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Hydrogel Contact Lenses Surface Roughness and Bacterial Adhesion

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Additional information is available at the end of the chapter

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1. Introduction

Contact lenses are a safe and effective mode of vision correction and today's industry offers wearers the choice of continuous wear, overnight orthokeratology, frequent-replacement or daily-disposable lenses among others. However, despite these options, including different care and maintenance systems, there are still features of contact lenses that could be improved such as possible microbial contamination (Weisbarth et al., 2007).

Microbial keratitis (MK) is a serious complication of contact lens (CL) wear that can lead to vision impairment (Buehler et al., 1992; Catalonotti et al., 2005; Leitch et al., 1998; Mah-Sadorra et al., 2005; Keay et al., 2009). Although the incidence of CL-related MK is only 0.02–0.5% (Cheng et al., 1999; Holden et al., 2005), the use of CL is so wide-spread that the problem may affect several millions of people and must therefore be considered a major health threat.

The CL surface is a suitable substrate for bacterial adhesion and biofilm formation, and can sustain the growth of microorganisms in prolonged contact with the cornea (Elder et al., 1995). In addition, CL wear may impair the immune response of the cornea by distorting its epithelial barrier function, and thus promote MK (Liesegang, 2002). To improve the corneal/CL interface, new soft hydrogel lens materials incorporate several co-polymers, including silicone polymers for increased oxygen permeability and phosphoryl-choline to increase biocompatibility. Further, the new modalities of wear, such as daily disposable (DD) hydrogel CL, avoid the need for regular cleaning and storage, which are known to be an important cause of microbial contamination (Laughlin-Borlace et al., 1998). However, several studies have surprisingly shown that users of DD and silicone hydrogel CL do not show a reduced risk of MK (Dart et al., 2008; Stapleton et al., 2008; Willcox et al., 2010). In effect, in the paper by Dart et al., differences in soft CL design and/or the composing

polymer rather than the mode of wear were found to determine susceptibility to MK (Dart et al., 2008).

The process of initial adhesion of bacteria to the CL surface has been extensively examined in terms of the physical and chemical properties of both the bacterial cell and CL surface, such as hydrophobicity and roughness. Thus, the results of several *in vivo* studies suggest that a rougher CL surface is prone to more extensive bacterial adhesion (Bruinsma et al., 2002; Bruinsma et al., 2003) since imperfections in the lens surface is where deposits are likely to form (Hosaka et al., 1983). Also, depending on the surface thermodynamics, hydrophilic strains seem to preferentially adhere to hydrophilic surfaces, while more hydrophobic strains have a preference for hydrophobic surfaces (Bos et al., 1999; Bruinsma et al., 2001). Apart from lens surface factors, adhesion is also conditioned by features of the bacterial surface including flagella and fimbriae (Fletcher et al., 1993a; Fletcher et al., 1993b; Gupta et al., 1994; Gupta et al., 1996; Willcox et al., 2001; Donlan, 2002; Donlan, 2002; Kogure et al., 1998; Morisaki et al., 1999) or the presence or release of extracellular substances such as polysaccharides, proteins and biosurfactants (Mack et al., 1999; Mack et al., 1996; Mack et al., 1994).

Occasionally, a contact lens wearer will suffer an adverse response to a lens. These problems are frequently caused by bacterial contamination of the contact lens surface, and MK is one of the most feared complications (Patel and Hammersmith, 2008; Stapleton et al., 2008). Contact lenses absorb tear film proteins and lipids and this induces lens contamination and deterioration. Moreover, the build-up of tear film components on contact lenses can cause discomfort and inflammatory complications such as giant papillary conjunctivitis (GPC) (Skotnitsky et al., 2002; Skotnitsky et al., 2006), and this may occur with any type of daily or extended wear lenses (Donshik PC, 2003). This adsorption depends mainly on the contact lens material, and varies according to the tear secretion rate and certain pathological conditions. Research on conventional poly-HEMA-based lens materials has shown that the deposition of lysozyme and albumin depends upon the polymer's composition (Bohnert et al., 1988), charge (Garrett et al., 2000; Soltys-Robitaille et al., 2001) and water content (Garrett et al., 1999). Silicone-hydrogel materials give rise to different deposition profiles to those associated with the use of conventional poly-HEMA hydrogel lenses in that they induce less protein deposition and more lipid deposition (Jones et al., 2003; Subbaraman et al., 2006; Carney et al., 2008). Surface roughness also need to be considered since deposits are more likely to form on imperfections of the lens surface (Hosaka et al., 1983). It was also previously demonstrated that as surface roughness increases, the biofilm deposited on the lens also increases (Baguet et al., 1995) and that bacterial transfer from a contact lens is determined by the roughness and hydrophobicity of the surface receiving the bacteria (Vermeltfoort et al., 2004).

Further, a smooth surface is essential for the optical quality of a contact lens since reduced scattered light improves the performance of an optical system (Bennett, 1992). Developments in soft contact lens materials continue to be an important issue, since the performance and comfort of a contact lens will depend on the material, its surface

architecture and the quality of the lens manufacturing process (Lorentz et al., 2007; Riley et al., 2006; Guillon and Maissa, 2007). In addition, the performance of contact lenses does not remain constant over time and lens surface changes induced by wear will affect their performance and determine a need to replace the lens.

The aim of this chapter was to qualitatively and quantitatively characterize the surfaces of unworn hydrogel contact lenses using Atomic Force Microscopy (AFM) and White Light Optical Profiling (WLOP), and to analyze how these surface characteristics affect on bacterial adhesion.

2. Contact lens surface roughness

2.1. Roughness parameters

The actual geometry of a surface is very complex (Gadelmawla et al., 2002). Even areas considered "very smooth" show a complex mix of geometric features. Surface roughness is becoming increasingly important for applications in many fields (Bennett, 1992). Among other factors, surface roughness of devices in direct contact with living systems will influence their biological reactivity. How a surface is finished is an important factor for a good operation of many types of products, which include optical products (Bennett, 1992), related to engineering (Blunt, 2006), food (Sheen et al., 2008; Wang et al., 2009) and biomedical products (Hooton et al., 2004; Hooton et al., 2006; Linneweber et al., 2007; Lee et al., 2009). The surface of any body or object is the part which interacts with the surrounding environment. Roughness is a biological factor that affects in a molecular scale, the manner in which bacteria adhere to surfaces, above all for initial adhesion. (Mitik-Dineva et al., 2008; Mitik-Dineva et al., 2009). The real geometry of a surface is so complex that only by increasing the number of parameters used can a more accurate description be obtained (Gadelmawla et al., 2002). Surface parameters can be considered as height and shape parameters:

2.1.1. Height parameters

The parameters generally used to quantify roughness include height parameters such as average roughness (R_a), mean-square-roughness (R_{ms}) and Maximum Roughness (R_{max}) (Baguet et al., 1993; Guryca et al., 2007; Bhatia et al., 1997; Hinojosa Rivera and Reyes Melo, 2001; Lira et al., 2008; Gonzalez-Meijome et al., 2009; Giraldez et al., 2010a; Giraldez et al., 2010c; Gonzalez-Meijome et al., 2006a). R_a is the average deviation or arithmetic mean of the profile from the mean line; it is universally accepted and is the most used international parameter of roughness. R_{ms} is the standard deviation from the mean surface plane. Although R_a and R_{ms} seem to be the most informative and consistent parameters used to define the surface topography of contact lenses (Gonzalez-Meijome et al., 2006a), they both show a dependency on sample length (Hinojosa Rivera and Reyes Melo, 2001; Kiely and Bonnell, 1997; Kitching et al., 1999). Degree of their variation with sample length could be representative of how homogeneous a surface is in its irregularities distribution. R_{max} is the

maximum peak-to-valley height identified within the observed area. It could be affected by local imperfections or sample contamination leading to higher values than expected, so material characterization based on this parameter could be unreliable.

2.1.2. Shape parameters

Two statistical parameters of roughness, not generally used to analyze contact lens surfaces, are kurtosis (R_{ku}) and skewness (R_{sk}). R_{ku} is a measure of the sharpness of the profile about the mean line that provides information on the distribution of spikes above and below the mean line. Thus, spiky surfaces will have a high kurtosis value ($R_{ku} > 3$) and bumpy surfaces a low value ($R_{ku} < 3$). R_{sk} is a measure of the symmetry of the profile about the mean line, giving information on asymmetrical profiles for surfaces with the same values of R_a and R_{ms} . Negative values of R_{sk} indicate a predominance of troughs, while positive ones are observed for surfaces with peaks. The use of both shape parameters, R_{ku} and R_{sk} , which serve to distinguish between two profiles with the same R_a and/or R_{ms} , (Gadelmawla et al., 2002) has been reported in several biomedical fields (Hansson, 2000; Olefjord and Hansson, 1993; Yang et al., 2007; Linde et al., 1989; Zyrianov, 2005; Raulio et al., 2008; Szmukler-Moncler et al., 2004; Cehreli et al., 2008). Figure 1 shows the amplitude distributions/shape profiles of two surfaces with a similar R_a but different values of R_{sk} or R_{ku} (Gadelmawla et al., 2002).

The clinical applications of R_{ku} and R_{sk} in the contact lens field could be to provide a measure of the susceptibility of a contact lens surface to deposit formation or colonization by microorganisms. Also, different shapes could determine a greater specific surface area, and thus more available active sites for thermodynamic reactions. As two surfaces with similar R_a or R_{ms} could differ in shape (Figure 1), they may also differ in their performance.

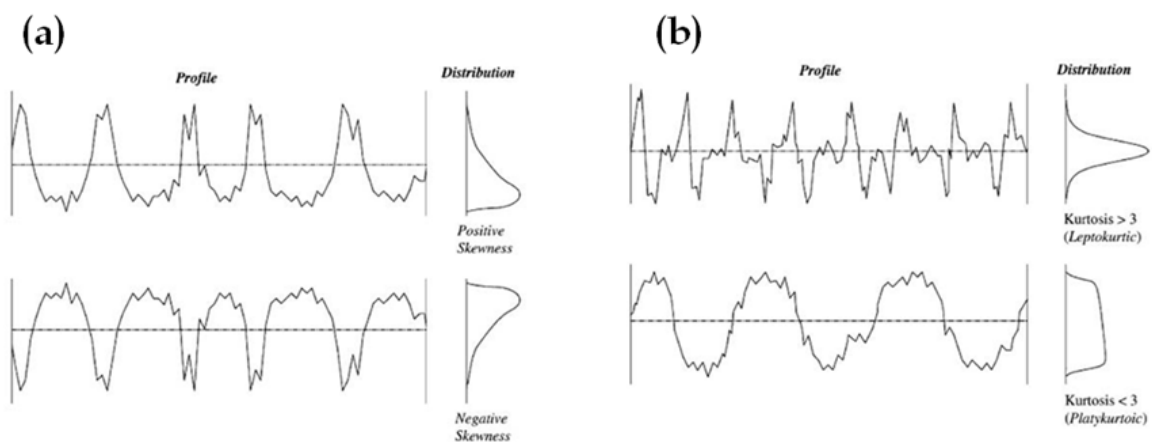


Figure 1. Amplitude distribution curve about the mean line for two surfaces showing similar R_a values but different values of R_{sk} (a) or R_{ku} (b).

2.2. Surface roughness measurement

A wide variety of methods are available for measuring surface roughness and the light scattering the roughness produces. As commented previously, the apparent surface

roughness depends upon the size of the sample area, so in order to provide a better description of the surface roughness, measurements must be acquired for a variety of sample sizes (Kiely and Bonnell, 1997; Kitching et al., 1999); with roughness parameters being calculated for areas with different location and size.

2.2.1. Atomic force microscopy

Atomic force microscopy (AFM) provides detailed information on the surface characteristics of contact lenses (Bhatia et al., 1997; Baguet et al., 1993; Baguet et al., 1995; Bruinsma et al., 2003; Lira et al., 2008; Guryca et al., 2007; Gonzalez-Meijome et al., 2006a; Gonzalez-Meijome et al., 2009; Teichroeb et al., 2008; Maldonado-Codina and Efron, 2005) and is a powerful tool for the high resolution examination of the structure of the hydrated contact lens surface. The method has the advantages that it avoids artefacts due to dehydration and coating (Bhatia et al., 1997; Kim et al., 2002), and allows for non-destructive surface topography and roughness measurements. AFM consists of a microscale cantilever with a sharp tip (probe) that is used to scan the specimen surface. The cantilever is typically made of silicon or silicon nitride with a tip radius of curvature of the order of nanometers. When the tip is brought into the proximity of a sample surface, forces between the tip and the sample cause the cantilever to deflect according to Hooke's law. (Lira et al., 2008) The advantage of AFM over conventional microscopy or scanning electron microscopy (SEM) is the high level resolution offered in three dimensions and that topographic information can be obtained in aqueous, nonaqueous or dry conditions, eliminating the need for sample preparation (e.g., dehydration, freezing or coating). In effect, AFM has proved useful for characterizing tear deposits on worn soft contact lens surfaces (Baguet et al., 1995; Rebeix et al., 2000) or characterizing the rigid gas permeable contact lens surface (Bruinsma et al., 2003). In fact, detailed information about the surface quality of CL has been studied previously by Atomic Force Microscopy (AFM) (Bhatia et al., 1997; Baguet et al., 1993; Baguet et al., 1995; Bruinsma et al., 2003; Gonzalez-Meijome et al., 2006a; Gonzalez-Meijome et al., 2009; Giraldez et al., 2010c) and Cryo-SEM (Gonzalez-Meijome et al., 2006b; Guryca et al., 2007). AFM is a very powerful tool for high resolution examination of hydrated CL surface structure. The method avoids artifacts due to dehydration and coating (Bhatia et al., 1997; Kim et al., 2002). However, when using AFM to analyse CL surface the area of measurement is very small, so it may be answered how representative of the total lens are R_a and R_{ms} obtained by AFM. Cryo-SEM, a modification of the Scanning Electron Microscopy (SEM), requires that the material be frozen in nitrogen before examination (Serp et al., 2002). In hydrogels, this usually means the destruction of the material, which is the main disadvantage of this technique.

2.2.2. White Light Optical Profilometer

White Light Optical Profilometer (WLOP) is one of the preferred methods of precision surface characterization in many fields (Caber, 1993; Windecker and Tiziani, 1999; Bennett, 1992; O'Mahony et al., 2003). WLOP is a topographic technique, that as well as AFM, enables the analysis of surface topography and roughness by means of a nondestructively

methodology. It is a powerful and well-established technique for non-contact measurement of surface topography for quickly determining three-dimensional surface shape over larger areas at high vertical and moderate lateral resolution (Bennett, 1992; O'Mahony et al., 2003; Novak et al., 2003). Two modes of operation are generally available for the optical profilers. For smooth surfaces the phase-shifting integrating bucket technique (PSI) is generally used since it gives sub-nanometer height resolution capability. For rougher surfaces, a vertical scanning coherence sensing technique can be used to give a nanometer height resolution over several hundred microns of surface height. WLOP allows analyze larger areas than techniques used before in contact lenses, so the values and statistics could be more representative of roughness distribution over the lens surface. Topographic information can be obtained from the surface in aqueous conditions.

2.3. Contact lens surface roughness characteristics

Surface topography and roughness parameters showed different characteristics depending on the type of contact lens (material, water content, manufacture system, replacement frequency). Moreover roughness varies with magnification, so the size of the measured area must be considered when comparing the results of different studies (Kiely and Bonnell, 1997; Kitching et al., 1999). R_a is the arithmetic mean of the departures of the profile from the mean line (Hinojosa Rivera and Reyes Melo, 2001). Thus, it should not vary with magnification for a surface with homogeneously distributed irregularities, regardless of how smooth or rough the surface is. However, the irregularities of most surfaces are not perfectly homogeneously distributed, and effectively differences in contact lens surface roughness values have been observed at different magnifications, with higher roughness scores obtained for larger areas more enlarged areas (Gonzalez-Meijome et al., 2006a). Hence, the amount of variation could reflect how homogeneous a surface is.

Contact lens surface characteristics determined by AFM and by WLOP are presented in the next sections.

2.3.1. CL surface roughness by AFM

Contact lens surfaces roughness and topography can be determined by AFM (Veeco, multimode-nanoscope V) in tapping mode™. (Giraldez et al., 2010c) Although the method used is the same as for dry conditions, a special cell could be necessary so measurements could be made on the lenses in their original shipping fluid (physiological saline) to keep CL hydrated during microscopy observation. All procedures and examinations must be conducted in the same room kept at 21°C and approximately 50% relative humidity. Then images have to be processed, for example, using the Vision®32 and Nanoscope v7.20 software packages.

Table 1 and table 2 shows height (R_a and R_{ms}) and shape (R_{ku} and R_{sk}) parameters of 6 hydrogel CL. The specific characteristics of these CL are provided in Table 3. They were all manufactured by cast-molding and had no surface treatment. Although all the lenses are

suitable for daily wear, manufacturers recommend a different replacement frequency (Table 1). Senofilcon A and comfilcon A are silicone-hydrogel contact lenses, while hioxifilcon (Osmo 2®), omafilcon A and ocufilcon B are hydroxyethylmethacrylate (HEMA) copolymers and nefilcon A is a polyvinyl alcohol (PVA). The main monomers of the material used to manufacture Osmo 2 contact lenses are those that comprise hioxifilcon (2-HEMA GMA; GMA, glycerylmethacrylate) plus MA (methacrylic acid).

| Contact lens | 25 μm^2 | | 196 μm^2 | |
|--------------------------|---------------------|---------------------|---------------------|---------------------|
| | R _a (nm) | R _q (nm) | R _a (nm) | R _q (nm) |
| Hioxifilcon-based | 4.31 \pm 0.59 | 5.50 \pm 0.58 | 5.91 \pm 0.65 | 7.90 \pm 0.89 |
| Omafilcon A | 1.90 \pm 0.39 | 2.78 \pm 0.45 | 4.66 \pm 2.05 | 6.80 \pm 2.74 |
| Nefilcon A | 11.25 \pm 0.38 | 15.41 \pm 1.26 | 12.99 \pm 0.05 | 18.34 \pm 0.25 |
| Ocufilcon B | 11.01 \pm 1.79 | 14.38 \pm 2.13 | 11.45 \pm 2.56 | 23.11 \pm 4.49 |
| Senofilcon A | 3.33 \pm 0.28 | 4.06 \pm 0.38 | 3.76 \pm 0.05 | 4.70 \pm 0.005 |
| Comfilcon A | 1.56 \pm 0.37 | 2.34 \pm 0.69 | 2.76 \pm 0.80 | 4.21 \pm 0.44 |

Table 1. Mean roughness parameters recorded for the hydrogel contact lenses using AFM on surface areas of 25 μm^2 and 196 μm^2

| | Hioxifilcon-based | Omafilcon A | Nefilcon A | Oculfincon B | Senofilcon A | Comfilcon A |
|-----------------------|-------------------|-------------------|-----------------|-----------------|-----------------|------------------|
| R_{ku} | 3.71 \pm 0.94 | 23.54 \pm 14.81 | 5.86 \pm 2.03 | 5.45 \pm 1.95 | 3.74 \pm 1.63 | 31.09 \pm 0.95 |
| R_{sk} | -0.22 \pm 0.17 | 2.04 \pm 1.07 | 1.43 \pm 0.32 | 0.98 \pm 0.17 | 0.74 \pm 0.41 | 2.93 \pm 0.82 |

Table 2. Mean R_{ku} and R_{sk} values recorded for the hydrogel contact lenses using AFM on a 25 μm^2 surface area

| Brand name | Material Generic name | Charge | Water content (%) | Type of hydrogel | Replacement Frequency* |
|------------------------|--------------------------|-----------|----------------------|-------------------|---------------------------|
| Osmo 2 | Hioxifilcon-based | Non ionic | 72 | HEMA copolymer | Three months |
| Proclear | Omafilcon A | Non ionic | 62 | HEMA copolymer | One month |
| Focus Dailies | Nefilcon A | Non ionic | 69 | Polyvinylalcohol | One day |
| Frequency 1 day | Ocufilcon B | Ionic | 52 | HEMA copolymer | One day |
| Acuvue Oasys | Senofilcon A | Non ionic | 38 | Silicone hydrogel | Two weeks |
| Biofinity | Comfilcon A | Non ionic | 48 | Silicone hydrogel | One month |

* Manufacturer's recommendation

Table 3. Specifications of the contact lenses analyzed by AFM.

The corresponding 3-D image of the lenses with the lowest (comfilcon A and omafilcon A) and highest (nefilcon A and ocufilcon B) roughness scores are shown in figure 2. Figure 3 and 4 show the corresponding image for senofilcon A and hioxifilcon CL respectively.

A different surface roughness in a new lens can be the result of the manufacturing method and the material's properties. The spin casting method generates contact lenses with the

smoothest surfaces, followed by cast-molding and then lathe-cut lenses (Guryca et al., 2007; Grobe, 1996). All the lenses presented here were cast-molded, and their roughness parameters were similar to the ranges reported for other non surface-treated cast-molded lenses (Guryca et al., 2007). Thus, the roughness differences between lenses cannot be attributed only to the manufacturing procedure. Besides the mode of elaboration, other authors have linked the presence of methacrylic acid (MA) (Baguet et al., 1993) or a reduced water content (Guryca et al., 2007; Vermeltfoort et al., 2004) to a greater lens surface roughness.

Daily replacement hydrophilic contact lenses (nefilcon A and ocufilcon B), showed the highest roughness values for both surface areas analyzed. In contrast, comfilcon A showed the smoothest, or flattest surface ($R_a = 1.56$ nm), followed closely by omafilcon A ($R_a = 1.90$ nm). Similar roughness values were observed for the hioxifilcon-based material and senofilcon A, yet their surface appearance was different (figures 3 and 4). Although the hioxifilcon-based contact lens contains MA, which should determine a greater surface roughness, its similar R_a to senofilcon A could be attributed to its high water content. As may be observed in Figure 3, senofilcon A shows a granulated surface structure, which is similar to that previously reported for the AFM observation of senofilcon A (Teichroeb et al., 2008), of galyfilcon A (Lira et al., 2008) and for the cryogenic SEM visualization of the latter. (Gonzalez-Mejome et al., 2006b) Galyfilcon A is a non surface-treated silicone hydrogel contact lens that contains PVP as an internal wetting agent.

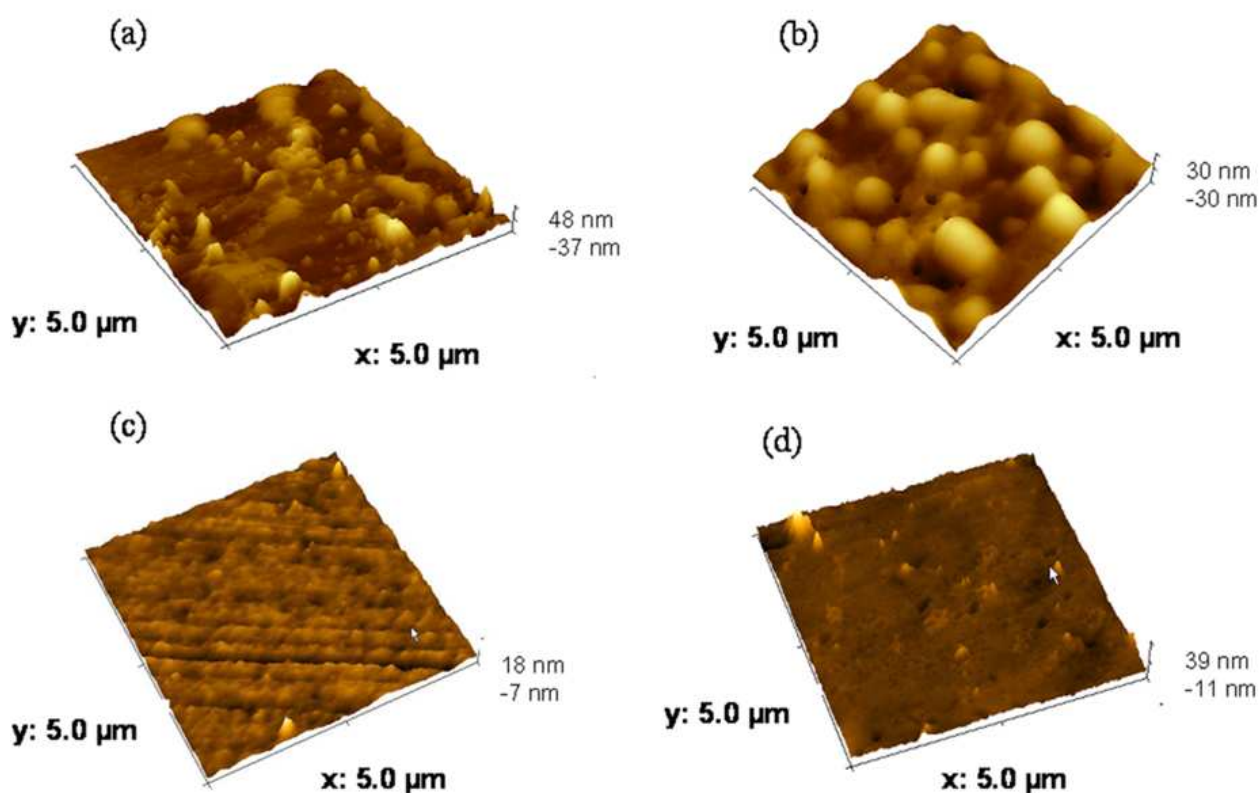


Figure 2. Three-dimensional images generated by the AFM analysis of a $25 \mu\text{m}^2$ area of nefilcon A (a), ocufilcon B (b), comfilcon A (c) and omafilcon A (d).

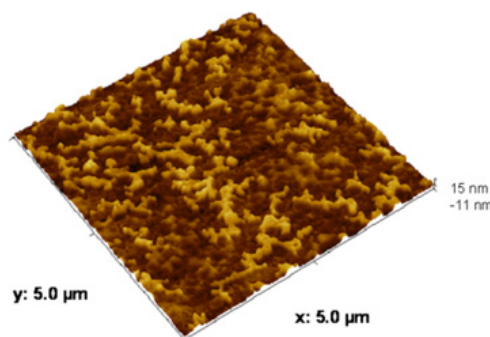


Figure 3. Three-dimensional image generated by the AFM analysis of senofilcon A over a $25 \mu\text{m}^2$ area.

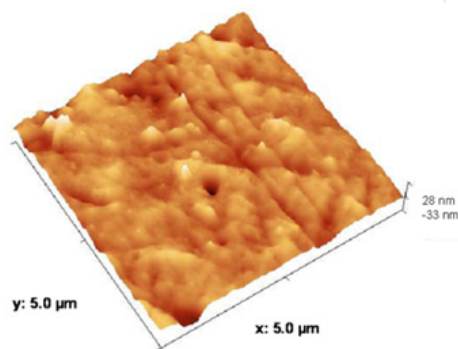


Figure 4. Three-dimensional image generated by the AFM analysis of hioxifilcon over a $25 \mu\text{m}^2$ area..

Silicone-hydrogel contact lenses exhibit different surface characteristics depending on their chemical composition and surface treatments (Nicolson PC, 2003). Surface treatments are targeted at obtaining wettable surfaces (Jones L and Dumbleton K, 2002), although the surfaces of the silicone-hydrogel contact lenses presented here were untreated. Thus, senofilcon A incorporates an internal wetting agent (polyvinyl pyrrolidone) that apparently leaches to the lens surface, and the Aquaform™ technology used in comfilcon A minimizes lens dehydration by forming hydrogen bonds with water molecules, creating a naturally hydrophilic contact lens that retains water inside the lens (Szczotka-Flynn L, 2007; Whittaker G, 2008). The roughness parameters obtained for these lenses were similar to those observed previously in silicone-hydrogel contact lenses lacking surface treatment, such as galyfilcon A and comfilcon A (Lira et al., 2008; Gonzalez-Meijome et al., 2009), but lower than those reported for surface-treated designs (Gonzalez-Meijome et al., 2006a; Guryca et al., 2007). Despite the similar surface appearance of silicone hydrogels included here and those examined by others, (Teichroeb et al., 2008; Gonzalez-Meijome et al., 2009) Teichroeb et al. observed higher roughness parameters for senofilcon A than Comfilcon A when measuring a $25 \mu\text{m}^2$ area. These differences could be related to the fact that the lenses were analysed after drying in ambient conditions for 15 minutes.

2.3.2. CL surface roughness by WLOP

The issue of measurement area is an important point to be considered in all surface roughness measurements (Bennett, 1992; Blunt, 2006; Hinojosa and Reyes, 2001; kiely and

Bonnell, 1997; Kitching et al., 1999). WLOP allows analysing larger areas than other techniques used before in CL. In this regard, the maximum Hydrogel CL area studied by AFM was $400 \mu\text{m}^2$ (Gonzalez-Mejome et al., 2006a), which means that for a 14.00 mm diameter CL, only about $2.6 \times 10^{-4} \%$ of the entire CL surface area would be analyzed. When using WLOP we were able to determine roughness parameters in areas as large as $67646 \mu\text{m}^2$, which is almost 170 higher than the greatest area evaluated by AFM, so values and statistics are supposed to be more representative of the total CL surface (Giraldez et al., 2010a).

WLOP measurements can be obtained with the interference microscopy Wyko[®]-NT1100, a tool that combines a microscopy and an interferometer into the same instrument and which was previously used for hydrogel CL surface analysis. (Giraldez et al., 2010a)

Table 4, 5 and 6 shows values for R_a , R_{ms} and R_{max} parameters of 4 hydrogel CL obtained from WLOP analysis for $625 \mu\text{m}^2$, $2500 \mu\text{m}^2$, $10829 \mu\text{m}^2$ and $67646 \mu\text{m}^2$ areas. The specific characteristics of these CL are provided in Table 7. All these CL were manufactured by cast-moulding and had no surface treatment. Although all lenses are indicated for daily wear, different replacement frequency is recommended by manufacturer (table 1). According with material, hioxifilcon, omafilcon A and ocufilcon B are hydroxyethylmethacrylate (HEMA) copolymers and nefilcon A is a polyvinylalcohol (PVA). Osmo 2 contact lens material is based in hioxifilcon, as their main monomers are those from hioxifilcon (2-HEMA GMA; GMA, glycerylmethacrylate) and MA (methacrylic acid). Lenses were obtained in the original containers filled with a physiological saline solution. As an example, surface appearance of hydrogel contact lenses at different magnification is shown in figure 5.

| | $625 \mu\text{m}^2$ | $2500 \mu\text{m}^2$ | $10829 \mu\text{m}^2$ | $67646 \mu\text{m}^2$ |
|--------------------------|---------------------|----------------------|-----------------------|-----------------------|
| Hioxifilcon-based | $31,04 \pm 1,75$ | $32,88 \pm 2,18$ | $42,26 \pm 7,92$ | $47,89 \pm 3,97$ |
| Omafilcon A | $17,62 \pm 2,50$ | $22,18 \pm 0,55$ | $49,84 \pm 9,83$ | $67,12 \pm 12,59$ |
| Ocufilcon B | $31,11 \pm 3,03$ | $35,68 \pm 2,50$ | $30,70 \pm 4,50$ | $173,11 \pm 95,55$ |
| Nefilcon A | $25,04 \pm 5,04$ | $54,73 \pm 17,31$ | $114,93 \pm 7,29$ | $323,77 \pm 16,11$ |

Table 4. Average Roughness (R_a) of hydrogel contact lenses determined by WLOP for $625 \mu\text{m}^2$, $2500 \mu\text{m}^2$, $10829 \mu\text{m}^2$ and $67646 \mu\text{m}^2$ areas. Mean and Standard Deviation are shown. Values are in nanometers (nm).

| | $625 \mu\text{m}^2$ | $2500 \mu\text{m}^2$ | $10829 \mu\text{m}^2$ | $67646 \mu\text{m}^2$ |
|--------------------------|---------------------|----------------------|-----------------------|-----------------------|
| Hioxifilcon-based | $40,07 \pm 2,24$ | $44,94 \pm 4,25$ | $61,54 \pm 13,32$ | $63,25 \pm 4,22$ |
| Omafilcon A | $22,41 \pm 3,22$ | $28,20 \pm 0,88$ | $65,99 \pm 16,08$ | $89,37 \pm 17,87$ |
| Ocufilcon B | $46,04 \pm 3,74$ | $52,92 \pm 2,28$ | $53,07 \pm 5,80$ | $307,61 \pm 178,88$ |
| Nefilcon A | $39,08 \pm 12,71$ | $97,89 \pm 30,97$ | $175,03 \pm 5,40$ | $508,47 \pm 49,04$ |

Table 5. Root-Mean-Square (R_{ms}) of hydrogel contact lenses determined by WLOP for $625 \mu\text{m}^2$, $2500 \mu\text{m}^2$, $10829 \mu\text{m}^2$ and $67646 \mu\text{m}^2$ areas. Mean and Standard Deviation are shown. Values are in nanometers (nm).

| | 625 μm^2 | 2500 μm^2 | 10829 μm^2 | 67646 μm^2 |
|--------------------------|---------------------|----------------------|-----------------------|-------------------------|
| Hioxifilcon-based | 433,98 \pm 27,40 | 869,04 \pm 117,33 | 1996,67 \pm 426,18 | 2306,67 \pm 1259,61 |
| Omafilcon A | 280,67 \pm 59,22 | 353,57 \pm 35,63 | 1303,86 \pm 528,49 | 2646,67 \pm 2019,53 |
| Ocufilecon B | 583,65 \pm 103,34 | 854,75 \pm 43,99 | 1401,80 \pm 352,84 | 18196,67 \pm 10208,47 |
| Nefilcon A | 620,39 \pm 94,48 | 1800,00 \pm 612,20 | 2723,33 \pm 583,12 | 22970,00 \pm 4690,00 |

Table 6. Maximum Roughness (R_{max}) of hydrogel contact lenses determined by WLOP for 625 μm^2 , 2500 μm^2 , 10829 μm^2 and 67646 μm^2 areas. Mean and Standard Deviation are shown. Values are in nanometers (nm).

| Brand | Manufacturer | Material (USAN) | Charge | Water content (%) | Principal monomers | Replacement Frequency* |
|------------------------|---------------|-------------------|-----------|-------------------|--------------------|------------------------|
| Osmo 2 | MarkEnnovy | Hioxifilcon-based | Non ionic | 72 | 2-HEMA GMA MA | Three months |
| Proclear | Cooper Vision | Omafilcon A | Non ionic | 62 | HEMA, PC | One month |
| Frequency 1 day | Cooper Vision | Ocufilecon B | Ionic | 52 | 2-HEMA EGDMA | One day |
| Focus Dailies* | Ciba Vision | Nefilcon A | Non ionic | 69 | PVP NAAADA | One day |

* Manufacturer recommendation

+All Day Comfort (with enhanced lubricating agents)

Table 7. Specifications of the contact lenses analyzed by WLOP.

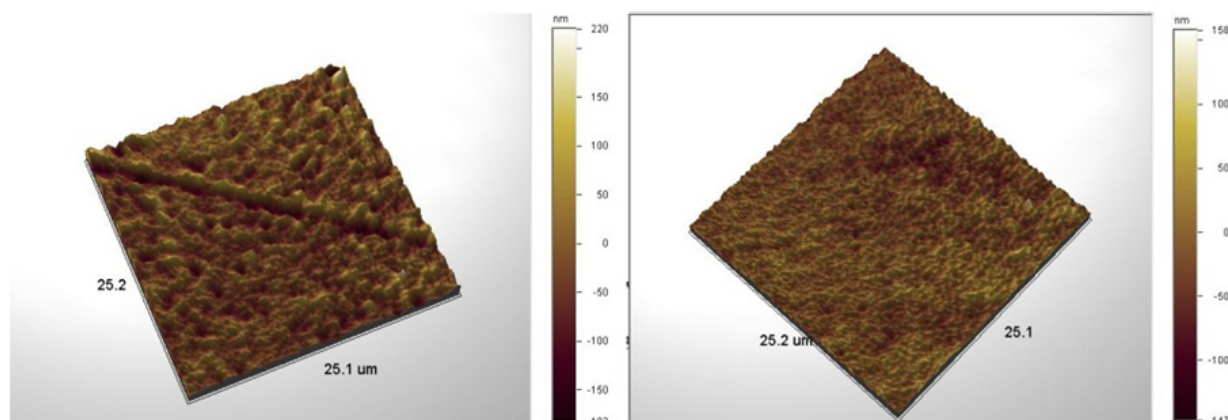


Figure 5. Surface topography of hioxifilcon and omafilcon A contact lenses (surface area: 625 μm^2) obtained by WLOP.

According with the 625 μm^2 and 2500 μm^2 area, ocufilecon B and hioxifilcon based CL showed statistical rougher surface scores than those obtained by omafilcon A, although differences between lenses were not large enough to be clinically relevant. However, when higher areas were considered, it could be observed that daily CL showed an important increase in their roughness values, which is not observed in hioxifilcon based and Omafilcon A lenses (Figures 6 and 7). According to this, analyzing higher areas could assist to detect differences between lenses surface characteristics, which may be not so obvious if smaller areas are studied.

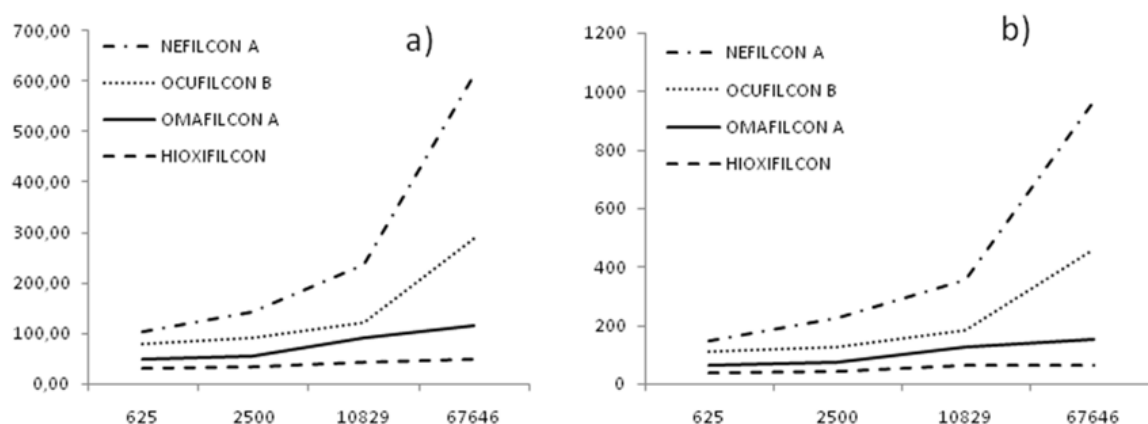


Figure 6. Variation of R_a (a) and R_{ms} (b) parameters for different scanning surface areas. Y-values represent nanometers (nm). X-values represent μm^2 .

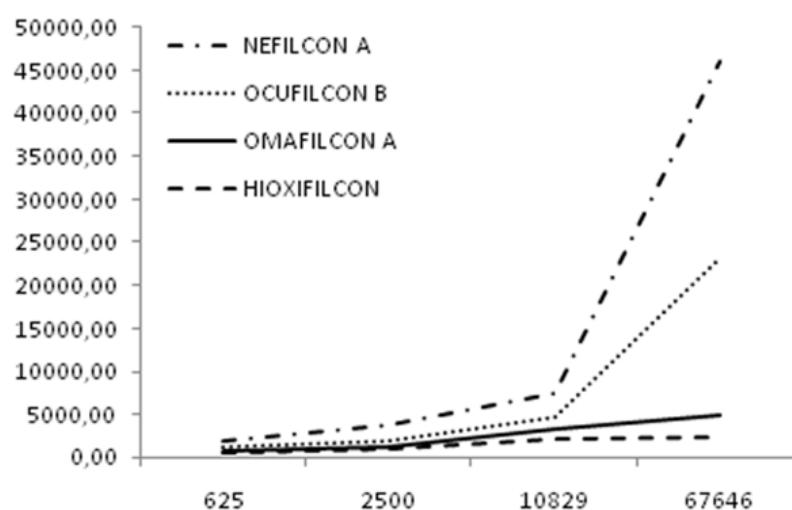


Figure 7. Variation of Maximum Roughness (R_{max}) for different scanning surface areas. Y-values represent nanometers (nm). X-values represent μm^2 .

As can be observed, roughness analysis varies with the magnification. R_a is the arithmetic mean of the departures of the profile from the mean line. So, when a surface presents irregularities homogeneously distributed, R_a should not vary with magnification, irrespective of its roughness degree. However, this is not the usual situation, as most of surfaces are not perfectly homogeneous in their irregularities distribution. In fact, there has been reported differences in CL surface roughness values at different magnifications using AFM technique, showing higher roughness scores in higher areas (Gonzalez-Meijome et al., 2006a; Giraldez et al., 2010c). Degree of variation of roughness parameters when increasing size of the measured area could be representative of how homogeneous a surface is. From the data presented here, hioxifilcon based CL has the most homogeneous surface, showing the lower R_a and R_{ms} variation when comparing values from different areas (Figure 6 and 7). Conversely, Nefilcon A showed the highest increase in roughness, displaying the less homogeneous surface of the study.

Local imperfections or sample contamination could affect R_a , R_{ms} and R_{max} values. However, their effect on R_a and R_{ms} is supposed to be lower than that on R_{max} , since R_a and R_{ms} are average values that should be less affected by local imperfections when higher areas are considered. On the other hand, R_{max} might show higher values than expected when imperfections are present, as it indicates maximum peak to valley distance in a measured area, independently of its size. When comparing CL presented here, R_{max} variation with area size had a similar pattern than that observed in R_a and R_{ms} for all CL. This can be easily observed when comparing figures 6 and 7. This finding could indicate that the higher R_{max} values observed in larger areas, especially in daily CL, would not be due to local imperfections or sample contamination, but rather due to the actual surface roughness of the CL.

Roughness parameters values obtained by WLOP are significantly higher than those previously observed in other hydrogel CL by AFM. This difference between techniques could be related to the effect of the measured area size on the R_a and R_{ms} values, as they tend to be higher when the analyzed area increases (Hinojosa and Reyes, 2001; Kiely and Bonnell, 1997; Kitching et al., 1999).

CL surface roughness degree is an important issue as imperfections in the lens surface is where deposits are likely to form (Hosaka et al., 1983). It was also previously demonstrated that the surface roughness increase, the biofilm deposited on the lens increase (Baguet et al., 1995), and that bacterial transfer from a CL is determined by the roughness and hydrophobicity of the surface receiving the bacteria (Vermeltfoort et al., 2004). Daily replacement CL in present study are supposed to acquire more deposits during wear as they had the highest increase in roughness values when higher areas are considered. So, strict replacement regime must be followed in Nefilcon A and OcuFilcon B CL wear. By gaining a better understanding of the surface roughness of different types of CL, practitioners will be better placed to prescribe the most suitable lens for any given patient and to interpret the clinical performance of lenses they prescribe in relation to patient symptoms and ocular surface signs.

3. Bacterial adhesion to contact lenses

The process of initial adhesion of bacteria to the CL surface has been extensively examined in terms of the physical and chemical properties of both the bacterial cell and CL surface such as hydrophobicity and roughness. Thus, depending on the surface thermodynamics, hydrophilic strains seem to preferentially adhere to hydrophilic surfaces, while more hydrophobic strains have a preference for hydrophobic surfaces. (Bos et al., 1999; Bruinsma et al., 2001) Also, the results of several *in vivo* studies suggest that a rougher CL surface will be prone to more extensive bacterial adhesion (Bruinsma et al., 2002; Bruinsma et al., 2003) since imperfections in the lens surface is where deposits are likely to form. (Hosaka et al., 1983)

Microbial colonization can be quantified by enumerating colony-forming units (CFU) using different bacterial strains, as the *P. aeruginosa* strain CECT 110 or *S. epidermidis* strain CECT 4184 (both from the Spanish Type Culture Collection). Adhesion can be determined by

immersing each CL, convex side up, in 1 ml of a cell suspension of *P. aeruginosa* or *S. epidermidis* whose concentration of 1.2×10^9 CFU/ml (adjusted to McFarland scale No.4) is determined by dilution in sterile saline solution (SS) and spreading on Tryptic Soy Agar (TSA) plates. Following incubation of the bacterial suspension for 2 h at 37°C with continuous shaking (15 rpm), each CL has to be carefully removed and washed 3 times in sterile SS. Next each lens is placed in 2 ml of sterile SS and sonicated using a Bronson Sonifier 250 for 1 min. The suspensions then spread on TSA-1 plates and CFU enumerated after 24 h of incubation at 37°C.

3.1. Microbial keratitis on contact lens wear

The adhesion of bacteria to contact lenses (CL), notably that of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, is considered a primary risk factor of serious corneal problems (Buehler PO et al., 1992; Catalonotti P et al., 2005; Leitch EC et al., 1998). The CL surface is a suitable substrate for bacterial adhesion and biofilm formation, and can sustain the growth of an inoculum of organisms in prolonged contact with the cornea (Elder Mj et al., 1995). In addition, corneal interaction with the CL can override the protective mechanisms of the cornea, augmenting the capacity of microbial cells to adhere to the cornea and progress to microbial keratitis (MK). To improve the corneal/CL interface, several co-polymers have been incorporated into soft hydrogel lens materials, including silicone polymers for increased oxygen permeability and phosphoryl-choline to increase biocompatibility. Further, the new modalities of wear, such as daily disposable (DD) hydrogel CL, avoid the need for regular cleaning and storage, which are known to be an important cause of microbial contamination (Laughlin-Borlace et al., 1998). Notwithstanding, studies have shown that users of DD and silicone hydrogel CL do not show a reduced risk of MK (Dart et al., 2008; Stapleton et al., 2008). In the paper by Dart et al., differences in soft CL design and/or the composing polymer rather than the mode of wear were found to determine susceptibility to MK (Dart et al., 2008).

Several microbial strains have been isolated from clinical samples of MK. Approximately two thirds of these strains are Gram-negative bacterial strains, most notably *Pseudomonas aeruginosa* but also some *Serratia* species, while one third comprises Gram-positive cocci, including *Staphylococcus aureus* and *Staphylococcus epidermidis* (Catalonotti P et al., 2005; Leitch EC et al., 1998; Seal et al., 1999). *S. epidermidis* is one of the microorganisms most frequently isolated from the normal microbiota of the human eye surface (Ayoub M et al., 1994; Doyle A et al., 1995; Hara J et al., 1997). Despite this, this bacterium has been held responsible for infections such as chronic blepharitis, conjunctivitis and keratitis, especially in immunocompromised hosts (Pinna A et al., 1999), and may account for 45 per cent of all cases of bacterial keratitis (Nayak et al., 2007; Nayak and Satpathy, 2000). In CL wearers, *S. epidermidis* finds itself in a privileged position to act as an opportunistic pathogen, colonizing the lens surface from the eye and surrounding areas. The microorganism also shows an adhesion preference for foreign materials and has the capacity to produce an extracellular substance comprised of polysaccharides (slime) (Perilli et al., 2000).

Pseudomonas aeruginosa is a common Gram-negative bacillus that acts as an opportunistic pathogen under several circumstances (Lyczak et al., 2000). As a Gram-negative bacterium, the lipopolysaccharides (LPS) composing its outer membrane act as key virulence factor, promoting infection by interfering with the host immune response (Wilkinson, 1983; Cryz, Jr. et al., 1984). Other virulence factors encoded by *P. aeruginosa* could help bacterial survival on the ocular surface. These factors are those needed for strategies such as biofilm formation, resistance against killing, communication between bacteria (e.g., quorum sensing), invading epithelial cells and surviving within them, destroying tear components, breaking down cell-to-cell junctions and extracellular matrices, and injecting toxins into cells (Alarcon et al., 2009; Angus et al., 2008; Evans et al., 2007; Fleiszig et al., 1994; Fleiszig, 2006; Hauser, 2009; Lyczak et al., 2000; Wagner and Iglewski, 2008; Willcox, 2007; Zolfaghar et al., 2003; Zolfaghar et al., 2005; Zolfaghar et al., 2006). *Pseudomonas aeruginosa* also possesses factors that are highly immunogenic (initiate inflammation) while being able to evade the immune responses they initiate (Choy et al., 2008; Evans et al., 2007; Hazlett, 2007; Lyczak et al., 2000). Interestingly, *P. aeruginosa* virulence factors can also confer resistance to contact lens disinfectants (Lakkis and Fleiszig, 2001).

3.2. Effect of hydrophobicity and surface roughness

Bacterial adhesion to a biomaterial is thought to depend on the hydrophobicity of the biomaterial, such that adhesion decreases with the water content of the CL (Ahanotu et al., 2001; Kodjikian et al., 2004; Magnusson, 1982). The effect of surface roughness on bacterial adhesion to a CL is still far from being well understood. According to prior work, it seems clear that surface roughness is related to deposit formation and microorganism colonization of the surface (Baguet et al., 1995; Vermeltfoort et al., 2004). Greater surface roughness determines a greater specific surface area, thus creating more available active sites for thermodynamic reactions. Bacterial adhesion initiates on surface irregularities that serve as microenvironments where bacteria are sheltered from unfavorable environmental factors and then promote their survival (Shellenberger and Logan, 2002; Chae et al., 2006; Jones and Velegol, 2006). The effects of surface roughness have been examined over a wide range of physical scales (Bruinsma et al., 2001; Li and Logan, 2004; Li and Logan, 2005; Emerson et al., 2006; Mitik-Dineva et al., 2008; Park et al., 2008) and previous studies suggest that nanoscale surface roughness may greatly influence bacterial adhesion (Mitik-Dineva et al., 2008).

3.2.1. *Staphylococcus epidermidis*

Initial adhesion of *S. epidermidis* to unworn or worn conventional hydrogel CL has been reported to be strain and substrate related, the hydrophilic nature of the lens being a key factor (George et al., 2003; Henriques et al., 2005). The incorporation of silicone in a hydrogel polymer achieves high oxygen permeability but on the other hand reduces hydrophilicity (Tighe B, 2009). According with previous studies (Giraldez et al., 2010b), unworn silicone hydrogel CL (more hydrophobic) show a greater susceptibility to *S. epidermidis* adhesion

than the conventional hydrogel CL (Figure 6). This observation is consistent with the established relationship between microbial adhesion and lens surface hydrophobicity. Notwithstanding, Santos et al. (Santos et al., 2008) were unable to detect any difference in microbial adhesion when comparing unworn silicone hydrogel and conventional hydrogel CL. This discrepancy could be explained by the different extents of microbial colonization observed for different *S. epidermidis* strains, and/or the different methodologies employed (Henriques et al., 2005; Kodjikian et al., 2007). In both hydrophobic and hydrophilic groups, the lenses showing the lowest R_a values (omafilcon A and comfilcon A) also returned the lowest numbers of *S. epidermidis* CFU, despite their high R_{ku} and R_{sk} values. Roughness values corresponding to these lenses are shown in tables 1 and 2.

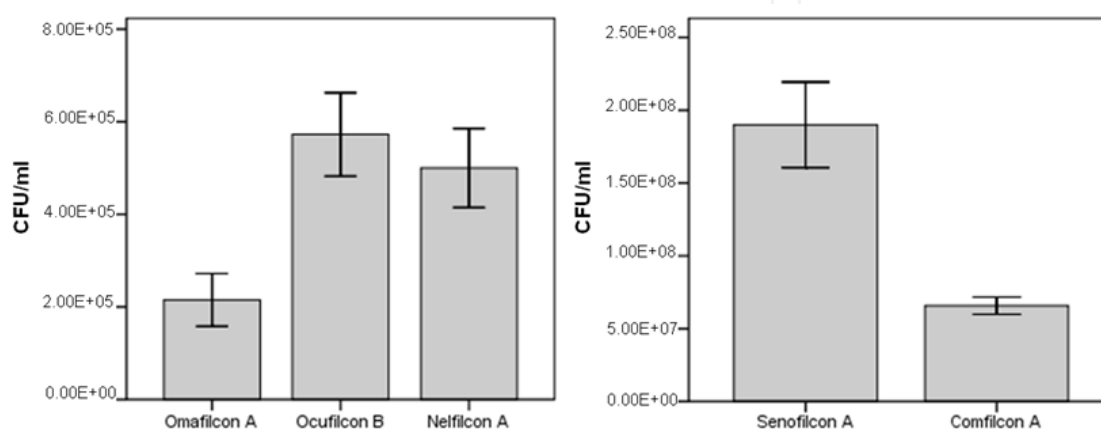


Figure 8. Adhesion of *S. epidermidis* CECT 4184 to hydrophilic (a) and hydrophobic (b) hydrogel contact lenses.

3.2.2. *Pseudomonas aeruginosa*

Figure 7 provides the quantities, in CFU, of *P. aeruginosa* that adhered to six unworn CL (4 silicone hydrogel and 2 conventional hydrogel CL). In these lenses, it can be observed no substantial preference of *P. aeruginosa* to adhere to unworn hydrophilic or hydrophobic CL. Although this is consistent with other studies for other bacterial strains (Borazjani et al., 2004; Santos et al., 2008), it challenges the established relationship between microbial adhesion and lens surface hydrophobicity (Pritchard et al., 1999; Doyle, 2000; Young et al., 2002; van Oss, 2003; Giraldez et al., 2010b). This discrepancy could be explained by the different extents of microbial colonization observed for different bacterial strains, and/or the different methodologies employed (Henriques et al., 2005; Kodjikian et al., 2007). In fact, most *P. aeruginosa* strains have a more hydrophilic surface than *S. epidermidis* or other bacteria (Gottenbos et al., 2001; Mitik-Dineva et al., 2009). This could explain the scarce difference observed between *P. aeruginosa* adhesion to hydrophilic and hydrophobic contact lenses relative to previously observed *S. epidermidis* adhesion patterns (Bos et al., 1999; Bakker et al., 2002; Giraldez et al., 2010b).

In relation with roughness effect, the lenses showing the highest R_a values accompanied by low R_{ku} and R_{sk} values (for a $25 \mu\text{m}^2$ area, ocufilcon B: $R_a=11.01 \pm 1.79 \text{ nm}$, $R_{ku}=5.45 \pm 1.95$ and $R_{sk}=0.98 \pm 0.17$; and lotrafilcon B: $R_a=26.97 \pm 3.91 \text{ nm}$, $R_{ku}=4.11 \pm 1.28$ and $R_{sk}=-0.34 \pm$

0,07) also returned the lowest numbers of *P. aeruginosa* CFU. Nanomaterials are those with constituent dimensions smaller than 100 nm in at least one direction and have numerous biomedical applications (Park et al., 2008). Nanophase materials have greater surface areas, more surface defects, increased surface electron delocalization and greater numbers of surface grain boundaries. Since they show a higher percentage of atoms at their surfaces compared to conventional materials, the surface properties of nanophase materials differ and this results in higher surface reactivity to cell responses (Park et al., 2008; Mitik-Dineva et al., 2008). Although changes in metabolic responses have not been clearly defined, research has shown altered attachment rates for certain bacteria on nanophase surfaces, which could translate to enhanced or reduced adhesion (Park et al., 2008; Mitik-Dineva et al., 2008; Mitik-Dineva et al., 2009). Thus, while nanophase materials show reduced *Staphylococcus epidermidis* colonization compared to conventional materials (Colon et al., 2006; Giraldez et al., 2010b) they nevertheless show improved *P. aeruginosa* colonization (Mitik-Dineva et al., 2009; Webster et al., 2005).

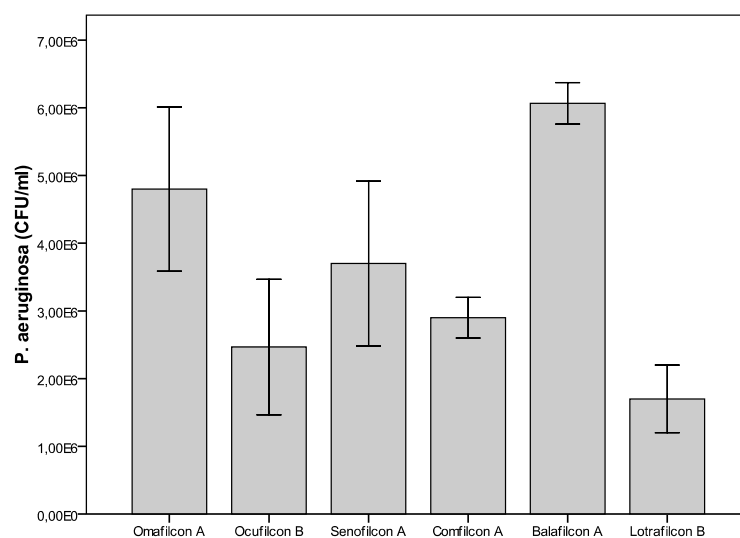


Figure 9. Adhesion of *P. aeruginosa* to both hydrophilic (omafilcon A and ocufilcon B) and hydrophobic (senofilcon A, comfilcon A, balafilcon A and lotrafilcon B) contact lenses.

4. Conclusion

Surface hydrophobicity and roughness are critical factors for bacterial adhesion; the surface of any body or object is the part which interacts with the surrounding environment. Hydrophobicity effect on bacterial adhesion to contact lenses is different in depending on bacterial strains; it seems to have a higher influence in *S epidermidis* than in *P aeruginosa* adhesion. Moreover, roughness is a biological factor that affects the manner in which bacteria adhere to surfaces, above all for initial adhesion; so by gaining a better understanding of the surface roughness of different types of CL, practitioners will be better placed to prescribe the most suitable lens for any given patient and to interpret the clinical performance of lenses they prescribe in relation to patient symptoms and ocular surface signs.

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