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Effects of bacterial adhesion with respect to the type of material, structure and design of intraocular lenses

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The properties of the biomaterials used to constitute lenses are important factors choosing a lens for human implantation because these can influence in posterior clinical evolutions of patients. In this study, different characteristics of intraocular lenses such as chemical composition, surface roughness and lens design have been investigated in terms of their influence into a pathological environment. Eight commercial lenses were tested by optical profiling, Infrared spectra with Fourier transformation (FTIR), water-material contact angle and scanning electron microscope (SEM) to know their chemical composition and structural characteristics. These lenses were then exposed to infectious conditions in order to evaluate their responses to the bacterial environment.

1. Introduction

Intraocular lens implantation has become the procedure of choice for many ophthalmologists treating aphakia because the lenses perform remarkably well. While these implants are usually successful there are many clinical reports of infections in patients who have intraocular lenses.

Many studies of IOL implant related infections involve the investigation of surgical complications [1–6]; few of these reports however are concerned with bacterial infections which are aided by the design and structural properties of the lens itself [7].

The biomaterial constituting an intraocular lens is important to the success of the implant [8]. However, product surface finish and the design of the optic-haptic seals of IOLs are examples of other parameters which should be considered for successful implantation. We attempt to show in this study that they are critical factors in avoiding bacterial colonization. Material hydrophilic capacity, roughness, edges and extractable products can influence microorganism adhesion and proliferation after an implantation period.

With the objective of better understanding these relationships, different commercial IOLs have been compared in terms of design and behavior in infectious conditions in this study.

2. Materials and methods

Eight types of lenses were studied, with six types of tests being carried out on each lens: surface roughness, chemical composition, wettability, bacterial adhesion and biodeterioration. Commercial lens specifications are shown in Table I.

2.1. Surface roughness

This test was carried out to determine the surface roughness of the lens. An optical UBM profilometer with a microfocus sensor was used. A line profile measurement was performed on the surface of the optical part of the lenses [9]. Five different profiles were acquired on each lens with a length profile of 1.0 mm and a resolution of 400 points/mm.

2.2. Chemical composition

An infrared spectra with a Fourier transformation (FTIR) spectrometer (Magna IR 750 Nicolet/ATR) was used and the tests were carried out as per ASTM-E 1252 "Techniques for qualitative infrared analysis" [10].

2.3. Wettability according to contact angle

The contact angle of the surface of the materials was measured by computerized image analysis following the protocol provided by GBX Scientific Instruments. The apparatus (DIGIDROP, GBX Scientific Instruments) consisted of a syringe, a drop microcontroller, video card, CCD camera a zoom lens and software for image analysis and contact angle calculation. The measurement range was from 2 to 160° with a margin of

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TABLE I Marker lenses specifications

Lens	Optical material	Total diameter	Specific weight	UV interference at 10% of T	Haptic material	Haptic angle
1	Poly(methyl methacrylate)	11.5 mm	1.19	374 nm (−10 diop) 388 nm (+34 diop)	Poly(methyl methacrylate)	Modified C
2	Poly(dimethyl siloxane)			404 nm (6 diop) 409 nm (27 diop)	polyvinylidene fluoride	Modified C
3	Poly(dimethyl siloxane)	12 mm	1.10	392 nm (12 diop) 394 nm (28 diop)	polyvinylidene fluoride	Modified C
4	Poly(hydroxyethyl methacrylate)	12.5 mm	1.19	404 nm (6 diop) 409 nm (27 diop)	Poly(methyl methacrylate)	Modified C
5	Poly(hydroxyethyl methacrylate)	10.5 mm	1.19	374 nm (10 diop) 388 nm (27 diop)	Poly(hydroxyethyl methacrylate)	
6	Poly(ethyl acrylate)	13 mm		378 nm (10 diop) 383 nm (27 diop)	Poly(methyl methacrylate)	Modified C
7	Poly(benzyl methacrylate)	13 mm	1.10	398 nm (10 diop) 400 nm (30 diop)	Poly(methyl methacrylate)	Modified C
8	Poly(benzyl methacrylate)	12.5 mm	1.19	404 nm (6 diop) 409 nm (27 diop)	Polybenzyl methacrylate	Modified L

error of $\pm 0.5^{\circ}$. Deionized water was used for all calculations. Uniform drop size was controlled with a calibrator. Pressure was exerted on the syringe to deposit a 20 μl drop on the surface of the lens. An image was taken at the moment of contact.

The differences in the radius of curvature of the lenses were corrected using the manual method of the software. With this method we can calculate the angle between drop and surface independently the surface was curved.

2.4. Optical surface

A scanning electron microscope SEM (GXA 8600, Jeol) was used to image the optical surface before and after biodegradation tests.

2.5. Bacterial adhesion

This test measured the number of bacteria adhered to the surfaces of the different lenses, after a specific period of contact [11, 12].

For this assay we selected *Pseudomona aeruginosa* because a common cause of endophthalmitis following cataract surgery and lens implantation is the infection caused to adherence of *Pseudomona aeruginosa* to intraocular lenses [13–16].

Pseudomonas aeruginosa CECT 108 was grown in Nutrient Broth no. 2 media (Oxoid), for 24 h at 37 °C. The suspension was rinsed 3 times with a washing buffer ((K_2HPO_4 0.56% (Panreac, 0710J), KH_2PO_4 0.22% (Panreac, 1101), ClNa 0.8% (Panreac, 1105F)) in distilled water), by centrifugation at 3000 rpm for 10 min. Finally, the pellet was resuspended in washing buffer. This media avoids bacterial growth because it has not nutrients. In this way we can only evaluate bacterial adhesion.

Each intraocular lens was placed in 20 ml of the *P. aeruginosa* suspension (1×10^6 cfu/ml) for 48 h at 37 °C while being shaken. The lenses were then washed with washing buffer 3 times for 10 s to remove bacteria that did not attach to them. Adherent bacteria were extracted with extraction buffer (washing buffer with 0.001% Tween 80 (Panreac, 152050 (60)) for 1 h at 25 °C in a shaker.

Progressive dilutions were made in washing buffer from the extracted bacterial solution. An aliquot of each of these dilutions was seeded on *Pseudomonas aeruginosa* selective media plates (Medium King B (Pronadisa, Hispanlab S. A) with 1% Glycerine (Probus, 20895)). The plates were incubated at 37 °C for 48 h then bacterial colonies on each plate were counted (Bacteria counter, IVL).

2.6. Biodeterioration

This test identified the degree of microbial deterioration that the lens could experience in the presence of a bacterial population. *P. aeruginosa* is suspended in washing buffer without source of carbon, in this way the bacteria can only grow at the expense of the material of lenses. We can observe the damage caused for bacterial exposition, and in conclusion determinate the most vulnerable lens in presence of *P. aeruginosa* [17].

After obtaining a *Pseudomonas aeruginosa* suspension as described in Section 2.5 each lens was placed in a suspension of *P. aeruginosa* (1×10^6 cfu/ml) for 25 days at 37 °C while being shaken. The lenses were then treated with the extraction buffer (referenced in Section 2.5) for 1 h at 25 °C in a shaker to eliminate the bacteria adhered to the lenses. Once dry, the lenses were assessed by SEM (GXA 860, Jeol).

3. Results

3.1. Surface roughness

Table II summarizes the roughness and uniformity parameters of surface lenses.

The lens referenced as 6 shows the lowest roughness average ($R_a = 0.14$ μm) and the lowest maximum roughness depth ($R_{max} = 0.028$ μm), which indicates that this sample has not only the smoothest surface but also a surface without irregularities. On the other hand, the lens referenced as 5 has the highest roughness average (R_a) and highest maximum roughness depth (R_{max}) with values of 0.028 μm and 0.211 μm, respectively,

TABLE II Results of the 8 comercial lens in related on surface roughness and uniformity, chemical structure, wettability and bacterial adhesion

Lens	Surface roughness			Wettability Contact angle	
Reference	R_a[1] (μm)	R_{max}[2] (μm)	Chemical structure	(Grades)	Bacterial adhesion c.f.u.[5]
1	0.014 ± 0.003	0.088 ± 0.014	Poly(methyl methacrylate)	80.0 ± 3.3	$10.5 \times 10^4 \pm 7.1 \times 10^3$
2	0.025 ± 0.001	0.105 ± 0.073	Poly(dimethyl siloxane)	108.5 ± 0.6	$33.8 \times 10^4 \pm 35.4 \times 10^3$
3	0.020 ± 0.001	0.041 ± 0.016	Poly(dimethyl siloxane)	115.8 ± 2.0	$45.8 \times 10^4 \pm 21.2 \times 10^3$
4	0.015 ± 0.005	0.162 ± 0.108	Poly(hydroxyethyl methacrylate)[3]	66.7 ± 1.8	$3.0 \times 10^4 \pm 14.1 \times 10^3$
5	0.028 ± 0.006	0.211 ± 0.235	Poly(hydroxyethyl methacrylate)	74.7 ± 5.7	$55.6 \times 10^4 \pm 56.6 \times 10^3$
6	0.014 ± 0.000	0.028 ± 0.003	Poly(ethyl acrylate)	84.0 ± 5.1	$17.7 \times 10^4 \pm 7.1 \times 10^3$
7	0.020 ± 0.003	0.149 ± 0.084	Poly(benzyl methacrylate)	77.8 ± 1.7	$89.2 \times 10^4 \pm 56.6 \times 10^3$
8	0.017 ± 0.001	0.157 ± 0.026	Poly(benzyl methacrylate)	83.8 ± 2.5	$19 \times 10^4 \pm 7.1 \times 10^3$

[1] R_a—Average roughness: is the area between the roughness profile and its mean line, or the integral of the absolute value of the roughness profile height over the evaluation length.
[2] R_{max}—Maximum roughness depth: is the vertical distance between the maximum peak and the lowest valley within a single sample length. The roughness parameter data shown correspond to the average of five different profiles.
[3] It may contain small proportion of Poly(methyl methacrylate). An RMN spectrum should be obtained to confirm this.
[4] Three measurements of contact angle were taken for each lens. The data shown in the table are the average value and standard deviation of these units.
[5] Colony Forming Units (cfu) data are the average of the c.f.u. were attached on three plates for each lens.

indicating that this lens has the roughest and probably, most irregular surface of the analyzed lenses.

3.2. Chemical composition

Infrared study of the samples is summarized in Table II. The infrared spectroscopy used to verify the composition of the lens, and it could be seen that each type of lens generally corresponds to a defined chemical composition (data not shown).

Sample 1, defined as poly (methyl methacrylate) by the makers, was confirmed that it was PMMA. Samples 2 and 3 were polysiloxanes, as stated by the manufacturers. Samples 4 and 5 were stated to be hydrophilic (poly (hydroxyethyl methacrylate)) but sample 4 may have contained a small proportion of poly (methyl methacrylate). An RMN spectrum should be obtained to confirm this data. Sample 6 was ethyl polyacrylate and samples 7 and 8, which contained the same composition, were poly (benzyl methacrylate).

3.3. Wettability

Fig. 1 and Table II show the behavior of the water on the polymers. Greater values of contact angle correspond to less wettability.

The hydrogels contain hydroxyl groups which contribute to increase the attraction between the water and the gel. Using our wettability assay we determined that lens 4 had a wettability value of 66.7° and lens 5 was 74.7°.

The presence of non-polar groups (methyl, ethyl, and aromatic groups) on the surface of the polymer weakens the interactions between the material and the water, giving lenses 1, 6, 7 and 8 wettability values of 80.0°, 84.0°, 77.8° and 83.8° respectively. With the polysiloxanes this hydrophobicity is more marked, due to the replacement of carbon atoms by silicon. Lenses 2 and 3 had values greater than 100°.

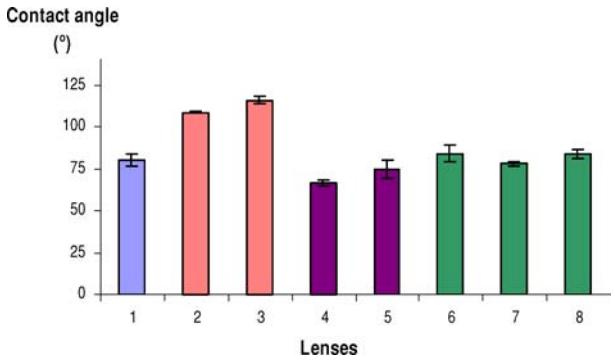

Figure 1 Behaviour of the lens surfaces on the water. The bars indicate the value of angle which a drop of water does in contact with the polymer surface (Data showed in Table II).

3.4. Optical surface

The micrographs in Fig. 2 show the different characteristics of the surface lenses before contacting bacteria (a–h). It is interesting to note that lens 3 contain a broken optical component (image c).

The images i–p in Fig. 2 of the optic-haptic junction zones clearly show two types of junctions: those formed by different materials for the optical and the haptical (samples 2, 3, 4, 6 and 7) and those that were a single block, optical-haptical of the same material (1, 5 and 8).

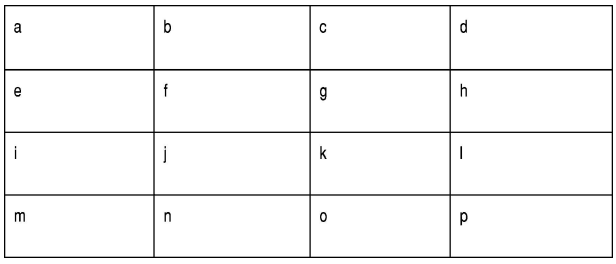

Figure 2 Micrographs of surface lenses by SEM. Images marked as a–h correspond to optical surface lenses and these named as i–p are the optical-haptical junctions in the lenses 1–8 respectively. While the most optical surfaces were uniform the broken surface of lens 3 showed in image c stood out specially. Among the images i–p two types of junction were differentiated: those formed by different materials for the optical and the haptical (lenses 2, 3, 4, 6 and 7 as j, k, l, n and o respectively) and those that were a single block, optical-haptical of the same material (1, 5 and 8 as images l, m, and p).

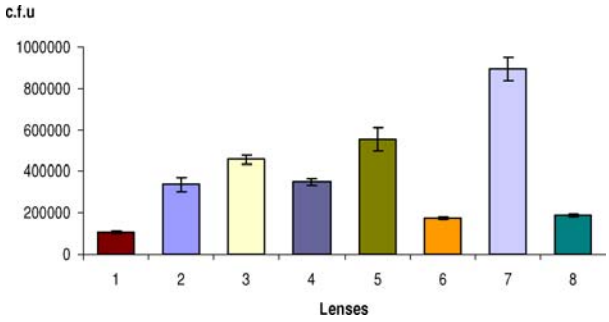

Figure 3 Bacterial adhesion onto the lenses after placed in a suspension of *Pseudomonas aeruginosa* (1×10^6 cfu/ml) for 48 h at 37 °C. Progressive dilutions from bacterial extraction solution were seeded and incubated at 37 °C for 48 h. The bars indicate the value of colony forming units grown in these cultures, showed in the Table II. These values are the average of three replicates made.

3.5. Bacterial adhesion

The results are shown in Table II and Fig. 3.

Lens 1 showed the least tendency for adhesion, being the only item made from PMMA and having no optic-haptic junctions. As seen in the profiling test (3.1 Section), the surface of lens 1 was smooth and homogeneous.

Bacterial adhesion also was very low in Lenses 6 and 8, both made from hydrophobic materials. The roughness profile of both lenses is low, although lens 8 showed some surface irregularities.

It is known that the rougher the material, the greater the contact surface and the greater the possibility of bacterial deposits, as shown in lens 5. However, lens 7 showed the greatest bacterial load of all the samples tested. This led us to deduce that the optic-haptic junction of this lens is possibly a zone that is susceptible to bacterial colonization. These seal areas of optic-haptic could also be colonization nuclei in the lenses 2 and 3, which being from hydrophobic material, such as polysiloxanes, showed high bacterial levels.

3.6. Biodeterioration

Electron microscopy was used to show the structure of the optical surfaces of the lenses after they had been exposed to deteriorating conditions (Fig. 4). The lenses were only exposed to bacteria so the damage that we can see is due to bacterial deterioration. The micrographs showed the most hydrophilic lenses suffered more damage from bacteria (lens 4 in image d and lens 5 as image e). With lens 4, the affected zones (dark patches) showed deeper bacterial deterioration. Lens 5 was covered with bacterial biomass and showed deep cracks over its whole structure colonized by bacteria.

The opposite was true of the silicon lenses (lenses 2 and 3, images b and c respectively), which resisted bacterial contaminations and were practically unaffected by biodeterioration. Lens 1 and lens 3 showed accumulations of salts and organic material that could originate from bacterial remains but without material damage. With the rest of the acrylates (lenses 6, 7 and 8 as images f, g, and h respectively), bacterial deterioration affected them to varying degrees, with lens 6, made of ethyl polyacrylate, showing both organic and bacterial remains, although the damage seemed to be superficial.

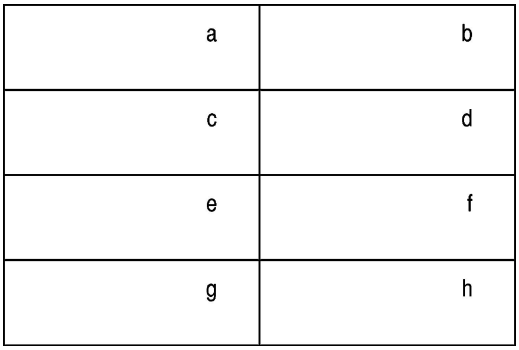

Figure 4 Scanning electron micrographs of biodeterioration of optical surfaces after the incubation of the lenses in a suspension of *Pseudomonas aeruginosa* (1×10^6 cfu/ml) for 25 days at 37 °C. Micrographs named as a–h correspond to lenses 1–8 respectively.

4. Conclusions

This study clearly shows that material wettability grade, surface uniformity and design of lens are three main characteristics that influence in bacterial adhesion and deterioration of the lenses.

Roughness and irregularity are properties that increase the contact surface, making bacterial deposition on the material easier. After the obtained results, it is possible to confirm that as general rule, there is a relationship between surface roughness and bacterial adhesion in the first 48 hs of incubation (high roughness, more susceptible of adhesion), although the composition of the lens material is the determining factor for its degree of biodeterioration in the long term. It should also be pointed out that the optic-haptic junctions may become susceptible zones to bacterial colonization, depending on the design and composition of the material of both parts.

In order to avoid bacterial adhesion and damage the ideal characteristics for IOLs are a hydrophobic surface, low roughness and no junctions between optic-haptic components. These premises are only obeyed by one sample, lens 1, and as the results showed was the less colonized and damaged lens. Lens 8, avoiding some irregularities on its surface could be also a good candidate.

With the rest of samples the valuation is more difficult given the complexity of the interactions of the different factors so the advantages and drawbacks of each product should be considered individually.

References

1. T. A. MACKY, L. WERNER, M. M. SOLIMAN, S. K. PANDEY, A.M. IZAK and D. J. APPLE, *Ophthalmic Surg Lasers Imaging.* **34**(3) (2003), 197.
2. S. SAIKA, T. MIYAMOTO and Y. OHNISHI, *J. Cataract Refract Surg.* **29**(6) (2003) 1198.
3. F. KOC, Z. AKMAN, S. KURUOGLU, I. OGE and M. GUNAYDIN *Eur. J. Ophthalmol.* **11**(1) (2001) 31.
4. J. FEYS, J. P. EMOND, A. SALVANET-BOUCCARA and A. DUBLANCHET, *J. Fr. Ophthalmol.* **26**(3) (2003) 255.
5. J. FEYS, J. P. EMOND, D. MEZIANE and A. SALVANET-BOUCCARA, *J. Fr. Ophthalmol.* **22**(2) (1999) 213.

6. A. MISTLBERGER, J. RUCKHOFER, E. RAITHEL, M. MULLER, E. ALZNER, S. F. EGGER and G. GRANER, *J. Cataract Refract Surg.* **23**(7) (1997) 1064.
7. H. B. DICK, M. TEHRANI, P. BRAUWEILER, E. HAEFLIGER, T. NEUHANN and A. SCHARRER, *Ophthalmologe.* **100**(6) (2003) 465.
8. A. W. LLOYD, R. G. A. FARAGHER and S. P. DENYER, *Biomaterials.* **22** (2001) 769.
9. Surface Profile Parameters, "Surface Metrology Guide" (www.predev.com/smg/parameters.htm).
10. American Standards for Testing Materials ASTM E-1252. "Techniques for Qualitative Infrared Analysis." Vol. 1.06. West Conshohocken, P. 1996.
11. E. W. NG, G. D. BARRETT and R. BOWMAN *J. Cataract Refract Surg.* **22**(2) (1996) 1331.
12. M. D. P. WILLCOX, N. HARMIS, B. A. COWELL, T. WILLIAMS and B. A. HOLDEN *Biomaterials.* **22** (2001) 3235.
13. M. CRUCIANI, M. MALENA, G. AMALFITANO, P. MONTI and L. BONOMI, *Clin Infect Dis.* **26**(2) (1998) 330.
14. M. M. GABRIEL, D. G. AHEARN, K. Y. CHAN and A. S. PATEL *J. Cataract Refract Surg.* **24**(1) (1998) 124.
15. C. A. LAWIN-BRUSSEL, M. F. REFOJO and K. R. KENYON, *ibid.* **18**(6) (1992) 598.
16. D. N. GERDING, B. J. POLEY, W. H. HALL, D. P. LEWIN and M. D. CLARK, *Am. J. Ophtalmol.* **88**(5) (1979) 902.
17. International Standard ISO 846-1978, Plastics- Determination of Behaviour Under The Action of Fungi and Bacteria-Evaluation By Visual Examination or Measurement of Change in Mass or Physical Properties. 1978-09-01.

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