurement of the plasma-treated surfaces was repeated over a period of 30 days. As shown in Figure 5, the contact angle was practically unchanged over time for plasma-treated surfaces, and variations were not significant ($P = 0.25 > 0.05$). This is important because it has been reported that treatment with ionized gases, in most cases, was not of lasting nature and the effect of treatment normally disappeared within days or even hours.[22]

Also, it can be seen that collagen matrices crosslinked using GA-PEODA system had a lower contact angle (more hydrophilic) compared with those crosslinked using GA system (Fig. 5). This was likely due to hydrophilic nature of PEODA that also enhanced cell attachment.

Surface morphology of plasma-treated and untreated polymers was studied by AFM. Figures 6 and 7 represent AFM topographical images of the surface of the polymers crosslinked using GA and GA-PEODA systems, respectively. For both cases, the surface roughness was increased after argon plasma treatment. Upon plasma treatment, the AFM mean surface roughness significantly increased from 30 ± 2.5 to 35 ± 1.7 nm, and from 24.0 ± 1.9 to 40.0 ± 2.5 nm for collagen matrices crosslinked by GA and by GA-PEODA, respectively, (Fig. 8). This increase may be due to the etching that took place at the artificial cornea surfaces during plasma treatment. Also, untreated samples crosslinked by GA-PEODA system demonstrated a smoother surface compared with those crosslinked by GA, while it was the opposite for the plasma treated samples. Incorporation of PEODA into the collagen matrices may have filled up the gaps between collagen chains resulting in a smoother surface. As depicted in Figure 8, the increase in surface roughness was more significant for GA-PEODA crosslinked materials ($P = 0.002$) compared with that for GA crosslinked materials ($P = 0.049$). We have hypothesized that high-energy plasma treatment may have caused the scission of PEODA chains as well as removal of low-molecular-weight materials from the surface of the corneas resulting in a higher increase in the roughness.

The mechanism of cell adhesion to biomaterial surfaces has been extensively studied and documented.[23,24] There are two theories on how cells interact with biomaterials surfaces. The surfaces are

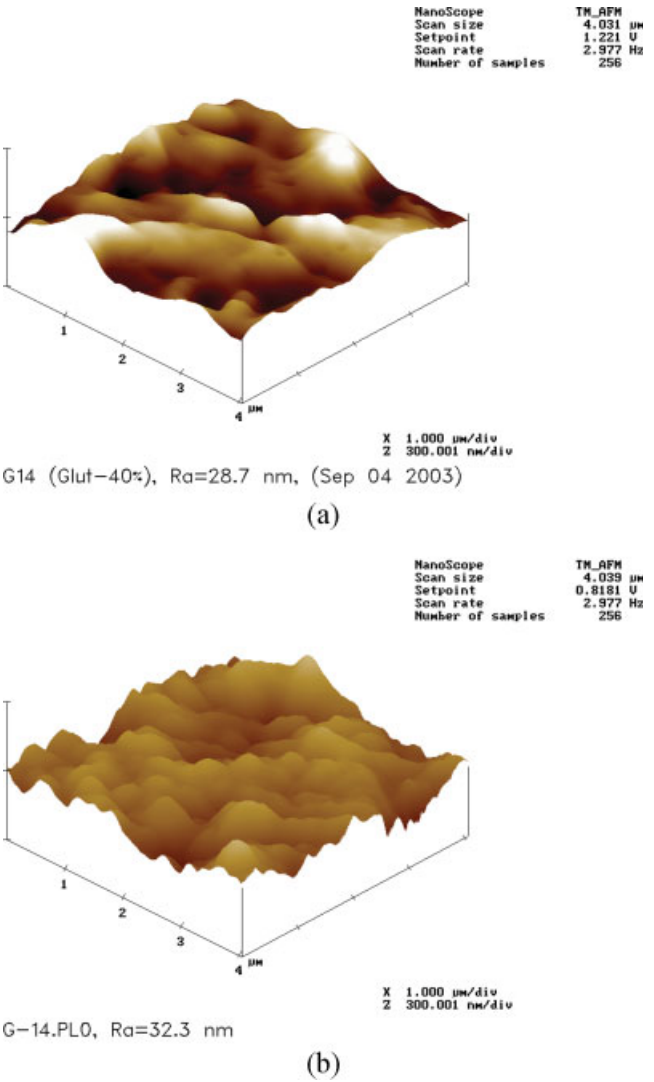

Figure 6 AFM images representing the effect of argon plasma treatment on surface morphology of matrices crosslinked by GA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

either capable of (1) selectively adsorbing intact, functional proteins in the biological environments (i.e., cell culture medium) that in turn can interact with receptors from a targeted cell type or (2) directly interacting with cell surface receptors through the functional polar groups or ligand motifs.[6] Our results are consistent with the second theory although further surface studies should be conducted to confirm our findings.

It is reported that the increase in surface energy (reduced contact angle) after argon plasma treatment is caused by the formation of hydroxyl (OH), carbonyl (CO), and carboxyl (COOH) functional groups that exhibit negative charges.[5] Also, cells are normally believed to exhibit polarity. This polarity is important because normal cell function can only be expressed if the cell has the correct orientation.[25] Therefore, it was hypothesized in this study that enhanced cell attachment was the result of polar interactions (i.e., hydrogen bonding) between hydrophilic functional groups at the polymer surface and cell membrane proteins. The functional groups at the polymer surface were either created by plasma or already existing particularly when the polymer matrices contain PEODA component.

More specifically, the cells may be held to the polymer surface through hydrogen bonding between the oxygen of the CO or OH functional groups at the polymer surface and the hydrogen of NH_2 or COOH groups in the polypeptide chains of cell membrane proteins. Hydrogen bonding is found very often in proteins, especially those having α-helical and/or β-pleated structures. In these structures every peptide bond is essentially involved in at least one hydrogen bond.[26] In addition to surface chemistry and polar interactions, surface morphology has been a factor in cell attachment mechanism. For example,

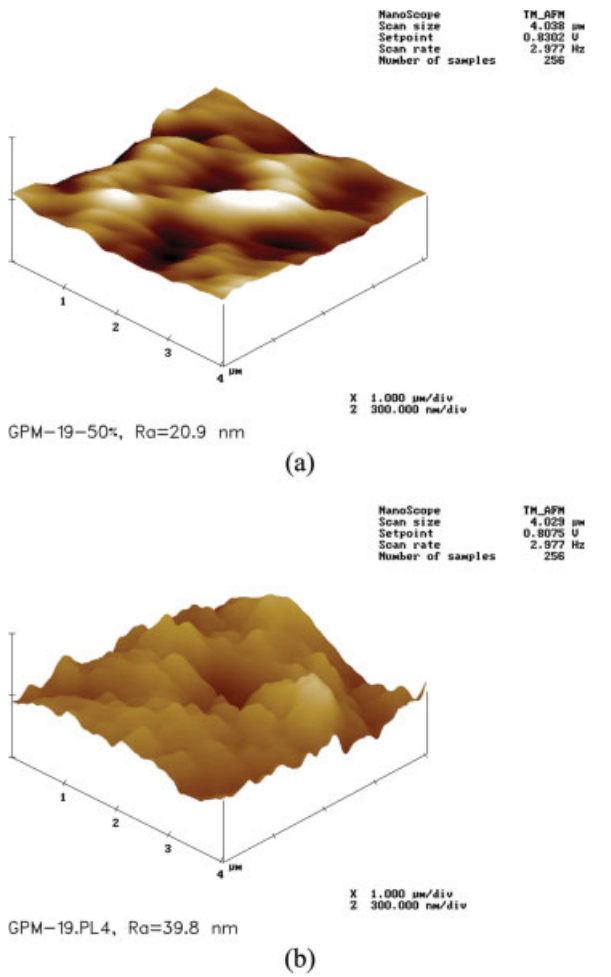

Figure 7 AFM images representing the effect of argon plasma treatment on surface morphology of matrices consecutively crosslinked by GA-PEODA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

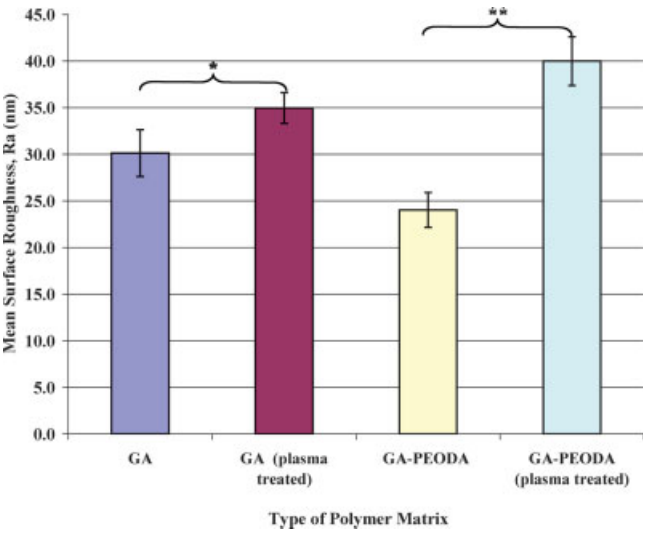

Figure 8 Effect of plasma treatment and type of polymer matrix on surface roughness measured by AFM. The statistical difference between data sets was analyzed using one-way ANOVA. $*P = 0.049 < 0.05$; $**P = 0.002 < 0.05$. Three replicate samples were used in determining the mean value and standard deviation for each data set. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the increase in surface roughness increased the effective surface area resulting in more sites available for the interaction between the cells and the polymer surfaces.

The results presented here suggest that the epithelial cell attachment to artificial cornea surfaces was influenced by the combined effects of plasma-induced surface chemistry (i.e., surface energy), polar interactions between functional groups at the polymer surface and cell membrane proteins, and polymer surface morphology (i.e., surface roughness).

CONCLUSIONS

In conclusion, plasma surface modification of collagen-based artificial cornea was found to be a promising technique to

- Enhance epithelial cells attachment to collagen-base surfaces
- Enhance surface hydrophilicity
- Enhance surface roughness.

More specifically, the following conclusions are drawn from this work:

1. Enhanced attachment of epithelial cells to collagen-based artificial cornea surfaces was observed after argon plasma treatment when compared with the untreated surfaces.
2. Among plasma treated surfaces, those crosslinked by consecutive application of GA and PEODA crosslinkers showed better cell attachment compared to those crosslinked with GA only.
3. Equilibrium contact angle significantly decreased from 70° to 32° on average after argon plasma treatment.
4. The contact angle of the plasma-treated samples did not change over a period of 30 days.
5. Upon plasma treatment, the mean surface roughness increased from 24.0 ± 1.9 to 40.0 ± 2.5 for collagen matrices crosslinked by GA-PEODA, and from 30 ± 2.5 to 35 ± 1.7 nm for collagen matrices crosslinked by GA.
6. Increased epithelial cells attachment to plasma-treated surfaces is most likely due to the increase in number of hydrophilic functional groups (increased hydrophilicity), and increased roughness, endowing the surface with increased effective surface area that is made available for specific interactions with corneal cells.

The author is grateful to Dr. Dave Carlsson for his careful review of this article.

References

1. Chirila, T. V.; Hicks, C. R.; Dalton, P. D.; Vijayasekaran, S.; Luo, X.; Hong, Y.; Clayton, A. B.; Ziegelaar, B. W.; Fitton, J. H.; Platten, S.; Crawford, G. J.; Constable, I. J. Prog Polym Sci 1998, 23, 447.
2. Eye Bank Association of America. 1999 Eye Banking Statistical Report; Washington: EBAA, 1999.
3. Carlsson, D. J.; Li, F.; Shimmura, S.; Griffith, M. Curr Opin Ophthalmol 2003, 14, 192.
4. Leibowitz, H. M.; Trinkaus-Randall, V.; Tsuk, A.; Franzblau, C. Prog Retinal Eye Res 1994, 13, 605.
5. Latkany, R.; Tsuk, A.; Sheu, M. S.; Loh, I. H.; Trinkaus-Randall, V. J Biomed Mater Res 1997, 36, 29.
6. Koenig, A. L.; Grainger, D. W. In Methods of Tissue Engineering; Atala, A., Lanza, R. P., Eds.; Academic Press: San Diego, 2002; Chapter 66.
7. Ratner, B. D.; Chilkoti, A.; Lopez, G. P. In Plasma Deposition: Treatment and Etching of Polymers; d'Agostino, R., Ed.; Academic Press: San Diego, 1990; p 463.
8. Schroder, K.; Meyer-Plath, A.; Keller, D.; Ohl, A. Plasmas Polym 2002, 2, 103.
9. Ratner, B. D.; Hoffman, A. S.; Schoen, F. J; Lemons, J. E. Biomaterials Science: An Introduction to Materials in Medicine; Academic Press: San Diego, 1996.
10. Nitschke, M. Surface Modification by Low Pressure Plasma Techniques. Presented at the Deutsche Ophthalmologische Gesellschaft (DOG) Symposium, Nov. 17, 2002. Available at www.dog.org/termin/biomaterials.html#intro.
11. Sipehia, R.; Garfinkle, A.; Jackson, W. B.; Chang, T. M. S. Biomater Artif Cells Artif Org 1990, 18, 643.
12. Dewez, J. L.; Doren, A.; Schneider, Y. J.; Rouxhet, P. G. Biomaterials 1999, 20, 547.
13. Schakenraad, J. M.; Busscher, H. J.; Wildevuur, C. R.; Arends, J. In Biological and Biomechanical Performance of Biomaterials;

Christel, P., Meunier, A., Lee, A. J., Eds.; Elsevier: Amsterdam, 1986; p 263.
14. Vogler, E. A. Colloids Surf 1989, 42, 233.
15. Busscher, H. J.; Stokroos, I.; Golverdingen, J. G.; Schakenraad, J. M. Cells Mater 1991, 1, 234.
16. Altankov, G.; Groth, T. J Biomater Sci Polym Edn 1997, 8, 299.
17. Saltzman, W. M.; Parsons-Wingerter, P.; Leong, K. W.; Shin Lin, J. Biomed Mater Res 1991, 25, 741.
18. Kowalczynska, H. A.; Kaminski, J. J Cell Sci 1991, 99, 587.
19. Lee, H. J.; Jung, H. W.; Lee, H. B. Biomaterials 1994, 15, 705.
20. Curtis, A. S. G.; Forrester, J. V.; Clark, P. J Cell Sci 1986, 86, 924.
21. Singh, S.; Khulbe, K. C.; Matsuura, T.; Ramamurthy, P. J Membr Sci 1998, 142, 111.
22. Decker, W.; Pirzada, S.; Michael, M.; Yializiz, A. In Proceedings of the 43rd Annual Technical Conference; Society of Vacuum Coaters: Albuquerque, NM, 2000; Vol. 7188, p 505.
23. Hubbell, J. A. J Biotechnol 1995, 13, 565.
24. Hammer, D. A.; Tirrell, M. Annu Rev Mater Sci 1996, 26, 651.
25. Silver, F. H.; Christansen, D. L.; Biomaterials Science and Biocompatibility; Springer: New York, 1999.
26. Hein, M.; Best, L. R.; Pattison, S.; Arena, S. Introduction to General, Organic, and Biochemistry; Brooks Cole: Toronto, 1993.