Comparison of poly (methyl methacrylate) and acrylic hydrophobic intraocular lens surface irregularities using atomic force microscopy

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Background: Posterior capsule opacification (PCO) is a very frequent complication of phacoemulsification surgery in patients undergoing cataract extraction. This has led to modifications in design and material of intraocular lenses (IOLs). There is increasing evidence suggesting that foldable acrylic hydrophobic IOLs are associated with lower PCO incidence. However, traditional rigid poly methyl methacrylate (PMMA) IOLs are still in widespread use due to the large number of manual small-incision cataract surgeries performed in developing countries. The amount of surface irregularities on an IOL is linearly correlated to the rate of lens epithelial cell (LEC) migration and hence to the occurrence of PCO. Objective: Surface roughness parameters of PMMA and acrylic hydrophobic IOLs were compared using atomic force microscopy (AFM). Topographical characteristics of 7 PMMA and 4 acrylic hydrophobic IOLs obtained from various manufacturers were evaluated with AFM in tapping mode. A V-shaped, silicon nitride cantilever with a tip curvature <10 nm, length 115-135 µm and a spring constant in the range of 0.20-80 N/m was used. All images were acquired with a resolution of 256×256 data points per scan at a scan rate of 0.5 Hz per line and a scan size of 10×10 µm. Results: AFM images of IOL optic surfaces showed a collection of pores, grooves, ridges, very small peaks and surface irregularities. Acrylic hydrophobic IOLs showed significantly less surface roughness when compared with PMMA IOLs. Conclusions: AFM is a powerful technique for topological characterization of IOLs. Acrylic hydrophobic IOLs appear to be more suitable in preventing PCO compared with PMMA.

Keywords: Intraocular lens (IOL); PMMA; acrylic hydrophobic; atomic force microscopy (AFM); surface roughness

1. Introduction

Phacoemulsification and posterior chamber intraocular lens (IOL) implantation is the preferred surgical correction for cataract, which is one of the most common causes of blindness worldwide [1]. Long term post-operative complication includes posterior capsular opacification (PCO) with an incidence of nearly 20-50% within 5 years of surgery [2]. Although numerous factors contribute towards the development of PCO, lens epithelial cells (LEC) are the major cellular precursors of this process. It is well established that PCO is caused by the proliferation, differentiation, and migration of LEC onto the IOL surface leading to the opacification of the posterior capsule [3, 4].

The influence of IOL surface properties on the adhesion and migration of LEC has been extensively investigated [5,6]; however, the effect of IOL material on PCO is still not very well understood. Several attempts have been made to prevent postoperative migration and proliferation of LEC, by improving IOL designs and materials [4,7]. IOLs composed of various optic materials have been developed which range from the high water content hydrophilic acrylic material, low water content hydrophobic acrylic material, to hydrophobic silicone material and the traditional poly methyl methacrylate (PMMA) or hydroxy ethyl methacrylate (HEMA).

Although several research groups have examined the surface of IOLs using scanning electron microscopy (SEM) [8, 9], there are limited reports on the assessment of IOL surface characteristics using atomic force microscopy (AFM) [10]. AFM has emerged as an excellent tool for exploring biomaterial surface properties at a nanometer level [11]. Briefly, in AFM, a sharp tip at the end of a cantilever scans over the surface of the material to be investigated. Plotting the deflections of the cantilever tip in response to the surface profile yields its topological image [12]. This technique also has the added advantages of acquiring 3D topographic images, quantitatively assessing surface roughness characteristics and exploring samples in ambient air or liquid environment without involving elaborate sample preparation [13]. Dogru et al. (2000) have examined explanted IOL from a PCO patient using optical microscopy and AFM [14]. This technique has also been used to explore the variations in interfacial properties of hyaluronan coated PMMA IOLs [15]. Micron-level polishing effect of a latex posterior capsule facsimile on PMMA IOLs has been evaluated using AFM [16]. Morphological characteristics of a novel coating on IOL to prevent PCO formation has been studied using AFM [17]. Topographic features of soft contact lenses have also been explored using this technique [18-20].

The incidence of PCO is known to differ according to the optic material of the implanted IOLs [21]. There is increasing evidence suggesting that acrylic IOLs are less damaging to the corneal endothelium than PMMA IOLs and prevent LEC from migrating and forming PCO [7,22 and 23]. This may be attributed to the fact that acrylic material tends to adhere to the lens capsule to a greater extent than PMMA [24]. The objective of the present study is to investigate and compare the surface micro-roughness characteristics of acrylic hydrophobic and PMMA IOLs using AFM.
2. Material and Methods

Topological characteristics of 7 PMMA and 4 acrylic hydrophobic IOLs obtained from various manufacturers were examined. The IOLs were removed from their sterile packs with atraumatic forceps and placed on a magnetic stainless-steel sample holder using double sided adhesive tape for AFM. The person operating the AFM was kept unaware of the IOL model used to prevent biased observations.

2.1 Atomic force microscopy

A commercial AFM (CP II, Veeco Instruments Inc., USA) was used in the tapping mode to measure the surface topological features of the IOL optics. A V-shaped, silicon nitride cantilever (MMPb11123, Veeco Instruments Inc., USA) with a tip curvature <10 nm, length 115-135 µm and a spring constant in the range of 0.20-80 N/m was used. All images were acquired with a resolution of 256×256 data points per scan at a scan rate of 0.5 Hz per line and a scan size of 10×10 µm.

Two samples of each IOL model were used for analysis. The area scanned was limited to a maximum of 100 µm² owing to the gross curvature of the optics surface. At least six sites taken from different areas close to the center of the optics surface were scanned to confirm the reproducibility of the observed features. Each single area on the optics was imaged twice to ensure that the force exerted by the tip did not damage the sample surface and cause artifacts. All images were processed and analyzed using Image Processing and Data Analysis software (version 2.1.15; copyright TM Microscopes USA) which included 4th order flattening to remove the background slope caused by the nonlinearities of the piezoelectric scanner. Region analysis of the scanned images was performed to measure the surface roughness.

2.2 Data analysis

Mathematical tools help in extracting quantitative information on surface roughness from AFM images. Various roughness parameters such as peak–valley height difference (R_{pv}), average roughness (R_{a}), root mean square roughness (R_{q}) and height distribution were calculated. R_{a} and R_{q} are given by:

\[
R_a = \frac{1}{n_x n_y} \sum_{i=1}^{n_x} \sum_{j=1}^{n_y} |Z(i,j) - Z_{ave}| \quad R_q = \sqrt{\frac{1}{n_x n_y} \sum_{i=1}^{n_x} \sum_{j=1}^{n_y} [Z(i,j) - Z_{ave}]^2}
\]

where Z (i, j) denotes the topography data for the surface after specimen tilt-correction, Z_{ave} is the average surface height, i and j correspond to pixels in x and y direction. The maximum number of pixels in the two directions are given by n_x and n_y [11]. In the present study, n_x = n_y = 256. R_{q} is the standard deviation of the Z values within a given area whereas R_{a} is the mean roughness of the surface relative to the centre plane.

2.3 Skewness and Kurtosis

AFM images were imported in ASCII format to commercial mathematical software, Matlab 7.1.0 (service pack 3, Mathworks Inc.) for statistical analysis. Moments including skewness (S) and kurtosis (k) describing the probability distribution were calculated from the data:

\[
S = \left(\frac{1}{R^3_{q}}\right) \left(\frac{n_x n_y}{n_x n_y}\right) \sum_{i=1}^{n_x} \sum_{j=1}^{n_y} (Z(i,j) - Z_{ave})^3 \quad k = \left(\frac{1}{R^4_{q}}\right) \left(\frac{n_x n_y}{n_x n_y}\right) \sum_{i=1}^{n_x} \sum_{j=1}^{n_y} (Z(i,j) - Z_{ave})^4
\]

where, i and j are coordinates on the xy plane of a point on the surface Z(i, j) with height=h.

2.4 Roughness analysis

A surface profile is composed of a superposition of spatial waves of increasing frequencies due to the multi-scale nature of roughness. Hence, to characterize such a profile it becomes necessary to determine the amplitude of the roughness component at each spatial frequency. This is typically accomplished by calculating the power spectrum (PS) of the roughness profile using the relation:

\[
PS(f) = \frac{1}{L} \int_0^L dx e^{2\pi f x} \left[ h(x) - \langle h \rangle \right]^2
\]

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where, \( \text{PS}(f) \) is the power of the surface roughness wave of frequency \( f \) and has the units of \( \mu\text{m}\,\AA^2 \). \( L \) represents the total scan length in \( \mu\text{m} \) and \( x \) is the spatial variable in \( \mu\text{m} \). \( \text{PS} \) of a single image, i.e. five line spectra separated equidistantly on the optics surface, were recorded and averaged.

2.5 Statistical analysis

Surface roughness parameters are summarized as mean ± SEM. Data were compared using one way variance analysis (ANOVA). \( P \leq 0.05 \) was considered to be significant. Results were statistically analyzed using version 2.0 beta 13 software (software developed by Koichi Yoshioka and available online: http://www.woundedmoon.org/win32/kyplot.html), SPSS software (version 11; SPSS, Inc., Chicago, IL) and MATLAB 7.1.0 (service pack 3, Mathworks Inc., USA).

3. Results and Discussion

3.1 Topological characteristics of IOLs

Two-dimensional AFM images of PMMA and acrylic hydrophobic IOLs are represented in Figure 1. Three-dimensional AFM images of the two lenses are shown in Figure 2. AFM images were color mapped to represent height distribution, with the dark areas depicting dents on the surface and the bright areas representing protuberances. PMMA IOLs showed significantly higher surface irregularities when compared with acrylic hydrophobic IOLs (\( P \leq 0.001 \)).

Figure 1: Two dimensional images of IOLs A) PMMA and B) Acrylic hydrophobic

Table 1 summarizes the surface roughness and height parameters of PMMA and acrylic hydrophobic IOLs.
Table 1: Quantitative analysis of surface roughness parameters of PMMA and acrylic hydrophobic IOLs using AFM

<table>
<thead>
<tr>
<th>Roughness parameters</th>
<th>PMMA</th>
<th>Acrylic Hydrophobic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{p-v}$ (nm)</td>
<td>100.22±9.42</td>
<td>28.92±2.17</td>
<td>p≤0.001</td>
</tr>
<tr>
<td>RMS Roughness $[R_q]$ (nm)</td>
<td>5.66±0.57</td>
<td>3.25±0.41</td>
<td>p≤0.01</td>
</tr>
<tr>
<td>Average Roughness $[R_z]$ (nm)</td>
<td>3.92±0.43</td>
<td>2.53±0.31</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Mean Height (nm)</td>
<td>31.14±2.86</td>
<td>13.73±0.91</td>
<td>p≤0.001</td>
</tr>
<tr>
<td>Median Height (nm)</td>
<td>31.04±2.86</td>
<td>13.61±0.87</td>
<td>p≤0.001</td>
</tr>
</tbody>
</table>

Data represents mean ± SEM.

PMMA: poly-methyl methacrylate

Skewness and kurtosis of the AFM images, representing the real area of contact, were assessed and is represented in Figure 3.

3.2 Power spectrum analysis

Figure 4 shows average PS of PMMA and acrylic hydrophobic IOLs on a log scale. The trend of the graph supports our findings summarized in Table 1; a significant increase in the spatial roughness of PMMA IOLs compared to acrylic hydrophobic IOLs was observed (p≤0.001).

Figure 4: Average power spectra (PS) of PMMA and acrylic hydrophobic IOLs on a log scale indicating decreased spatial roughness of acrylic hydrophobic IOLs
During the last 50 years, the IOL has undergone several modifications in terms of design and material. Earlier, the rigid PMMA lens was the most commonly used IOL in clinical practice [25]. Advent of phacoemulsification highlighted the need to identify foldable lens material which would allow small-incision surgery [26]. This resulted in the development of a series of new foldable materials such as acrylic hydrophilic, acrylic hydrophobic and silicone group [7]. While hydrophobic acrylic IOLs have gained considerable popularity owing to their low PCO rates, PMMA IOLs are still in widespread use in the developing world due to the large number of manual small-incision cataract surgeries performed in these countries.

It is well accepted that different physical properties of the IOL material influence LEC adhesion due to its direct interaction with intraocular tissue, proteins and inflammatory mediators [27]. Adhesion of LEC on the surface of IOLs generally indicates postoperative inflammatory reaction and the limited biocompatibility of the IOLs [26]. However, conflicting views exist regarding the efficacy of different IOL materials in preventing PCO [7].

Recently, AFM has become an effective tool in the investigation of surface roughness of biomaterials, as it provides microscopic information with a spatial resolution relevant to the size of polymeric functional group proteins and cells [28]. Valuable information on height distribution can also be obtained from AFM images. Several groups have suggested AFM to be the most preferred technique for exploring IOL surfaces due to its high surface sensitivity and simplicity of sample preparation, unlike SEM [14, 29]. Further, there is less destruction of the IOL biomaterial, therefore allowing the lenses to be examined in three dimensions in their physiological environment, and under near-use conditions.

AFM images of IOL optic surfaces showed a collection of pores, grooves, ridges, very small peaks and surface irregularities. Numerous distinct grooves were present throughout the PMMA lens surface while ridge-like structures were irregularly distributed over the entire surface optic. Our results are consistent with the findings of Lombardo et al. (2006) who have also reported the occurrence of infrequent and distinct depressions on the posterior surface of PMMA IOLs [29]. Further, pores of varying densities and sizes were seen on acrylic hydrophobic IOLs. Occasional crater-like features were also observed on their optic surface. Similar distinct depressions on the surface of acrylic hydrophobic IOLs are reported by other groups [14, 29]. Differences in the topological features of IOL materials may be attributed to the differences in their physical and chemical properties [23].

AFM studies showed statistically significant differences in the surface micro-roughness properties of PMMA and acrylic hydrophobic IOLs (Table 1). PMMA IOLs exhibited significantly higher surface roughness and irregularities as compared to acrylic hydrophobic IOLs (Figure 1-2). Our findings are supported by a similar study by Lombardo et al. (2006) where highest roughness parameters of PMMA IOLs are reported as compared to other IOL biomaterials [29]. Surface profile differences between various IOLs may be attributed to the differences in manufacturing procedures, intrinsic polymerization [1, 30] and dissimilarities in fabrication processes [29].

Two additional amplitude parameters, skewness and kurtosis were evaluated to establish the topological differences between the two IOL biomaterials. While skewness specifies the degree of symmetry in the height distribution about the mean, kurtosis characterizes the peakedness of the height distribution and measures the number of isolated. Surfaces with positive skewness and higher kurtosis signify lower real area of contact and hence, lower friction [11]. Positive skewness (1.118) in acrylic hydrophobic IOLs imply lesser friction due to minimal undulations on the surface. Similarly, increased kurtosis (0.030) in PMMA IOLs reflects increased micro-friction due to surface irregularities [Figure 3]

PS offers quantitative information not only on the height deviation of the roughness profile, but also on its lateral distribution (the spatial extent of the height variations in the roughness profile). PS analysis, therefore, gives a more definite description than the standard roughness parameters [11]. Spatial roughness of PMMA IOLs was found to be significantly increased as compared to acrylic hydrophobic IOLs [Figure 4]

It is evident that the amount of surface irregularities is linearly correlated to the number of inflammatory cells adhering to the IOL optic surface and to the rate of LEC migration [5, 6 and 10]. Our AFM results indicate that surface roughness of acrylic hydrophobic IOLs is significantly less than PMMA IOLs. This possibly prevents the adhesion of LEC to the acrylic surface, thereby reducing the incidence of PCO.

4. Summary

AFM is a powerful technique for the topological characterization of IOLs. Surface micro-roughness properties of commonly used acrylic hydrophobic and PMMA IOLs were extensively investigated using AFM. Surface roughness parameters of acrylic hydrophobic IOLs were significantly less as compared to PMMA IOLs. It is, therefore, tempting to consider acrylic hydrophobic IOLs over PMMA as the ideal biocompatible material for decreasing LEC proliferation, thereby lowering PCO incidence. Further AFM measurements of micromechanical properties, such as elasticity and adhesion may be useful in providing additional information on the biocompatibility of the IOLs.