AFM in Peak Force mode applied to worn siloxane-hydrogel contact lenses

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Running head: AFM-Peak Force applied to the study of worn Siloxane-Hydrogel Lenses

Abstract

The objective of this work is to apply Atomic Force Microscopy in Peak Force mode to obtain topographic characteristics (mean roughness, root-mean-square roughness, skewness, kurtosis) and mechanical characteristics (adhesion, elastic modulus) of Siloxane-Hydrogel Soft Contact Lenses (CLs) of two different materials, Lotrafilcon B of Air Optix (AO) and Asmofilcon A of PremiO (P), after use (worn CLs). Thus, the results obtained with both materials will be compared, as well as the changes produced by the wear at a nanoscopic level. The results show significant changes in the topographic and mechanical characteristics of the CLs, at a nanoscopic level, due to wear. The AO CL show values of the topographic parameters lower than those of the P CL after wear, which correlates with a better comfort qualification given to the former by the wearers. A significant correlation has also been obtained between the adhesion values found after the use of the CLs with tear quality tests, both break-up-time and Schirmer.

Keywords: worn siloxane-hydrogel contact lenses, Atomic Force Microscopy-Peak Force mode, surface topography, Young modulus, adhesion

Highlights

The Peak Force mode of AFM provides topographic and mechanical properties.

The worn CLs show significant changes in respect to the un-worn CLs.

The AFM results can be correlated with clinical test results.

The elastic modulus is not the only parameter to consider regarding CL comfort.

1. Introduction

Contact lenses (CLs) are in common use for visual defect corrections and, as they are in contact with the eye, the biocompatible properties of the materials used and their topographic and mechanical characteristics are of major importance in order to fulfil the ocular tissues' requirements, and not provoke ocular damage. Manufacturers of CLs try to obtain materials with low elastic modulus values and good permeability to oxygen, as well as a good level of wettability, all attributes necessary for wearing contact lenses healthily and in comfort. In this way, new siloxane-hydrogel (Si-H) materials have been created [1] and manufacturers have continued producing new materials to improve their characteristics.

Topographic and especially mechanical characteristics of the CL surface at a nanometric level have been less studied. To provide these studies, the Atomic Force Microscopy (AFM) technique is useful [2] and in an earlier work Baguet et al. [3] investigated lachrymal component accumulation on a soft CL surface after wear using AFM. In this study the CL was of a hydrogel material and the work presents AFM images and values of roughness, R_a and R_{max}, and identified two types of deposits. Deposits of lipids and proteins can provoke changes in roughness, elastic modulus and adhesion, and can influence the comfort and the cleaning of the CLs. Bathia et al. [4] also used AFM to observe the topography of several hydrogel CLs, and Goldberg et al. [5] used AFM in combination with scanning electron microscopy (SEM) and optical microscopy to study the deposit formation on hydrogel CLs.

From the development of the new siloxane-hydrogel (Si-H) materials, studies of this type of contact lenses were made. Studies on worn Si-H CLs and using the AFM technique are those of Gonzalez-Meijome et al. [6], Lira et al. [7], and Bettuelli et al. [8]. Other studies on un-worn Si-H CLs are those of Guryca et al. [9] and Giraldez et al. [10], but none of them used the Peak Force Quantitative Nanomechanics mode to obtain nanomechanical properties, such as adhesion or the Young modulus, also known as elastic modulus. Gonzalez-Meijome et al. [6] found that the mean roughness increased in all the worn CLs studied, in respect to the un-worn state, but the degree of change depends on the Si-H material. Lira et al. [7] point to changes in the appearance of the surface of the CL after wear, as well as changes in R_a, R_q and R_{max} roughness values. Changes are greater for Galyfilcon A, followed by Balafilcon A, while for Lotrafilcon B they were less significant. The researchers concluded that the surface treatment of Si-H CLs can play a role in the prevention of a significant increase in roughness. The surface properties and wear performance of a new Si-H CL material have been studied by Bettuelli et al. [8] using AFM. These authors found an increase in R_q values due to wear, with two types of morphologies that they call sharp type and smooth type, the former presenting higher values for topographic parameters (R_q, R_{ku}, R_{sk}). They also found a correlation between the change in the clinical test OSDI (ocular-surface-disease index) before and after wear and the lens surface characteristics. Studies using AFM on worn CLs were also done with other materials than Si-H. For instance, Bruinsma et al. [11] used Rigid Gas Permeable lenses (RGP Boston-ES). They concluded that surface properties of RGP lenses change slightly during the first 10-50 days of wear, but end-stage lenses all had increased surface roughness, concurrent with increased bacterial adhesion.

In a previous paper [12] the applicability of the Peak Force mode of AFM to study un-worn CL was proved. Thus, the main goal of this paper is to obtain the adhesion and the Young modulus of two worn Si-H CL made of different materials using the Peak Force mode, and to compare these values. The treatment also provides topographic images and statistical values of roughness. The work provides these values for two different CL materials using the same technique and procedure, which affords a higher level of confidence in comparing them. These values are compared with those obtained in un-worn CLs. Clinical tests of the tear film of the subjects of the study were done. A clinical comfort test of the subjects wearing the CLs was also done, and the results were correlated with those obtained from the AFM study. A clinical investigation of siloxane-hydrogel CLs was previously performed by Lakkis and Vincent [13], but no correlation was established there with an AFM study.

2. Materials and methods

Soft CLs of two different siloxane-hydrogel materials, Asmofilcon A commercialized as PremiO (P) by Menicon and Lotrafilcon B commercialized as Air Optics (AO) by CIBA Vision, were studied. The basic characteristics of the materials are presented in Table S1 of supplementary material. PremiO CLs were used during a period of 15 days, while Air Optix CLs were used during 30 days, according to manufacturers' specifications, and then stored for AFM characterization. Care and cleaning of the CLs during wear were those suggested by the manufacturers, and instructions were explained to the wearers. CLs should be used a minimum of 6 hours per day and a maximum of 10 hours per day, and should never be used during sleep. One P CL and one AO CL, one in each eye, were used for each wearer, in a blind trial, that is, the wearer did not know which type of CL he was wearing in each eye. The CLs were stored and cleaned using Hidrohealth SiH solution, from DISOP.

At the end of the wearing period, the CLs were removed from the eye and stored in a preservative saline solution for a further study using AFM. No more than 3 days passed between removal and AFM characterization.

Subject selection and Clinical tests

For the study, a population of 10 subjects (20 eyes) was selected from young people wearing CLs (between 21 and 27 years old). Five of the subjects were men and five were women. An inclusion criterion for selection was to have normal ocular conditions (no dry eye problem, no ocular damage). A visual exam, an ocular exploration and ocular tests such as Schirmer, break-up-time (BUT), non-invasive-BUT (NIBUT), height of lachrymal meniscus and Ocular Surface Disease Index (OSDI) were performed on all subjects before CL fitting. The cut off values are: 5.5 mm for the Schirmer test [14], 0.1 mm for lachrymal meniscus [15,16], 10 s for NIBUT test [17], 5 s for BUT test [18] and score of 21 for the OSDI test [19]. In addition, a comfort test was completed after CL wear. The comfort test simply consists in asking the wearer to give a score between 0 and 5, being 0 the best comfort and 5 the worse comfort. The statistical analysis was done with common statistical programs, and the significance of the hypothesis was established with the *P* value ($P<0.05$ indicating statistical significance).

Sample preparation for AFM

The samples were prepared taking the CL from the CL case, washing it gently with water, cutting a small piece of the central part, fixing it with glue on a clean Teflon support mounted on a magnetic plate, and placing 3-4 drops of saline solution (0.9% NaCl) on top of the sample. Prepared samples were left 30 min before imaging so as to ensure hydration equilibrium. Finally, the sample was mounted in a liquid cell for the AFM experiment.

AFM technique and equipment

AFM was done in Peak Force mode using Multimode 8 and Nanoscope V electronics (Bruker). The study was carried out in liquid using tips of silicon nitride of low spring constant (0.1-1 N/m) and resonant frequency in liquid between 2-20 kHz. The selected holder was for scanning small areas. The room was maintained at controlled ambient temperature (20ºC) and humidity (50%). For image treatment Nanoscope Analysis v1.2 software was used. The equipment was calibrated prior to the sample measurements with the thermal noise method and using a mica surface. This calibration allows us to obtain a precise spring constant and, consequently, quantitative values of the Young modulus. The Peak Force mode permits us to obtain nanomechanical properties such as Young modulus and adhesion, and at the same time a topographic image of the surface sample. The Peak Force mode covers a wide range of Young modulus values, between 1 MPa and 50 GPa, and adhesion values, between 10 pN and 10 μN. The Peak Force mode operates in Tapping mode, controlling the maximum applied force and performing force curves at each point.

For the topographic analysis, four parameters have been used [20]: mean roughness (R_a), root-mean-square roughness (RMS or R_q), skewness (R_{sk}) and kurtosis (R_{ku}). More details were reported in reference [12].

The Young modulus, or elastic modulus, Y_m, measures the response, the deformation, of a material when a force per unit area is applied. Adhesion, Adh, or adhesion force, is a measure of the binding interaction between two material surfaces as a result of intermolecular forces. More details were reported in reference [12].

3. Results and discussion

3.1 Results

Clinical tests were done on the subjects of the study and the results (see Table S2 in supplementary material) indicate that none of the subjects present dry eye, according to the initial requirements. On the other hand, no significant differences (P>0.05) were observed between the two eyes. P values for the different tests were: P=0.068 for Schirmer, P=0.343 for NIBUT, P=0.273 for BUT and the meniscus values are the same for both eyes.

The characteristics of un-worn P and AO CLs were studied in a previous work [12], and they are summarized in Table 1 for the case of being immersed in saline solution.

Table 1. Mean values and standard deviations, in (), for the un-worn PremiO (P) and Air Optix (AO) CLs, in saline solution.

CL	R_a (nm)	R_q (nm)	R_{sk}	R_{ku}	Y_m (MPa)	Adh (nN)
P	4.1 (0.9)	5.03 (0.04)	0.65 (0.50)	7.5 (3.1)	3.0 (4.7)	0.02 (0.03)
AO	4.5 (2.2)	6.4 (4.7)	0.6 (2.3)	16.1 (16.5)	10.8 (17.0)	0.11 (0.13)

Figure 1 shows the AFM topographic images of several worn CLs, corresponding to several subjects, for both Si-H CL types. In Figures 2 and 3 the Young modulus and adhesion maps of these lenses, respectively are also shown. In Figure S1 of the supplementary material, images of the un-worn CLs have also been included for comparison. Tables 2 and 3 present the values for the topographic and nanomechanical parameters of both worn P and AO CLs, respectively, obtained from the analysis of the AFM images. Table 4 contains the results of the comfort test. The cells in the table contain the number of eyes and the percentage, in (), that deserves the giving score from 0 to 5. The last column in Table 4 contains the total number of eyes.

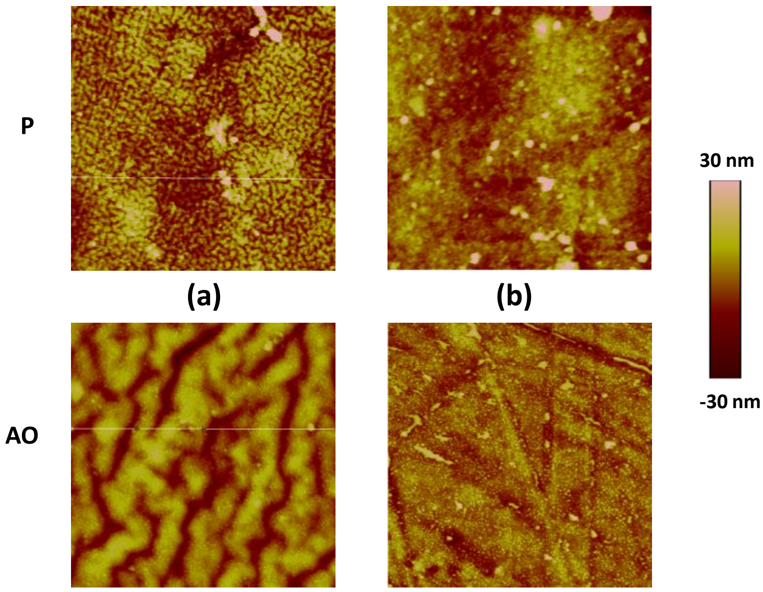

Figure 1. AFM topographic images of the surface of PremiO (upper row) and Air Optix (lower row) worn CLs corresponding to subject 2 (a) and subject 5 (b). The image area is 5 μm x 5 μm. For image AO (a) the colour bar is from -90 to 90 nm.

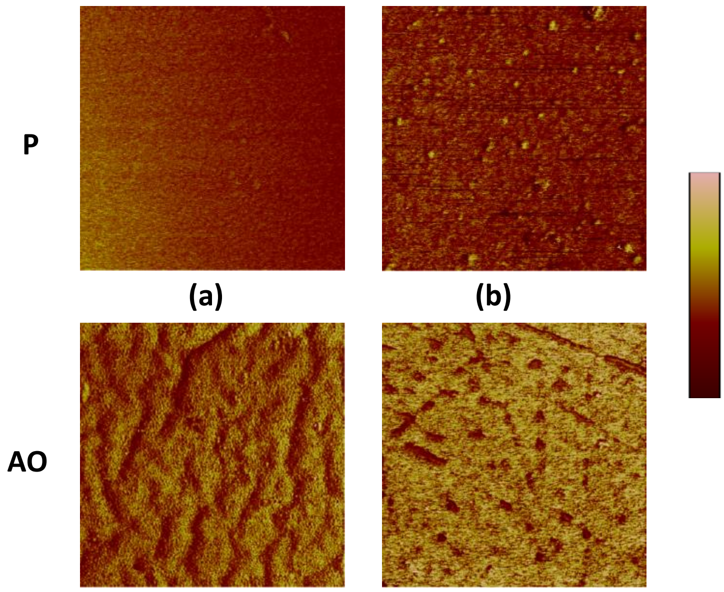

Figure 2. Young modulus maps for PremiO (upper row) and Air Optix (lower row) of worn CLs corresponding to subject 2 (a) and subject 5 (b). Images 5 μm x 5 μm. Colour bar from 0 to 4 MPa for P (a) and (b), from 0 to 20 MPa for AO (a), from 0 to 60 MPa for AO (b).

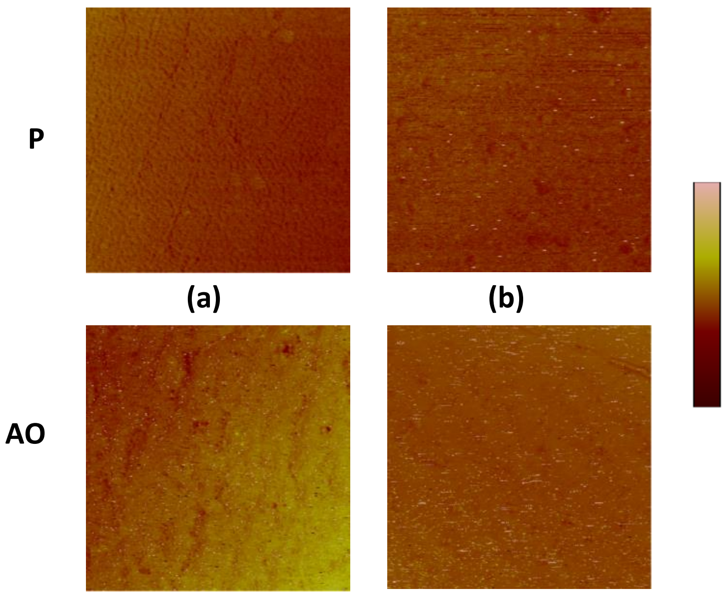

Figure 3. Adhesion maps for PremiO (upper row) and Air Optix (lower row) of worn CLs corresponding to subject 2 (a) and subject 5 (b). Images 5 μm x 5 μm. Colour bar from -0.3 to 0.6 nN for P (a), P (b), from 0 to 2 nN for AO (a), from -2 to 2 nN for AO (b).

Table 2. Values from the topographic and mechanical analysis for worn PremiO CLs.

PremiO CL	R_a (nm)	R_q (nm)	R_{sk}	R_{ku}	Y_m (MPa)	Adh (nN)
S1	4.7	8.3	6.7	92	0.9	0.1
S2	7.4	9.8	0.9	8.9	0.8	0
S3	20	42.3	5.2	40.1	1.1	0.1
S4	82.2	104	0.2	3.1	4.5	0.5
S5	5.7	7.8	1.3	8.3	1.4	0.1
S6	4,4	6	1.7	14.6	1.2	0.2
S7	211	261	-0.2	2.9	1	0.2
S8	35.9	67.2	3.9	21.6	1.1	0.02
S9	120	143	0.3	2.3	2	0.04
S10	8	20	6.5	59.2	1.1	0.08
Mean	49.9	66.9	2.6	25.3	1.5	0.1
Confidence Interval (95%)	49.9±39.9	66.9±47.9	2.6±1.6	25.3±17.3	1.5±0.6	0.1±0.08

Table 3. Values from the topographic and mechanical analysis for worn Air Optix CLs.

Air Optix CL	R_a (nm)	R_q (nm)	R_{sk}	R_{ku}	Y_m (MPa)	Adh (nN)
S1	34.7	44.9	0.3	3.7	4.4	3.9
S2	19.2	23.7	-0.6	3	16.5	1.4
S3	24.8	35.3	1.5	9.6	10.4	3.5
S4	27.6	35.1	-1	4	15.5	0.4
S5	4.7	6.1	0.5	4.4	34.8	0
S6	33	40.6	0.5	2.6	9.2	0.5
S7	32.9	43.2	-0.1	4.6	14.6	0.3
S8	3.6	4.7	1.3	10.7	25.4	0.6
S9	113	174	1.6	9	22.7	0.5
S10	30.4	39.1	-1	4	4.3	0
Mean	32.4	44.7	0.3	5.6	15.8	1.1
Confidence Interval (95%)	32.4±17.7	44.7±27.6	0.3±0.6	5.6±1.7	15.8±5.6	1.1±0.8

Table 4. Results of the comfort test as a function of the CL.

Score	0	1	2	3	4	5	
P CL	1 (5%)	1 (5%)	1 (5%)	3 (15%)	3 (15%)	1 (5%)	10 (50%)
AO CL	1 (5%)	4 (20%)	3 (15%)	1 (5%)	1 (5%)	0 (0%)	10 (50%)
Sum	2 (10%)	5 (25%)	4 (20%)	4 (20%)	4 (20%)	1 (5%)	20 (100%)

3.2 Discussion

Comparing the topographic images of worn CLs with those of un-worn CLs (Figure 1 and Figure S1), changes in the topography of worn CLs can be seen, which depend on the subject and on the type of CL. The presence of small deposits is observed but in some cases, as in the case shown in Figure 1 (a) lower row for an AO CL, the change is very important and a notable deposit has been formed that changes the original topography of the CL (see also Figure 4). For worn P CLs there were no problems with the engagement in Peak Force mode, which could be due to the modification in the surface characteristics as a consequence of the biofilms formed on the surface during use.

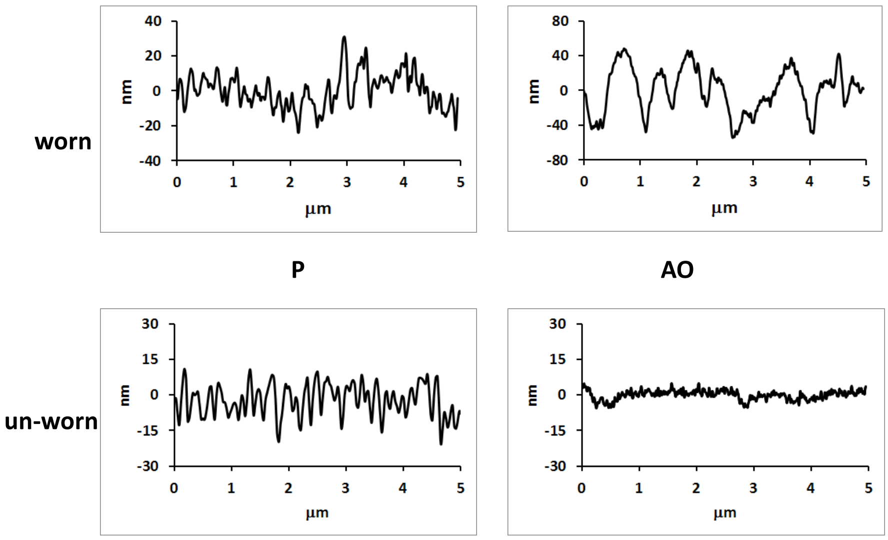

Figure 4. Profiles for AFM topographic images in Figure 1(a) and Figure S1, corresponding to worn and un-worn P and AO CLs.

The analysis of the values of worn CLs for all subjects (Tables 2 and 3) indicates that for the P CLs there are notable differences with respect to the un-worn CLs ($P<0.05$), the values of R_a, R_q, R_{sk} and R_{ku} being higher while the values of Y_m and Adh are lower. For the AO CLs the values of R_a, R_q and Adh are higher for the worn CLs, the values of Y_m are lower, while the values of R_{sk} and R_{ku} are not significantly different ($P>0.05$). Comparing both types of CLs, the worn P CLs present, on average, higher values for the topographic parameters in respect to the worn AO CLs, while the contrary occurs with the nanomechanical properties. In the particular case of the Young modulus the un-worn AO CLs already presented a notable higher value than the un-worn P CLs, and this tendency has not been changed by use.

It is also observed that in AO CLs presenting a notable deposit, which changes the original topography of the CL, the value of Y_m is decreased to a higher degree than in those without a notable deposit. Consequently, as the Peak Force mode works on a nanometric layer of the surface, when a notable deposit is formed, the nanomechanical tests not only inform of changes in the surface but also provide us with the properties of the formed deposit.

In tables 2 and 3 a notable standard deviation is observed, especially in the topographic values. As the values refer to worn CLs, we can conclude that the resulting values depend strongly on the subjects wearing the lenses. The standard deviations of the topographic parameters are also more important for the P CLs than for the AO CLs, while the contrary occurs for the nanomechanical properties. This indicates that the topography of the worn P CLs is more variable depending on the users' tears, but not the nanomechanical properties, which by the way are always lower for the P CLs.

The results reported on Table 4, determined with the comfort test, indicate that the AO CLs give a better level of comfort than the P CLs. This fact correlates well with the topographic values found for both worn CLs, the values of R_a, R_q, R_{sk} and R_{ku} of the AO CLs being lower than those of the P CLs. Also Lakkis and Vincent [13] worked with two Si-H CLs, named Asmofilcon A of PremiO (P) and Senofilcon A of Acuvue OASYS, and made a clinical investigation. They found good comfort level for both CLs, but with some cases of discomfort especially for the P CLs. This clinical investigation was conducted in a different way to our clinical tests, and for that reason the results of both works are not fully comparable. In the study of Lakkis and Vincent, the subjects had worn the P CLs during the full day (continuous use during 24 hours). In our study the P CLs were used a maximum of 10 hours per day, and were cleaned after each removal. Probably as a consequence of the continuous wear, the subjects in the study of Lakkis and Vincent evaluate the comfort of P CLs better than in our study. The continuous wear could create a layer or deposit on the surface of the CL that improved the initial discomfort of this kind of CLs. In this sense, it seems that this argument is corroborated in our study since the worn AO CLs present values of adhesion higher than those of the P CLs, due to the formation of a more uniform surface layer on the AO CLs. The fact that manufacturers look for materials with a lower Young modulus is not fully supported by the previous results, since the P CLs with lower values of this parameter give more discomfort than the AO CLs. Consequently, other facts such as wettability, adsorption, adhesion, and film and deposit formation contribute to the global comfort of the CLs.

Our results also show a good and statistically significant correlation between the adhesion and the BUT test (r=-0.660, P<0.05) and between the adhesion and the Schirmer test (r=-0.555, P<0.1), for the AO CLs. Lower values of BUT or Schirmer indicate a poor quality of the tear, and consequently facilitate the formation of deposits or layers on the surface of CLs. As commented before, this produces greater adhesion.

Our results for the AO CL can be compared with others reported in the literature. The value of R_a (32.4±17.7 nm) obtained by us is higher than those reported by Lira et al. [7] (4.96±4.1 nm) and by González-Meijome et al. [6] (8.42±4.14 nm). Also, the value of R_q (44.7±27.6 nm) obtained here is higher than those reported by Lira et al. [7] (7.3±5.5 nm) and by González-Meijome et al. [6] (11.59±4.91 nm). In our study the CLs were not washed at the end of the wearing period, only removed from the eye and stored in a preservative saline solution, in contrast to the other works commented. This fact has permitted the preservation of the last surface layer formed on the CL and for that the R_a and R_q values reported here are higher. Our observation that the Lotrafilcon B material (corresponding to the AO CL) presents, on average, fewer changes in topography after wear coincides with previous reported observations [6, 7]. No more values for the other parameters have been found in the literature for the AO CL, it being impossible to make more direct comparisons. On the other hand, no reported values have been found for worn P CLs.

Conclusions

The AFM in Peak Force mode has permitted us to obtain topographic as well as mechanical characteristics of the surface of CLs at the same time. Worn CLs have presented significant changes in respect to un-worn CLs in all the analyzed surface parameters. This study has also found influences of the material used, Asmofilcon A and Lotrafilcon B, on the parameter values obtained after use, and that the Young modulus is perhaps not the most important factor to be considered in the comfort response. Finally, some correlations have been found between clinical tests and the surface characteristics analyzed by AFM, and it can be concluded that the elastic modulus is not the only parameter to consider regarding CL comfort.

Acknowledgments

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Caption Figures

Figure 1. AFM topographic images of the surface of PremiO (upper row) and Air Optix (lower row) worn CLs corresponding to subject 2 (a) and subject 5 (b). The image area is 5 μm x 5 μm. For image AO (a) the colour bar is from -90 to 90 nm.

Figure 2. Young modulus maps for PremiO (upper row) and Air Optix (lower row) of worn CLs corresponding to subject 2 (a) and subject 5 (b). Images 5 μm x 5 μm. Colour bar from 0 to 4 MPa for P (a) and (b), from 0 to 20 MPa for AO (a), from 0 to 60 MPa for AO (b).

Figure 3. Adhesion maps for PremiO (upper row) and Air Optix (lower row) of worn CLs corresponding to subject 2 (a) and subject 5 (b). Images 5 μm x 5 μm. Colour bar from -0.3 to 0.6 nN for P (a), P (b), from 0 to 2 nN for AO (a), from -2 to 2 nN for AO (b).

Figure 4. Profiles for AFM topographic images in Figure 1(a) and Figure S1, corresponding to worn and un-worn P and AO CLs.

Caption Tables

Table 1. Mean values and standard deviations, in (), for the un-worn PremiO (P) and Air Optix (AO) CLs, in saline solution.

Table 2. Values from the topographic and mechanical analysis for worn PremiO CLs.

Table 3. Values from the topographic and mechanical analysis for worn Air Optix CLs.

Table 4. Results of the comfort test as a function of the CL.

Supplementary material

Table S1 presents the basic characteristics reported by manufacturers for the CLs used.

Table S1. Characteristics of the different contact lenses used in the study provided by manufacturers. Dk: oxygen permeability.

	PremiO	Air Optix
Name	Asmofilcon A	Lotrafilcon B
Manufacturer	Menicon	CIBA Vision
FDA Group	I	I
% water	40	33
Surface treatment	Nanogloss™	Plasma polymerization
Contact angle	27	78
$Dk \cdot 10^{11}$ $(cm^2/s) \cdot (mlO_2/ml \cdot mmHg)$	129	110
Young modulus (MPa)	0.9	1.2

Table S2 presents the clinical results of the ten subjects of the study. For the Schirmer test all subjects present values higher than 5.5 mm, for lachrymal meniscus measurement all subjects present values higher than 0.1 mm, for NIBUT test all subjects present values higher than 10 s, for BUT test all subjects present values higher than 5 s and for the OSDI test all subjects present values lower than 21.

Table S2. Values of the clinical tests. Sc: Schirmer, Me: height of lachrymal meniscus, BUT: Break-up time, NIBUT: non-invasive BUT, OSDI: Ocular Surface Disease Index. L: left eye, R: right eye.

Subject	Sc L (mm)	Sc R (mm)	Me L (mm)	Me R (mm)	NIBUT L (s)	NIBUT R (s)	BUT L (s)	BUT R (s)	OSDI
S1	9	8	0.15	0.15	11	10	7	6	0
S2	13	12	0.2	0.2	14	15	9	9	2.1
S3	8	8	0.15	0.15	12	10	8	6	2.1
S4	16	15	0.2	0.2	10	12	7	8	12.5
S5	19	19	0.2	0.2	11	11	8	8	6.3
S6	8	10	0.2	0.2	15	12	9	7	10.4
S7	28	26	0.3	0.3	18	16	12	10	0
S8	19	16	0.2	0.2	11	11	7	7	6.3
S9	7	6	0.15	0.15	11	10	8	7	0
S10	30	28	0.3	0.3	15	16	9	11	20.8

Fig. S1 presents the AFM topographic images, Young modulus and adhesion maps of un-worn P and AO CLs.

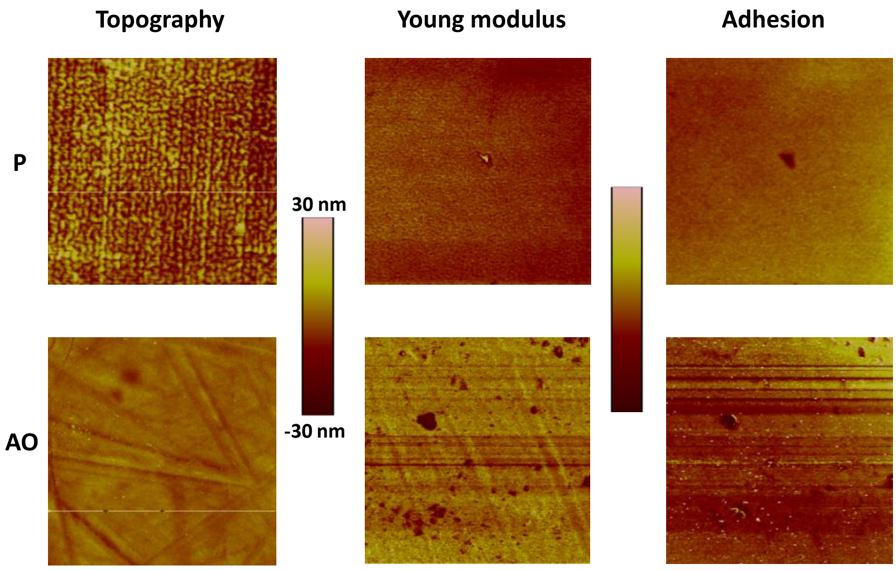

Figure S1. AFM topographic images, Young modulus and adhesion maps of P and AO un-worn CLs. Images 5 µm x 5 µm. Colour bar from 0 to 20 MPa for P, from 0 to 60 MPa for AO, from 0.6 to 2.2 nN for P, from -0.3 to 0.6 nN for AO.