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White light interferometry to characterize the hydrogel contact lens surface

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Abstract

Purpose: The aim of this study was to characterize, qualitatively and quantitatively, the surface morphology of four unworn conventional hydrogel contact lenses (Omafilcon, Hioxifilcon-based, Nefilcon A and Ocufilcon B) by White Light Optical Profiling (WLOP). WLOP is an ideal technique for sampling larger areas as well as for higher measurement speed compared with other topography techniques used in contact lens studies.

Methods: Surface roughness was assessed by WLOP in the Vertical Scanning Mode, with a Wyko® NT1100, which is a non-contact optical profiling system that provides high vertical resolution. Representative roughness parameters, the Average Roughness (R_a), Root-mean-square Roughness (R_{ms}), and Maximum Roughness (R_{max}), for areas of 625, 2500, 10829 and 67 646 μm^2 were calculated.

Results: Higher R_a, R_{ms} and R_{max} values were obtained for larger areas in all lenses. Daily disposable contact lenses (Nefilcon A and Ocufilcon B) presented the highest R_a, R_{ms} and R_{max} values, the larger changes in these parameters becoming apparent with the increase in the measured area. Differences between lenses were less obvious when data from 625 and 2500 μm^2 area were compared.

Conclusions: Daily disposable contact lenses showed the highest roughness surface. Analyzing larger areas might be adequate to detect differences between lenses in terms of surface characteristics, which may not be so obvious if smaller areas are studied.

Keywords: contact lenses, hydrogels, surface characterization, surface roughness, topography, white light optical profiling

Introduction

Spoilage of contact lenses (CL) by either proteins or lipids is an important factor in the biocompatibility of CL materials (Rebeix *et al*., 2000; Jones *et al*., 2003; Santos *et al*., 2007; Carney *et al*., 2008). It may produce tear film disruption, decreased vision, discomfort, intolerance, and bacterial adhesion (Leahy *et al*., 1990; Zhang *et al*., 2005; Lorentz *et al*., 2007; Urs and Ranganathaiah, 2008). The degree of surface roughness is an important issue as imperfections in the lens surface are where deposits are likely to form (Hosaka *et al*., 1983). It was 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 CL is determined by the roughness and hydrophobicity of the surface receiving the bacteria (Vermeltfoort *et al*., 2004). Moreover, a smooth surface is also essential to optimise the optical performance of the CL by reducing scattered light (Bennett, 1992).

Parameters generally used to quantify surface roughness include Average Roughness (R_a), Root Mean Square Roughness (R_{ms}) and Maximum Roughness

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(R_{max}) (Baguet *et al.*, 1993; Bhatia *et al.*, 1997; Hinojosa and Reyes, 2001; Gonzalez-Meijome *et al.*, 2006a; Guryca *et al.*, 2007; Lira *et al.*, 2008). R_a is universally recognized, and the most used international parameter of roughness that represents the average deviation or arithmetic mean of the profile from the mean line. On the other hand, R_{ms} reflects the standard deviation from the mean surface plane. Although R_a and R_{ms} appear to be the most helpful and consistent roughness parameters to characterize surface topography of CL (Gonzalez-Meijome *et al.*, 2006a), their values depend on the sample length (Bennett, 1992; Kiely and Bonnell, 1997; Kitching *et al.*, 1999; Hinojosa and Reyes, 2001). However, the variation of these parameters with sample length could be indicative of how homogeneous a surface is in its distribution of irregularities. The third parameter, R_{max}, is the maximum peak-to-valley height identified within the observed area. It may be affected by local imperfections or sample contamination leading to higher values than expected, so the material characterization based on this parameter could be unreliable.

Detailed information about the surface quality of CL has been studied previously by Atomic Force Microscopy (AFM) (Baguet *et al.*, 1993, 1995; Bhatia *et al.*, 1997; Bruinsma *et al.*, 2003; Gonzalez-Meijome *et al.*, 2006a, 2009) and Cryo-SEM (Gonzalez-Meijome *et al.*, 2006b; Guryca *et al.*, 2007). AFM is a very powerful tool for high resolution examination of the hydrated CL surface structure. The method avoids artefacts due to dehydration and coating (Bhatia *et al.*, 1997; Kim *et al.*, 2002). However, when using AFM the sampling area is very small, and there is some controversy about the representativeness of the R_a and R_{ms} values with regard to the complete lens surface. Cryo-SEM, a modification of the Scanning Electron Microscopy (SEM), requires that the material be frozen in nitrogen before examination (Serp *et al.*, 2002). The main disadvantage of this technique is that, in hydrogels, this usually means the destruction of the material.

WLOP is one of the preferred methods of precision surface characterization in many fields (Bennett, 1992; Caber, 1993; Windecker and Tiziani, 1999; O'Mahony *et al.*, 2003). In particular, the WLOP technique, has been successfully applied in the characterization of medical devices such as implants, prostheses, stents, and others (Filiz *et al.*, 2008; Wippermann *et al.*, 2008). It is a powerful and well-established technique for non-contact measurement of surface topography, which can quickly determine three-dimensional surface shape over larger areas at high vertical and moderate lateral resolution (Bennett, 1992; Novak *et al.*, 2003; O'Mahony *et al.*, 2003). This topographic technique, like AFM, enables the analysis of surface topography and roughness by means of a non-destructive methodology. 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 has sub-nanometer height resolution capability. For rougher surfaces, a vertical scanning coherence sensing technique can be used to give nanometer height resolution over several hundred microns of surface height. WLOP allows the analysis of larger areas than techniques used previously in CL, so the values and statistics should be more representative of roughness distribution over the lens surface. Topographic information can also be obtained from the surface in aqueous conditions.

The aim of this study is to analyze in detail the surface topography by WLOP for unworn hydrogel CL in physiological solution. As far as we know, WLOP has not been used before to characterize Hioxifilcon-based, Omafilcon A, Nefilcon A and Ocufilcon B CL surfaces.

Methods

Contact lenses

Four commercially available conventional hydrogel CL were examined, whose characteristics are shown in *Table 1*. All CL in the present study were manufactured by cast-molding and had no surface treatment. Although all lenses are indicated for daily wear, different replacement frequency is recommended by the manufacturer (*Table 1*). Osmo 2 CL material is based on Hioxifilcon, as their main monomers are those from Hioxifilcon (2-HEMA GMA) and MA, so the generic term used for this lens was Hioxifilcon-based (*Table 1*). According to the material composition, Hioxifilcon-based, Omafilcon A and Ocufilcon B are hydroxyethylmethacrylate copolymers, whereas Nefilcon A is a polyvinylalcohol. Lenses were obtained in the original containers filled with their original shipping fluid.

Interference microscopy

WLOP measurements were obtained with the interference microscopy Wyko®-NT1100 (Veeco Instruments Inc., Plainview, NY, USA), a tool that combines a microscope and an interferometer into the same instrument (*Figure 1*). In brief, it consists of a tungsten halogen lamp as a light source; a beamsplitter which delivers the light beam to the microscopy objective and an interferometer which generates an interferogram that will be recorded by a CCD detector (*Figure 2*). This is processed by the computer using interferometric phase-mapping software.

The interference principle used here works as follows: A white-light beam is filtered and passed through an interferometer objective to the test surface. The inter-

Table 1. Specification of contact lenses used in this study

Brand	Manufacturer	Material (USAN)	Charge	Water content (%)	Principal monomers	Replacement Frequency*
Osmo 2	Mark'Ennovy, Madrid, Spain	Hioxifilcon-based	Non ionic	72	2-HEMA GMA MA	3 months
Proclear	Cooper Vision Inc., Fareham, Hants, UK	Omafilcon A	Non ionic	62	HEMA, PC	1 month
Frequency 1 day	Cooper Vision	Ocufilcon B	Ionic	52	2-HEMA EGDMA	1 day
Focus Dailies[†]	Ciba Vision Corporation, Duluth, GA, USA	Nefilcon A	Non ionic	69	PVP NAAADA	1 day

HEMA, hydroxyethylmethacrylate; GMA, glycerylmethacrylate; MA, methacrylic acid; PC, phosphorylcholine; EGDMA, ethylene glycol dimethacrylate; PVA, polyvinylalcohol; NAAADA, N-acryloylaminoacetaldehyde dimethylacetal
*Manufacturer recommendation.
[†]All Day Comfort (with enhanced lubricating agents).

Figure 1. Wyko® NT1100 interferometric microscope.

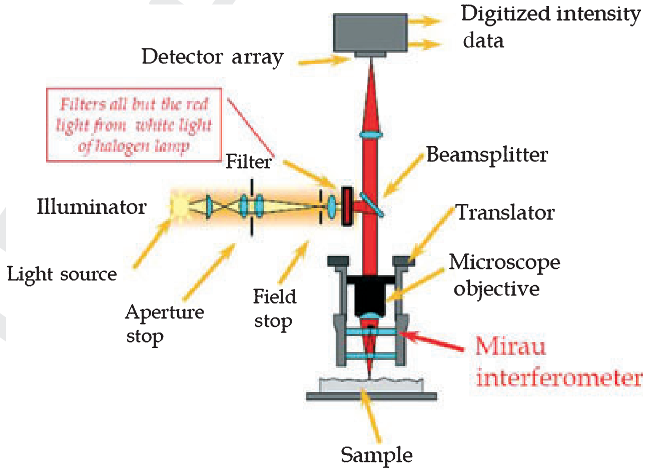

Figure 2. Schematic diagram of the interference microscope Wyko® NT1100.

ferometer beam splitter reflects half of the incident beam to the reference surface within the interferometer. The beams which have been reflected from the test surface and the reference surface recombine to form interference fringes. These fringes are the alternating light and dark bands that can be seen when the surface is in focus.

Two techniques are used by this interference microscopy; the Phase Shifting Interferometry (PSI) and the Vertical Scanning Interferometry (VSI). The basic interferometric principles are similar in both techniques but they have some differences. PSI mode uses a filter to obtain a monochromatic light source (wavelength of 632 nm), whereas VSI mode uses a white-light source filtered by a neutral density filter. Briefly, PSI mode is limited to fairly smooth, continuous surfaces, whereas VSI mode resolves rougher surfaces than PSI. A detailed description of the technique is reported elsewhere (Wyant and Creath, 1992). The interference microscope is equipped with three objectives of magnification: 5×, 20× and 50×. Each of them includes the interferometer whose type depends on the magnification employed. So, for small magnifications (5×) the Michelson interferometer is used (*Figure 3*), whereas for greater magnifications (20× and 50×), a Mirau interferometer is employed (*Figure 4*).

Procedure

Three CL of each material were included in the study, and only one measurement per lens was done. The same shipping fluid used to store the soft CL was added to the sample to maintain its hydration during microscopic observation. All procedures and microscopic examinations were carried out in the same room kept at 21°C and approximately 50% relative humid-

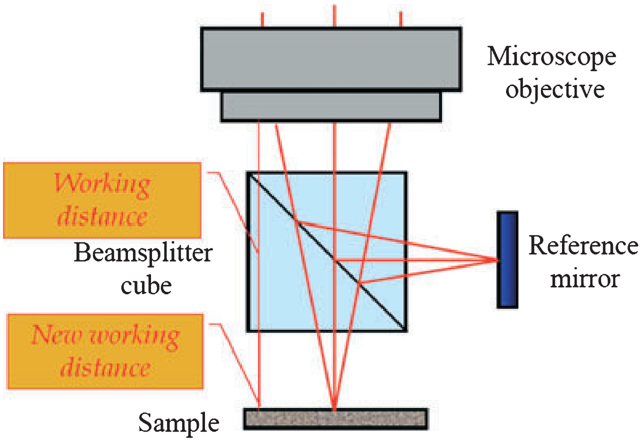

Figure 3. Schematic diagram of the Michelson Interferometer.

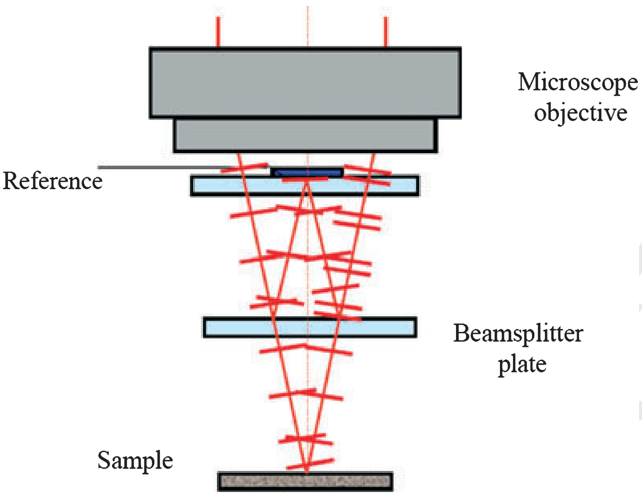

Figure 4. Schematic diagram of the Mirau Interferometer.

ity. Following the acquisition of images, surface roughness analysis was undertaken using the WycoVision®32 analytical software package. The roughness parameters were calculated after removal of curvature and tilt terms. This image processing step is necessary to remove the slight spherical curvature of the lens. Removing curvature causes spherical samples to appear flat, so that the 'real' surface features can be seen, instead of the dominant shape which does not represent the 'true' topography of the samples. As described 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 were acquired for a variety of sample sizes (Kiely and Bonnell, 1997; Kitching *et al.*, 1999); roughness parameters R_a, R_{ms} and R_{max} were calculated for 625, 2500, 10829 and 67646 μm^2 areas. Larger area size was determined by the magnification level used (50× and 20×). Roughness parameters, which are statistical values, were calculated from multiple measures in each area and were evaluated by one-way ANOVA test. Two distinct comparisons were made: between different areas within the same material and between lens materials. The Levene test was used to assess equality of variances in both cases, so Tukey or Games-Howell method was used depending on the equality of variances. For statistical analysis SPSS Professional Statistics 17 program (SPSS Inc., Chicago, IL, USA) was used.

Results

Mean roughness parameters R_a and R_{ms} obtained from WLOP analysis for 625, 2500, 10 829 and 67 646 μm^2 areas for all lenses are shown in *Table 2 and 3*. Three dimensional images of the surface topography of the hydrogel CL at different magnification are shown in *Figure 5*.

As can be seen in *Tables 2 and 3*, the R_a and R_{ms} values from small areas appear to be lower than those obtained from larger ones. In the smallest area (625 μm^2), Omafilcon A and Ocufilcon B lenses presented the lowest and highest roughness values respectively (*Tables 2 and 3*). Variation of R_a and R_{ms} for different scanning surface areas was found to be statistically significant in all lenses (One way ANOVA, $p < 0.05$) (*Figure 6*). Values obtained from the 67 646 μm^2 area were significantly higher than those from the smaller areas (625 and 2500 μm^2) in all lenses (Hioxifilcon-based contact lens, Omafilcon A and Ocufilcon B; Tukey test, $p < 0.05$: Nefilcon; Games-Howell test, $p < 0.05$), and are higher than those obtained from 10 829 μm^2 area in both Ocufilcon B (Tukey test, $p < 0.05$) and Nefilcon A lenses (Games-Howell test, $p < 0.05$). No differences between values from the 625 and 2500 μm^2 areas were observed in any lenses (Hioxifilcon-based, Omafilcon A and Ocufilcon B contact lenses; Tukey test, $p > 0.05$: Nefilcon; Games-Howell test, $p > 0.05$).

In order to compare surface characteristics for different lens materials, the R_a and R_{ms} differences between lenses were also analyzed. The most significant differences between lenses were observed at 10 829 and 67 646 μm^2. For these areas, Nefilcon A contact lenses showed a surface roughness higher than all other lenses (Tukey test, $p < 0.05$). Differences were also observed between Ocufilcon B and both Hioxifilon based and Omafilcon A CL(Tukey test, $p < 0.05$). For 625 and 2500 μm^2 areas there were only significant differences between Omafilcon A and Hioxifilcon-based; as well as between Omafilcon A and Ocufilcon B contact lenses (Tukey test, $p < 0.05$) (*Figure 6*).

R_{max} values found in this study are shown in *Table 4*. Lower values were observed in 625 μm^2 in

Table 2. Average Roughness (R_a) of hydrogel contact lenses determined by WLOP for 625, 2500, 10 829 and 67 646 μm^2 areas. Mean and Standard Deviation are shown. Values are in nanometers (nm)

	625 μm^2	2500 μm^2	10 829 μm^2	67 646 μm^2
Hioxifilcon-based	31.04 ± 1.75	32.88 ± 2.18	42.26 ± 7.92	47.89 ± 3.97
Omafilcon A	17.62 ± 2.50	22.18 ± 0.55	49.84 ± 9.83	67.12 ± 12.59
Ocufilcon B	31.11 ± 3.03	35.68 ± 2.50	30.70 ± 4.50	173.11 ± 95.55
Nefilcon A	25.04 ± 5.04	54.73 ± 17.31	114.93 ± 7.29	323.77 ± 16.11

Table 3. Root-Mean-Square (R_{ms}) of hydrogel contact lenses determined by WLOP for 625, 2500, 10 829 and 67 646 μm^2 areas. Mean and Standard Deviation are shown. Values are in nanometers (nm)

	625 μm^2	2500 μm^2	10 829 μm^2	67 646 μm^2
Hioxifilcon-based	40.07 ± 2.24	44.94 ± 4.25	61.54 ± 13.32	63.25 ± 4.22
Omafilcon A	22.41 ± 3.22	28.20 ± 0.88	65.99 ± 16.08	89.37 ± 17.87
Ocufilcon B	46.04 ± 3.74	52.92 ± 2.28	53.07 ± 5.80	307.61 ± 178.88
Nefilcon A	39.08 ± 12.71	97.89 ± 30.97	175.03 ± 5.40	508.47 ± 49.04

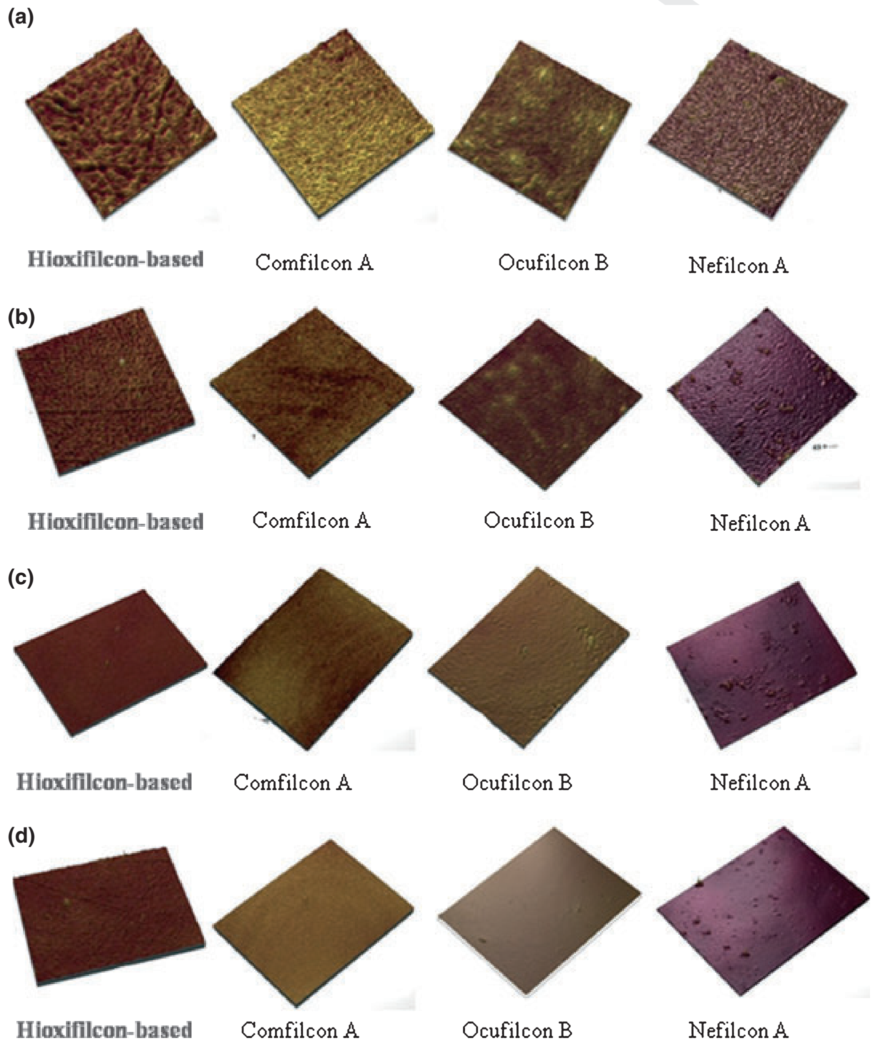

Figure 5. Surface topography of all contact lenses at different magnification. Surface areas: (a) 625 μm^2, (b) 2500 μm^2, (c) 10 829 μm^2, (d) 67 646 μm^2. Quantitative roughness parameters are presented in *Tables 2, 3 and 4*.

all lenses. The pattern of variation in R_{max} for different scanning surface areas was similar to that observed for R_a and R_{ms}, with an increase in R_{max} values with increasing scanning area (*Figure 7*). Values obtained from the 67 646 μm^2 area were significantly higher than those from the smaller areas (625 and 2500 μm^2)

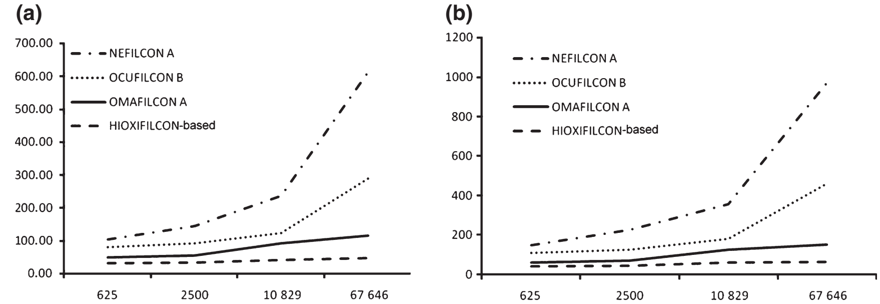

Figure 6. Variation of (a) R_a and (b) R_{ms} for different scanning surface areas. Y-values represent nanometers (nm). X-values represent μm^2.

Table 4. Maximum Roughness (R_{max}) of hydrogel contact lenses determined by WLOP for 625, 2500, 10 829 and 67 646 μm^2 areas. Mean and Standard Deviation are shown. Values are in nanometers (nm)

	625 μm^2	2500 μm^2	10 829 μm^2	67 646 μm^2
Hioxifilcon-based	433.98 ± 27.40	869.04 ± 117.33	1996.67 ± 426.18	2306.67 ± 1259.61
Omafilcon A	280.67 ± 59.22	353.57 ± 35.63	1303.86 ± 528.49	2646.67 ± 2019.53
Ocufilcon B	583.65 ± 103.34	854.75 ± 43.99	1401.80 ± 352.84	18 196.67 ± 10 208.47
Nefilcon A	620.39 ± 94.48	1800.00 ± 612.20	2723.33 ± 583.12	22 970.00 ± 4690.00

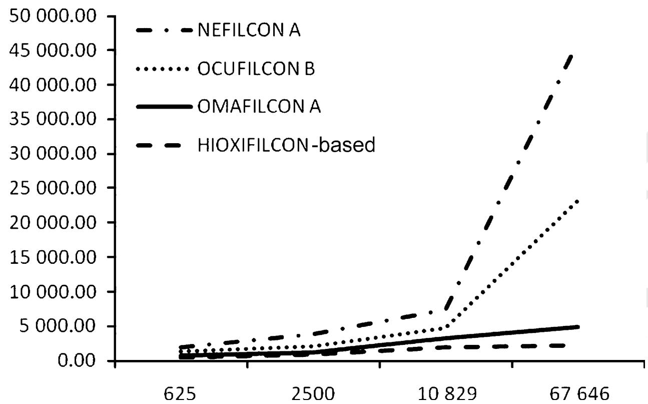

Figure 7. Variation of R_{max} for different scanning surface areas. Y-values represent nanometers (nm). X-values represent μm^2.

in all lenses (Hioxifilcon-based, Omafilcon A and Ocufilcon B contact lenses; Tukey test, $p > 0.05$: Nefilcon; Games-Howell test, $p > 0.05$), and higher than those obtained from 10 829 μm^2 in Ocufilcon B (Tukey test, $p > 0.05$) and Nefilcon A (Games-Howell test, $p > 0.05$). No differences between values from 625 and 2500 μm^2 areas were observed in any lenses (Hioxifilcon based, Omafilcon A and Ocufilcon B contact lenses, Tukey test, $p > 0.05$: Nefilcon; Games-Howell test, $p > 0.05$). Regarding differences between CL, daily CL (Nefilcon A and Ocufilcon B) showed significantly higher R_{max} values than those observed in Omafilcon A and Hioxifilcon-based lenses (Tukey test, $p < 0.05$), mainly for the largest area (67 645 μm^2). The R_{max} differences between Nefilcon A and Ocufilcon B; and between Omafilcon A and Hioxifilcon-based lenses were not statistically significant (Tukey test, $p > 0.05$).

Discussion

Improvement of hydrogel CL materials is a major focus of research in this field, even if a palliative solution can be found by using disposable CL to avoid complications associated with long time wearing (Rebeix *et al*., 2000). Surface properties of CL and interfacial interactions between lenses and the ocular surface may produce deposits and corneal damage and promote infection (Tripathi *et al*., 1991; Goldberg *et al*., 1997). CL provide a suitable substratum for bacterial adherence and biofilm formation, supplying an innoculum of organisms in prolonged contact with the cornea (Elder *et al*., 1995) New co-polymers have been incorporated into the soft hydrogel lens materials to increase biocompatibility, including phosphoryl-choline and polyvinyl alcohol. Furthermore, new CL modalities of wear (daily disposable) have been also introduced to reduce risks of CL spoilage. However, the risk of microbial contamination was not reduced in users of daily disposable lenses (Dart *et al*., 2008; Stapleton *et al*., 2008) It has been suggested that differences in soft CL design and/or polymer, rather than its method of wear, can modify susceptibility to microbial contamination (Dart *et al*., 2008).

The issue of measurement area is an important point to be considered in all surface roughness studies (Bennett, 1992; Kiely and Bonnell, 1997; Kitching *et al*., 1999; Hinojosa and Reyes, 2001; Blunt, 2006). WLOP allows the sampling of larger areas than other techniques used before in CL. In this regard, the maximum hydrogel CL area studied by AFM was 400 μm^2, (Gonzalez-Meijome *et al*., 2006a) which represents about an $2.6 \times 10^{-4\%}$ of the entire 14.00 mm diameter CL surface area. In the present study we were able to determine roughness parameters in areas as large as

67 646 μm^2, which is almost 170 times higher than the greatest area evaluated by AFM, so values and statistical results should be more representative with respect to the total CL surface.

If we consider the 625 and 2500 μm^2 area, Ocufilcon B and Hioxifilcon-based CL showed statistically rougher surface scores than those obtained from Omafilcon A, although the differences between lenses were not large enough to be clinically relevant. However, when larger 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*). Therefore analyzing larger areas could assist in detecting differences between lens surface characteristics, which may not be so obvious if smaller areas are studied.

Our results showed that 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 the degree of roughness. However, this is not the usual situation, as most of surfaces are not perfectly homogeneous in their distribution of irregularities. The calculated values of surface roughness parameter show dependence on the scan size and the particular area being scanned. This effect has also been observed in several studies with different techniques for topographic analysis: there is frequently an increase in the roughness values as the scan size increases (Duparre and Jakobs, 1996; Duparre *et al*., 2002). In fact, there have been reported differences in CL surface roughness values at different magnifications using the AFM technique, with higher roughness scores in larger areas (Gonzalez-Meijome *et al*., 2006a). The degree of variation of roughness parameters when increasing size of the measured area could be representative of how homogeneous a surface is. From the results in the present study, Hioxifilcon-based CL has the most homogeneous surface, showing the lower R_a and R_{ms} variation when comparing values from different areas (*Figures 6 and 7*). Conversely, Nefilcon A showed the highest increase in roughness, displaying the least homogeneous surface in 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} should be lower than that on R_{max}, especially when larger areas are considered. On the other hand, R_{max} might show higher values than expected when imperfections are present, as it indicate maximum peak to valley distance in a measured area, regardless of the size of the area. In the present study R_{max} variation with area size had a similar pattern to that observed in R_a and R_{ms} for all CL. This can be easily observed when comparing *Figures 6 and 7*. This result could indicate that the higher R_{max} values observed in larger areas, especially in daily CL, are not due to local imperfections or sample contamination, but are due to the actual surface roughness of the CL.

Roughness parameter values found in the present study were 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 (Kiely and Bonnell, 1997; Kitching *et al*., 1999; Hinojosa and Reyes, 2001) .

Profile analysis offers useful information about the quality of surfaces, showing whether the level of a surface roughness achieves the aim of the manufacturers who designed it. The roughness values obtained in this study were similar to those observed in normal machined surfaces, but higher than those generally obtained in commercially polished or superpolished glass optics (Bennett, 1992). When optical materials are manufactured, low scatter optical components are necessary to reduce scattered light and improve the performance of specialized optical systems. Since CL are optical devices used to correct refractive errors in ametropic subjects, further research should be conducted to determine the clinical implications of surface roughness on quality of vision.

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. Based on previous studies, it seems to be clear that surface roughness is related with deposit formation and microorganism colonization over that surface. (Baguet *et al*., 1995; Vermeltfoort *et al*., 2004). Greater surface roughness can increase the total surface area, therefore creating more available active surface sites for reactions by higher thermodynamic reaction potential. The degree of CL surface roughness is an important issue as imperfections in the lens surface are where deposits are likely to form (Hosaka *et al*., 1983). It was also previously demonstrated that as the surface roughness increase, the biofilm deposited on the lens also increases (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 disposable CL in the present study would be expected to acquire more deposits during wear as they had the greatest increase in roughness values when larger areas were considered. A strict replacement regime must therefore 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.

Conclusions

Our findings suggest that the WLOP is a highly suitable technique for sampling larger areas than other techniques previously used for topography studies in contact lenses. Analyzing larger areas is important to detect differences between lens surface characteristics, which may be not so obvious if smaller areas are studied.

Daily disposable CL in the present study would be expected to acquire more deposits during wear as they had the highest roughness surface. Further research would be necessary to elucidate this question.

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