Relating the Surface Properties of Intraocular Lens Materials to Endothelial Cell Adhesion Damage

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Relationships between corneal endothelial cell adhesion and intraocular lens (IOL) surface properties were studied to develop a lens surface with a lower potential to damage the corneal endothelium. The surfaces examined were poly(methyl methacrylate) (PMMA) and four types of plasma-deposited coatings on PMMA. These four films were prepared from perfluoropropane, ethylene oxide, 2-hydroxyethyl methacrylate (HEMA), and N-vinyl-2-pyrrolidone (NVP). These "monomers" were chosen to produce surfaces with a range in surface chemistry and surface energy. Each type of coating was characterized by electron spectroscopy for chemical analysis (ESCA) and contact angle techniques. In addition, these surfaces were contacted with rabbit corneal endothelium over a force range of -4000 to 20,000 dynes. The extent of endothelial cell damage was measured. Over the force range investigated, each modified surface was found to induce a significantly different degree of cell adhesion than that caused by PMMA. The perfluoropropane plasma film induced a constant lower degree of adhesion damage than the PMMA for all forces of contact. Although the HEMA and NVP hydrogel surfaces also induced lower adhesion damage than PMMA, the cell loss associated with each did increase as a function of force. The ethylene oxide film caused a significant increase in cell loss compared to the PMMA-induced losses. Based upon the correlation between the surface analysis data and the cell-surface contacting results, we suggest that a "soft" high-energy surface or a "rigid" low-energy surface is favorable for reduced cell adhesion. Also, the results indicate that cell adhesion increases for materials with increased hydrocarbon enrichment and for materials with lower (ether bonding)/(ester and ketone linkages) ratios. Invest Ophthalmol Vis Sci 30:853-860, 1989

Over 1,000,000 intraocular lenses (IOLs) are implanted in humans in the U.S. each year. During surgical implantation, contact between the most commonly used IOL material, poly(methyl methacrylate) (PMMA), and the corneal endothelium can cause severe permanent loss of endothelial cells. In clinical studies, cell loss has been directly related to the number of times the IOL contacts the endothelium during surgery: approximately a 20% loss may result from each contact. The nature of the implant surface is responsible for biological interactions such as cell adhesion. In several biomaterials studies, surface properties such as surface energy, surface chemistry and surface rigidity have been indirectly or directly correlated with cell growth and platelet adhesion. However, corneal endothelial cell adhesion has not been directly correlated with the surface properties of IOL materials.

Surface modification of the PMMA surface has been shown to alter its adhesiveness to cells. Knight and Link used gamma radiation to graft hydroxyethyl methacrylate (HEMA) and N-vinyl pyrrolidone (NVP) onto the PMMA surface. Using a static "touch test" between the lenses and rabbit corneas, they found the PMMA surface to induce 10-30% damage, the PMMA/NVP graft to induce less than 10% cell damage, and the PMMA/HEMA graft, about 10% damage. Unfortunately, "touch tests" such as these can yield only qualitative comparisons. Kassar and Varnell used an 18 g weight to contact the IOL and the rabbit cornea for 10 sec to obtain a consistent interactive force between the endothelium and IOL surfaces. They observed PMMA to cause "considerable" damage, silicone resin lenses to induce "less" damage than PMMA, and silicone elastomer to create "far less" damage. However, no quantitative comparisons of cell damage were made. Reich et al constructed and used an instrument for directly measuring the force of adhesion between rabbit corneal endothelium and IOL materials. The average stress calculated for PMMA (0.66 g/cm²) was

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shown to be the highest of all materials studied, whereas a plasma-deposited NVP coating on PMMA and a coating of Healon on PMMA each lowered the stress to 0.19 g/cm². The hydrogels, poly(HEMA) and Duragel, exhibited the lowest stresses: 0.09 and 0.14 g/cm², respectively. The method provided a direct quantitative comparison between materials.

In this work we examined the degree of cell adhesion induced by various materials. The surfaces studied were PMMA and four types of plasma-deposited polymer coatings on PMMA substrates. After contacting each material with rabbit endothelium at specific forces, we measured the extent of cell adhesion damage to the corneas. This method allowed a quantitative and consistent comparison among lens materials.

We studied relationships between surface properties of the materials and the degree of endothelial cell adhesion at specific forces of contact. In order to form these relationships, we characterized the nature of the surfaces by using electron spectroscopy for chemical analysis (ESCA) and a contact angle technique.

**Materials and Methods**

**Surface Preparation**

Coatings were deposited on 10 mm diameter PMMA disks (Perspex®, I.C.I. Ltd., London, UK) within a capacitively coupled radio frequency (RF) plasma reactor. The four “monomers” introduced were perfluoropropane (Matheson, East Rutherford, NJ), ethylene oxide (Matheson), N-vinyl-2-pyrrolidone (NVP) (Alfa Products, Danvers, MA), and 2-hydroxyethyl methacrylate (HEMA) (Hydromed Sciences, New Brunswick, NJ).

The plasma reactor consisted of a glass chamber (7.6 cm diameter) with 15.2 cm long capacitance plates encircling the center. An oscillating electric field was produced between these plates by means of a 13.56 MHz RF generator (Tegal). Sample disks were placed 5 cm downstream of the capacitance plates, etched with an argon plasma, and then coated with one of the four plasma-polymerized “monomers.” By defining the system parameters of RF power, system pressure and length of reaction, we controlled the nature and thickness of the deposition of each polymer.

**Surface Analysis**

The sample surfaces were characterized in terms of surface chemistry and surface energy by using the following techniques.

ESCA: Electron spectroscopy for chemical analysis (ESCA) was used to study the elemental composition and bonding states of the outermost 100 Å of the polymer surface. Both the Hewlett Packard Model 5950B ESCA system at the University of Utah and the Surface Science SSX-100 ESCA spectrometer at the University of Washington were used to analyze the surfaces. A survey scan (1–1000 eV) was taken to determine the various elements present. Scans in specified eV ranges were made to obtain high resolution spectra of the elements C, O, F and N. The C1s hydrocarbon peak was assigned to 285.0 eV and used as a reference peak to correct for any energy shifts.

Contact angle measurement: Critical surface tensions were determined by using the method of Zisman. Contact angles of various purified liquids on each type of surface were measured under atmospheric conditions using the Rame-Hart goniometer, Model 100-00-0NRL. Each critical surface tension was calculated from a plot in which the cosines of the measured angles were plotted as a function of the liquid–vapor surface tensions of the test liquids. Teflon® (Norton) and Mylar® (DuPont) were used as reference surfaces.

**Surface–Cell Adhesion Test**

The extent of damage resulting from contact between the corneal endothelium and a disk of sample material was measured in two steps. First, the disk and a freshly excised rabbit cornea were mounted in the device illustrated in Figure 1 and contacted at a measurable force. Second, the cornea was removed, stained and examined under a low-power light microscope to measure the number of damaged cells.

Each cornea, rimmed by 2–3 mm of sclera, was excised from a 2–3 kg New Zealand white rabbit and immediately placed in RPMI 1640 media with HEPES buffer, L-glutamine, and penicillin–strepto-
mycin (Grand Island Biological). The cornea in solution was placed in a Forma Scientific Hydrojac CO₂ incubator for at least 30 min. In preparation for a test, the cornea was removed from this solution, rinsed in a 0.9% NaCl solution, placed on a concave Teflon® block, and trephined to form a 9 mm diameter button. This cornea button was placed, endothelial side up, in a convex stainless steel holder. Thus, a 7 mm diameter circle of endothelium was exposed with the center projected 2 mm above the level of the holder edge. A 0.9% NaCl solution was dropped intermittently onto the cornea to keep the cells continuously moist.

The 10 mm diameter sample disk was mounted in a stainless steel holder which clamped the disk edges, leaving a 9 mm diameter exposed planar surface. This holder was then attached to the microforce detector (Deflection Sensor Cartridge, Model DSC3, Imperial Controls). The system was calibrated using 1–20 g weights, depending on the force anticipated for each test (range used: 4000–20,000 dynes).

The corneal holder was placed directly beneath the material sample, and the two surfaces were brought into contact by using the micrometer attachment. After 40–60 sec, the cornea and the sample were separated, and the cornea was immediately placed in a 0.9% NaCl solution. The initial, maximum force with which the contact was made was recorded.

Controls were corneal buttons which sat in the holder for 20 min and were kept moist with drops of a 0.9% NaCl solution. These controls were subjected to all of the above handling except for the contacting step.

The staining method of Spence and Peyman¹³ was used to inspect the endothelium. This method is a two-step staining procedure which uses a combination of Trypan Blue and Alizarin Red S stains (Sigma Chemical, St. Louis, MO). The cornea was examined under low-power (×100) microscopy. A central 9 mm² area divided into 900 grids was observed consistently for each cornea. Typical stained rabbit endothelial cells are shown in Figure 2.

This study was conducted in accordance with the AVRO Resolution on the Use of Animals in Research.

Results and Discussion

Surface Characterization

Surface chemistry: The surface composition of each material was determined by ESCA. In Table 1, the ESCA results of both conventional and plasma-deposited polymers are compared with the stoichiometry of the monomers. The untreated PMMA disk exhibits C/O ratios close to the expected stoichiometric ratio, and the fraction of the surface composed of each bonding environment is also as expected. HEMA and NVP plasma-deposited coatings show close resemblance in composition and bonding to the stoichiometry and model poly(HEMA) and poly(NVP) films centrifugally cast on glass. The peaks comprising the ESCA spectra of the model
Table 1. Elemental and bonding ratios

| Compound                  | C/O | C/N | C/F | C-H | C=O | O || O  |
|---------------------------|-----|-----|-----|-----|-----|---|---|
| 1. Stoichiometry based on monomer structure |     |     |     |     |     |   |   |
| MMA                       | 2.5 | —   | —   | 60  | 20  | — | — |
| HEMA                      | 2.0 | —   | —   | 50  | 33.3| — | 16.7|
| NVP                       | 6.0 | 6.0 | 50  | —   | —   | — | 100|
| Ethylene oxide            | 2.0 | —   | —   | —   | —   | — | — |
| Perfluoropropane          | —   | —   | 0.375| —   | 66.6| 33.3| — |
| 2. ESCA results           |     |     |     |     |     |   |   |
| PMMA                      | 3.0 | —   | —   | 58  | 23  | — | 19 |
| pHEMA*                    | 2.4 | —   | —   | 46  | 38  | — | 16 |
| HEMA†                     | 2.2 | —   | —   | 46.5| 31  | — | 23.5|
| pNVP†                     | 8.0 | 8.0 | —   | 43.2| —   | — | 29.7|
| NVP†                      | 5.7 | 8.1 | —   | 53  | —   | — | 34 |
| Ethylene oxide†           | 4.8 | —   | —   | 61  | 21  | 9 | — |
| Perfluoropropane†         | —   | —   | 0.52 | 4   | 28  | 27.6| 15.6|

* Poly(HEMA) centrifugally cast on glass coverslip (2% in DMF).
† Plasma-deposited film on PMMA disk.

Table 2. Critical surface tensions (γc) of samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>γc (erg/cm²)</th>
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<tbody>
<tr>
<td>Teflon (PTFE)</td>
<td>20.0 ± 1.3</td>
</tr>
<tr>
<td>Mylar</td>
<td>46.7 ± 0.3</td>
</tr>
<tr>
<td>PMMA</td>
<td>37.8 ± 3.2</td>
</tr>
<tr>
<td>Perfluoropropane</td>
<td>8.1 ± 3.1</td>
</tr>
<tr>
<td>Ethylene oxide plasma-deposited film</td>
<td>45.4 ± 2.7</td>
</tr>
<tr>
<td>HEMA plasma-deposited film</td>
<td>49.4 ± 4.1</td>
</tr>
<tr>
<td>NVP plasma-deposited film</td>
<td>48.0 ± 4.3</td>
</tr>
</tbody>
</table>

films are more distinct than those in the spectral envelopes of the plasma-deposited films. The more ill-defined spectra of the plasma-deposited films reflect a wider distribution of structures, an increased number of chemical species, and increased crosslinking, all of which are characteristic of plasma depositions. However, the general similarity of the HEMA and NVP plasma deposits to the model polymers indicates a higher level of molecular polymerization (ie, free radical polymerization through the double bond) than atomic polymerization. Because this similarity also suggests a comparable regularity of structure which is important to the hydrogel character of poly(HEMA) and poly(NVP), the coatings deposited by the HEMA and NVP plasmas are expected to exhibit hydrogel behavior.

Coatings deposited by ethylene oxide and perfluoropropane plasmas are vastly altered from the monomer structures. Apparently these plasmas undergo complex reactions involving atomic polymerization, a type of deposition in which the molecular structure of the monomer is not retained in the polymer. As a result, a polymeric regularity in the structure of the ethylene oxide plasma coating is not likely to exist because the bonding environments noted by ESCA bear little resemblance to those observed in anionically polymerized poly(ethylene oxide).

Surface energy: Experimentally determined values of the critical surface tension (γc) of several materials are listed in Table 2. Teflon and Mylar were examined as reference surfaces. The plasma-deposited surfaces on PMMA exhibited definite changes in wettability compared to the untreated PMMA substrate. The fluorinated plasma deposit converted the surface of the disk to one even more nonwettable than Teflon. The other three plasma-deposited surfaces showed an increase in wettability as suggested by the increased critical surface tension. These results are consistent with the trends noted by Baier et al, that is, wettability decreases with an increase in fluorination and increases with an increase in nitrogen or oxygen bonded to carbon.

Cell-Surface Interaction

In Figure 3–7, the percent cell damage is plotted as a function of initial force for each type of surface. The error bar in the x-direction represents the uncertainty in the initial force measurement, and the error bar in the y-direction represents a global error in percent.
cell damage, calculated as 10% of the average damage for each type of surface over the range of 9000–12,000 dynes. Solid lines drawn through the force-damage data represent the best fit for the data.

In Figure 8, the best fits for the five types of surfaces are drawn for the percent cell damage data as a function of initial contact force. The choice of fit was based on results from the "t test for chi-square," which determines whether adding a force-dependent term (least squares line) is an improvement from the average damage line. Because PMMA, ethylene oxide, NVP and HEMA surfaces displayed a trend of increasing damage with increasing force, the least squares fits are plotted. Since the slope of the perfluoropropane force-damage curve was not significantly different from zero, the average cell damage value is plotted. The control line, 3.5 ± 2.7% cell damage, represents the average damage associated with the control corneas.

The parameters and errors of the fits listed in Table 3 were used to calculate the significance of the differences in cell adhesion associated with the five surfaces. Relative to PMMA, each of the four coating data sets was found to have >97% probability that the difference between the fit for PMMA and the fit for an altered surface is significant.

The fluorinated surface induced the lowest endothelial damage over the entire force range investigated, and the damage appeared to be independent of force in that range. The HEMA and NVP surfaces were also associated with decreased cell damage compared to the unmodified PMMA. However, these two surfaces induced increasing cell damage with increasing force. The ethylene oxide coating caused significantly greater adhesion damage to the cornea compared to the untreated PMMA.

The scatter of the data can be attributed to several factors. As shown by the unique force versus cell damage trend of each surface, specific properties characteristic of each surface resulted in endothelial cell adhesion. However, there might have been instances in which mechanical damage (crushing), in addition to the adhesion-induced damage, caused the total injury to be greater than from adhesion alone. Other experimental factors to consider are the unique handling of each cornea, the age and sex of the rabbit, the batch of buffer solution used, the sequence of experiments in a day, and the batch of sample disks used. Controls were performed to estimate the magnitude of these factors on damage. Force versus percent cell damage data were plotted for specific cases, but no significant trend deviating from the overall fit was noted.

**Relationship between Surface Properties and Cell Adhesion**

The degree of cell adhesion damage was significantly altered by modifying the PMMA surface. The
surface chemistry and surface energy of each type of surface were documented by ESCA and contact angle studies. Trends relating the surface properties to cell adhesion damage are shown in Figures 9, 10 and 11.

As shown in Figure 9, the percent cell damage (represented here as the average value for the medium force range of 9000-12,000 dynes) increases with an increase in $\gamma_c$ for the PMMA, ethylene oxide, and perfluoropropane surfaces. However, the HEMA and NVP surfaces, both high energy surfaces, do not fit this trend and are associated with a low degree of damage. Weiss and Blumenson associated an increase in the critical surface tension with an increase in the degree of cell adhesion and spreading. Similarly, Yasuda et al found a linear relationship between total surface energy and the rate of cell adhesion to certain surfaces. However, Maroudas has noted that "soft" hydrogel surfaces are unable to support a concentrated load.

The separation of the HEMA and NVP surfaces from the others in Figure 9 suggests a distinct difference in their surface characteristics. As noted previously, the similarity in surface chemistry between the plasma-deposited HEMA and NVP polymers and the conventional HEMA and NVP polymers suggests that the plasma films also display the hydrogel characteristics of the conventional polymers. Therefore, because these two thin plasma films can be considered "soft" hydrogel surfaces, the separation from the "rigid" surfaces in Figure 9 agrees with the results from other studies involving hydrogel and "rigid" surfaces. Unfortunately, the hydrogel characteristics of these plasma deposits cannot be evaluated by swelling measurements because the extremely thin films result in large gravimetric errors.

In Figure 10, a separate trend for the HEMA and NVP surfaces is noted in the plot of cell damage as a function of the (carbon)/(other elements present) (C/X) ratio. The hydrogel characteristics of the HEMA and NVP coatings could explain this divergence from the trend of increased cell damage with hydrocarbon enrichment. We analyzed dry HEMA and NVP films to obtain this ESCA surface composition data. When these films are fully hydrated, as they were when used in cell-contacting experiments, their polymer chains possibly become flexible and able to orient polar groups to the surface. Thus, the correla-

Table 3. Best fits for the cell damage data

<table>
<thead>
<tr>
<th>Surface</th>
<th>Slope</th>
<th>Intercept</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA</td>
<td>0.0019 ± 0.0005</td>
<td>-1.65 ± 5.4</td>
<td>—</td>
</tr>
<tr>
<td>HEMA</td>
<td>0.0005 ± 0.0002</td>
<td>1.99 ± 2.2</td>
<td>—</td>
</tr>
<tr>
<td>NVP</td>
<td>0.0006 ± 0.0002</td>
<td>1.27 ± 2.7</td>
<td>—</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>0.0012 ± 0.0006</td>
<td>17.44 ± 6.1</td>
<td>—</td>
</tr>
<tr>
<td>Perfluoropropane</td>
<td>—</td>
<td>—</td>
<td>6.96 ± 3.83</td>
</tr>
</tbody>
</table>

Fig. 6. Percent cell damage as a function of the initial force of contact with the plasma-deposited NVP surface.

Fig. 7. Percent cell damage as a function of the initial force of contact with the plasma-deposited ethylene oxide surface.

Fig. 8. Comparison of the best fits of the force-cell damage data for PMMA and the four plasma-deposited films.
tion of hydrated HEMA and NVP elemental ratios with cell adhesion would be more appropriate. Ratner et al.18 studied the surface composition of several radiation-grafted polymers in both hydrated and dry states. They found that the C/O ratios of hydrated poly(HEMA) grafted to polyethylene were dramatically reduced (1.82) compared to the C/O ratios obtained for the dry grafts (3.53). If a similar trend in the C/O ratio held for our hydrated and dry NVP and HEMA films, the hydrated C/X surface ratios would be lower than the dry values indicated in Figure 10 and would follow more closely the line described by the other three surfaces. However, since the substrate polymer, PMMA, contains a considerable fraction of oxygen as compared to polyethylene that has little oxygen, a significant difference in elemental composition between the wet and dry material might not be observed.

In Figure 11, a decrease in the ratio of (ether bonding)/(ester and ketone bonding) is related to an increase in the percent cell damage induced by the PMMA and the plasma-deposited films of ethylene oxide and HEMA. This relationship somewhat corroborates the results of another study,17 which associates the ester functionality of materials with long-term cell growth, implying that there is strong cell adhesion to the substrate.

Significance for Intraocular Lens Implantation

The potential to damage the corneal endothelium during intraocular surgery has been greatly reduced by the introduction of viscoelastic agents (eg, hyaluronic acid). However, the possibility of accidental contact of an IOL with the cornea still exists. Also, the viscoelastic agents are expensive and can lead to transient complications. Permanently immobilized lens coatings that are nonadhesive to corneal endothelium could lead to a reduction in the amount of the viscoelastic agent used and the frequency of use.

Three permanent coatings that minimize contact damage to the corneal endothelium have been introduced here. One of the coatings, the C3F8 fluoropolymer deposition, should be the least damaging to corneal endothelium because an increased force of contact will not lead to increased cell damage. Also, the dense, crosslinked C3F8 polymer coating will serve as a barrier inhibiting the migration of any low molecular weight material from the IOL to the eye.

Thus, a change in surface properties by RF plasma deposition has been found to alter the degree of endothelial cell adhesion to the surface. The percent cell damage as a function of the initial force of contact for each modified surface was significantly different from that induced by PMMA. By examining these results, we suggest that a “rigid” low-energy surface such as the perfluoropropane plasma coating, or a “soft” high-energy surface such as the HEMA and NVP...
plasma coatings is desirable for reduced endothelial cell adhesion. Also, our results suggest that reduced cell adhesion is associated with a decrease in the hydrocarbon ratio and an increase in the ratio of (ether bonding)/(ester and ketone linkages).

Key words: intraocular lens, cell adhesion, surface properties, RF plasma deposition, corneal endothelium

References