Analysis of Surface Mechanical Properties of Unworn and Worn Silicone Hydrogel Contact Lenses Using Nanoindentation with AFM

González-Méijome JM[1], Almeida JB[1] and Parafita MA[2]

[1]Clinical & Experimental Optometry Research Lab, Department of Physics (Optometry), School of Sciences, University of Minho, Braga, Portugal
[2]Department of Surgery (Ophthalmology), School of Optics and Optometry. University of Santiago de Compostela. Spain

Introduction

Mechanical interaction between soft contact lenses (SCLs) and the ocular surface, particularly at the corneal level is one of the main critical concerns in modern contact lens material development. It is already known the close relationship between first generation Si-Hi materials and changes in the anterior corneal surface as superior epithelial arcuate lesion (SEAL), corneal flattening or debris in the post-lens space.[1-5] Beyond the inherent characteristics of the materials due to their content in siloxane moieties, significant changes regarding surface dehydration and microtopography have been identified[6;7], and these changes could influence the mechanical behavior of worn lenses.

However, the significance of the mechanical behavior of a CL material could be also reflected at the cellular level deep in the corneal stroma, not in direct contact with the contact lens surface. For example, Kallinikos *et al*.[8] have observed a loss of keratocytes in the corneal stroma with the use of Si-Hi contact lenses. The authors hypothesize that the mechanical stimulation of the corneal surface by the presence of the CL is able to release inflammatory mediators able to induce keratocyte apoptosis. The potential implication of mechanical stimulation of the corneal surface, due to the physical presence of a CL, in the release of inflammatory mediators as the likely cause of reduced keratocyte density or keratocyte redistribution associated with lens wear is being investigated.[8-10] On a similar line of thought but probably with different significance, Ladage *et al*.[11] have reported the proliferation of keratocytes under the corneal surface affected by the presence of post-lental debris in the form of mucin balls in rabbit corneas. This mechanism could also be the reflection of the mechanical impact of the mucin balls compressed under the contact lens and could be interpreted as a form of corneal response to localized mechanical interaction between the CL and the external epithelium.

Different methods have been used to evaluate the mechanical properties of contact lenses as will be later discussed. However, little has been reported on the use of atomic force microscopy (AFM) with this regard, and to the knowledge of the authors no reports on the potential changes in the mechanical properties of contact CL as measured with other techniques are available. We hypothesize that changes in the lens surface due to dehydration phenomena, deposits, and other kinds of spoliation could induce significant changes in the mechanical behavior of the CL surface. The AFM with its high resolution capability could be able to measure such changes at the nanoscale level.

The goal of this study was to evaluate the mechanical properties at the surface of several silicone hydrogel contact lenses before and after being worn on a daily wear schedule. These properties had been analyzed by nanoindentation with AFM and should not be interpreted as the classical mechanical properties reported by the industry concerning to the properties of the bulk of the lens usually obtained with macroscopic indenters or with instruments that induce a deformation on the whole sample, measuring the force needed to induce such a deformation, at a macroscopic scale.

Material and Methods

Unworn and Worn samples of Air Optix Night & Day™ (lotrafilcon A) and Air Optix™ (lotrafilcon B) from Ciba Vision (Duluth, VA), Purevision™ (balafilcon A) from Bausch & Lomb (Rochester, NY), Acuvue Advance™ (galyfilcon A) from Johnson & Johnson, (Jacksonvile, FL), and Biofinity™ (comfilcon A) from Coopervision (Irvine, CA) were used in this study. Other technical details are given in *table 1*. Ten samples of each material were used. All lenses had refractive power between -2.50 and -3.50 diopters (D). All lenses were worn for 30 days on a daily wear basis except for Acuvue Advance™ that was worn only for 15 days as recommended by the manufacturer. Lenses were worn for an average of 10-12 hours per day, followed by extraction and care including rub and rinse step and overnight disinfection. The same multipurpose solution (ReNu Multiplus, Bausch & Lomb, Rochester, USA) was used for daily care purposes with all lenses. No additional cleaning or disinfecting systems were used. Results were compared against brand new Si-Hi CLs equilibrated for at least 72 hours in the same solution used for the worn lenses, in order to wash out the original solution. All unworn samples had a power of -3.00 D.

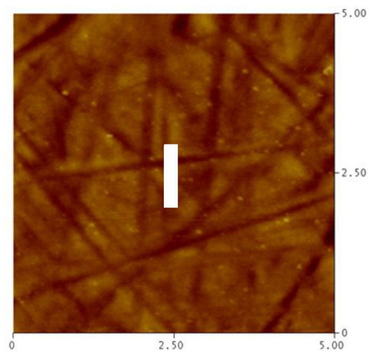


Figure 1. Microtopographic image of the contact lens surface with the area of indentation highlighted as white rectangle (0.100 x 0.500 μm).

Table 1. Technical details of Si-Hi contact lenses used in the study

	Air Optix Night & Day	Acuvue Advance	Air Optix	Purevision
USAN†	Lotrafilcon A	Galyfilcon A	Lotrafilcon B	Balafilcon A
Material‡ **(main monomers)**	TRIS+DMA+siloxane monomer	HEMA+PDMS+DMA+PVP	TRIS+DMA+siloxane monomer	TRIS+NVP+TPVC +NCVE+PBVC
Surface Treatment	Plasma Coating	No	Plasma Coating	Plasma Oxidation
Dk	140	60	110	99
t_c (mm @-3.00)	0.08	0.07	0.08	0.09
Dk/t (barrer/cm)	175	87	138	110
H_20 (%)	24%	47%	33%	36%
FDA	I	I	I	III
Power (D)	-3.00	3.00	-3.00	3.00
Diameter (mm)	13.8	14.6	14.2	14
Base Curve (mm)	8.6	8.7	8.6	8.6
Schedule/ Replacement	CW-DW/Monthly	DW/Bi-weekly	DW/Bi-weekly	CW-DW/Monthly

†USAN: United States Adopted Name Council; CW: continuous wear; DW: daily wear.

‡DMA: *N,N*-dimethl acrylamide; HEMA: 2-hydroxyethyl methacrylate; NCVE (*N*-carboxyvinyl ester); PC: phosphorylcholine; TRIS: 3-methacryloxy-2-hydroxypropyloxy propylbis (trimethylsiloxy)methylsilane; TPVC (tris-(trimethylsiloxysilyl) propylvinyl carbamate); PBVC (poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate])

Ten samples of five silicone hydrogel materials were used in order to measure their mechanical properties in response to nanoindentation with AFM in Contact Mode before and after being worn by indentation analysis in Tapping Mode according to the experimental protocol previously reported[12]. This process has demonstrated a high repeatability in qualitative terms.[13] *Figure 1* shows a 25 μm^2 micro-topographic image with the approximate points of the indentation highlighted (of 10 points over a square matrix of 0.5 x 0.1 μm).

Values of Young modulus for worn lenses were compared against those obtained for 10 samples of the same unworn materials (-3.00 D). Technical details of the lenses used in this study are listed in *table 1*. Purevision lens (balafilcon A) has been recently improved, including a slight change in the modulus of the material. However, all lenses used in the study (worn and unworn samples) corresponded to the older design.

Statistical analysis was performed using SPSS Software v.15.0 (SPSS Inc, IL). Normal distribution of variables was previously assessed by mean of the Kolmogorov-Smirnov test. Due to the small sample size and non normal distribution of data, Mann-Whitney non-parametric test for independent samples was carried out in order to compare mean values of Young Modulus between worn and unworn samples. Comparisons involving normally distributed variables were performed using independent samples T-test. In this case, Levene test was used to assess equality of variances. The level of statistical significant was set for $\alpha = 0.05$.

Results

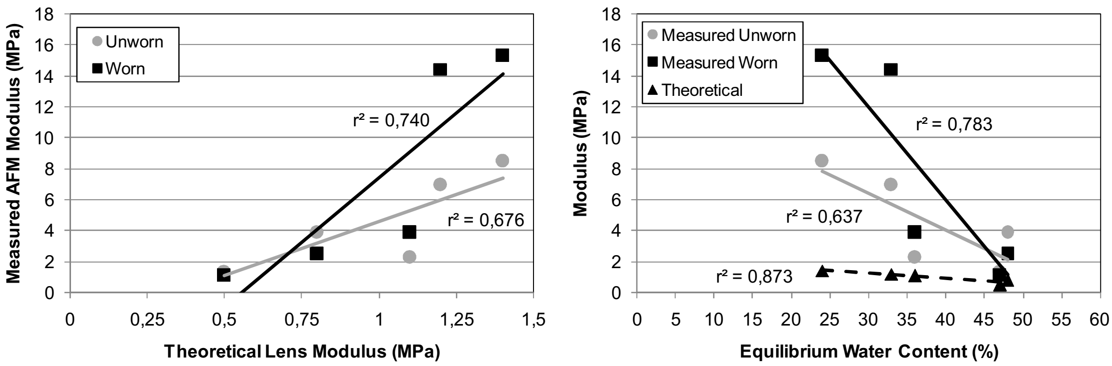
Table 2 present the average modulus of unworn and worn samples of the five materials. All materials displayed statistically significant differences between both groups suggesting an effect of lens wear on the mechanical properties of the surface. However, the effect is not the same for all lenses as can be seen in *figure 7*. The lenses with the higher EWC displayed a trend towards a decrease in modulus, while the opposite trend is observed in the remaining samples. Moreover, these changes are highly evident for the less hydrated samples lotrafilcon A and lotrafilcon B.

Table 2. Comparison of values of modulus for worn and unworn samples of the same CL materials. Units are MPa

Contact Lens	Unworn Samples (n=10)	Worn Samples (n=10)	Statistical Significance‡
Air Optix Night & Day *(lotrafilcon A)*	8.48 ± 1.08	15.28 ± 5.61	0.012†
Purevision *(balafilcon A)*	2.25 ± 0.19	3.85 ± 0.19	0.005†
Air Optix *(lotrafilcon B)*	6.96 ± 0.32	14.44 ± 3.16	<0.001‡
Acuvue Advance *(galyfilcon A)*	1.42 ± 0.04	1.06 ± 0.01	0.001‡
Biofinity *(comfilcon A)*	3.85 ± 0.11	2.47 ± 0.15	<0.001†

† Independent Sample T-Test; ‡ Mann-Whitney non-parametric test for independent samples

Values of modulus measured by nanoindentation in the present work for unworn and worn samples are significantly different form those reported from manufacturers. However, those values are highly correlated (Fig, 2a). Values of modulus measured before and after lens wear are also highly correlated with equilibrium water content. Furthermore, this correlation increased for worn lenses so that the lower the water content, the higher the increase in modulus after wear (Fig, 2b).



(A) (B)

Figure 2. Correlations between theoretical modulus as reported by the manufacturers using tensometers and measured by nanoindentation in the presnt work (A) and relationship of measured and theoretical modulus with equilibrium water content of the material (B).

Discussion

It is accepted that the singular mechanical properties of the Si-Hi contact lenses of the first generation are responsible, or at least important predisposing factors to explain several changes at the ocular surface level[14] including superior epithelial arcuate lesions,[1;15] mucin balls formation,[16] or slight topographic and refractive changes in the anterior corneal surface.[4;5] New Si-Hi materials have tried to address this evidence by lowering the modulus in order to reduce the impact of these changes and improve initial comfort. This is the case for second generation materials as galyfilcon A and senofilcon A (Johnson & Johnson, Jacksonville, FL), lotrafilcon B (Ciba Vision, Duluth, GA) and comfilcon A (Coopervision, Virginia). Recently, the lens made of balafilcon A has also been recently improved by the manufacturer (Bausch & Lomb, Rochester) in order to improve comfort.

Despite the important relevance of the Young modulus, it is presently unknown if the mechanical properties of the contact lenses can change overtime as a result of lens wear or different situations that can cause stress in the material as the use of certain care regimes. It could be hypothesized that rigidity of the CL surface could increase as a result of CL wear due to partial dehydration of the material overtime, an effect that has been observed with conventional hydrogels.[17] However, this assumption is not well supported because galyfilcon A and comfilcon A that would be expected to suffer more dehydration as a result of their higher EWC demonstrated in fact a lower modulus in worn samples. However, it is unknown the effect that could have the materials deposited on the contact lens surface on the mechanical relationship between the CL and the more superficial histological layers of the ocular surface, the corneal and conjunctival epithelium or even the underlying stroma. On this regard, the main concern with Si-Hi materials is with lipid deposits[18] and the impact of this contaminants on the contact lens surface mechanical properties are also unknown.

An increase in the rigidity of the contact lens, with reflection at the surface could be due to molecular changes at this level with reorientation of the hydrophobic elements to the outer surface that could lead to a dryer surface and consequently a more rigid interaction with the corneal epithelium. However, some authors argue that CL wear and the associated tear deposits can in fact increase the surface wettability of the contact lens, rather than decrease it. In this case, the nanoindentation procedure could reflect the mechanical properties of the deposits rather than the polymer. However, considering the depth of penetration in nanoindentation, that reaches 150 to 200 nm, it is hard to believe that deposited layers on disposable contact lenses reach such a thicker structure if properly care is taken by the patient according to the recommendations of the clinician. Moreover, our previous results showed how contact lens topography still reflect the surface issues that characterize new lenses, suggesting the thin structure of films deposited on the contact lens surface. For this reason, we could hypothesize that we are in fact measuring the properties of the surface of the contact lens, although an effect of the films deposited on it or those that had penetrated into the more superficial pores cannot be discarded. Furthermore, the presence of lipid deposits on the CL surface makes that less hydrophilic radicals are available to bind water at the surface, so, the presence of deposits itself certainly affect the mechanical response of the lens surface, and the CL surface should be considered as the combination of the CL surface properties (substrate for deposit formation) but also as the films deposited on it as well.

This study has revealed an increase in the elastic modulus of some Si-Hi CLs after being worn. This effect has been particularly evident in lenses with plasma treatment on their surfaces, but we cannot establish a direct link between both facts until further investigations could be conducted. However, this increase could be relevant because of the potential implications of mechanical presence of the CL on the ocular surface to explain some physiological facts at the level of the histology of the cornea.

It has been demonstrated that the presence of CL is able to induce an increase in concentration of Langerhans cells in the corneal epithelium of guinea pigs, and this effect has been attributed in part to a mechanical effect caused by the presence of the CL.[19] Also, Efron *et al.* in different studies conducted using confocal microscopy supported the mechanical aetiological factor to explain the lower keratocyte density in the stroma of corneas wearing Si-Hi CL.[8-10] This effect of the physical presence and the enhanced mechanical effect of certain CLs in the ocular surface has been postulated as an aetiological factor in the loss of keratocyte in the corneal stroma during lens wear had also been suggested in earlier investigations.[8;20] This fact could also be related with the stromal thinning[21] and overall corneal thinning[22] observed in long-term CL wearers. Long-term thinning of the stroma has been evidenced in low-Dk SCL wearers by modified optical pachometry (*unpublished data* from González-Méijome and Perez). The proof for this link will need to demonstrate this effect in long-term Si-Hi CL wearers, as the thinning effect on the cornea with low-Dk CL materials has been attributed to hypoxia in low-Dk CLs.

Another area of interest related with the mechanical impact of CL is contact lens giant papillary conjunctivitis (CLGPC). This is a relatively common contact lens complication and despite deposits on the surface are frequently considered as the etiological cause, and particularly denaturized protein deposits, it has also been suggested that mechanical trauma caused by the contact lens to the upper tarsal conjunctiva could be also an important factor to develop CLGPC in CL wearers. Donshik[23] stated that in addition to the chemistry of the CL polymer, other factors such as edge design and surface properties are also important variables in the pathophysiology of CLGPC. If we consider modulus as a potential factor to induce CLGPC in contact lens wearers in addition to the presence of denaturized lisozyme which has been considered as one of the main factors for the occurrence of this adverse response, we can find both entities converging in Si-Hi materials. The mechanical effect of CLs has also been pointed as a causative effect of CL-related corneal infiltrates.[24]

It is accepted that protein deposition on SCL is a material dependent process.[25] The ionic nature of FDA group IV containing methacrylic acid (MA) significantly adhere more proteins (particularly lisozyme) than copolymers of HEMA with NVP or acrylamide,[26] and this has been explained on the basis of an electrostatic affinity between the anionic material and the positively charged lysozyme at physiological pH.[27] Furthermore, the level of ionicity in the CL surface seems to be related with the amount of proteins deposited.[28] Despite higher levels of lipid deposits and lower levels of protein deposits have been found in Si-Hi materials, it has been also been observed that these new materials induce a higher degree of lysozyme denaturation.[18] Surprisingly, the higher incidence of lysozyme deposits on ionic materials compared to Si-Hi materials was associated with a lower incidence of denaturation in the same study. Despite lower deposits of protein in Si-Hi materials, the higher proportion of denaturized proteins, could be considered together with the higher modulus of first generation Si-Hi considered as the main factor for CLGPC in Si-Hi materals[29] to explain the increased occurrence of this entity with some Si-Hi materials.[30]

Although we were not able to know which reflection on the mechanical behavior of the CL surface could be expected from different types of deposits deposited on the lens surface, it could be hypothesized that denaturized proteins forming plaques could increase the rigidity of the contact lens surface beyond any level of superficial dehydration. An additional explanation to dehydration will be probably needed to explain the increase in surface modulus found in worn lenses as galyfilcon A and comfilcon A (materials with higher dehydration potential because of their higher EWC) did in fact decrease the modulus.

In summary, surface material changes due to contact lens wear of Si-Hi materials is commonly associated with some degree of increase in surface modulus. The results of the present study could be relevant to understand the mechanisms of certain ocular reactions to lenses covered with proteins, particularly when they are made of Si-Hi materials whose modulus could be increased as a consequence of wear. If these changes are significant enough to induce a higher incidence of papillary conjunctivitis or other reactions with a mechanical component on its etiology is still to be investigated.

Acknowledgments*:* This study was supported in part by a grant from the Science and Technology Foundation (FCT) - Ministry of Science and Superior Education (MCES) under contract 8281/2002 from the European Social Funding granted to JMG-M. We thank M. Planes and J.L. Moya (Polytechnic University of Valencia) for their assistance with atomic force microscopy analysis.

References

[1] Holden BA, Stephenson A, Stretton S et al. Superior epithelial arcuate lesions with soft contact lens wear. *Optom Vis Sci* 2001;78:9-12.
[2] Tan J, Keay L, Jalbert I, Naduvilath TJ, Sweeney DF, Holden BA. Mucin balls with wear of conventional and silicone hydrogel contact lenses. *Optom Vis Sci* 2003;80:291-297.
[3] Dumbleton K, Jones L, Chalmers R, Williams-Lyn D, Fonn D. Clinical characterization of spherical post-lens debris associated with lotrafilcon high-Dk silicone lenses. *CLAO J* 2000;26:186-192.
[4] Dumbleton KA, Chalmers RL, Richter DB, Fonn D. Changes in myopic refractive error with nine months' extended wear of hydrogel lenses with high and low oxygen permeability. *Optom Vis Sci* 1999;76:845-849.
[5] Gonzalez-Meijome JM, Gonzalez-Perez J, Cervino A, Yebra-Pimentel E, Parafita MA. Changes in corneal structure with continuous wear of high-Dk soft contact lenses: a pilot study. *Optom Vis Sci* 2003;80:440-446.
[6] Gonzalez-Meijome JM, Lopez-Alemany A, Almeida JB, Parafita MA. Surface AFM microscopy of unworn and worn samples of silicone hydrogel contact lenses. *J Biomed Mater Res B Appl Biomater* 2008;84:(in press).
[7] Gonzalez-Meijome JM, Lopez-Alemany A, Almeida JB, Parafita MA. Dynamic *in vitro* dehydration patterns of unworn and worn silicone hydrogel contact lenses. *J Biomed Mater Res B Appl Biomater* 2008;(*in press*).
[8] Kallinikos P, Efron N. On the etiology of keratocyte loss during contact lens wear. *Invest Ophthalmol Vis Sci* 2004;45:3011-3020.
[9] Kallinikos P, Morgan P, Efron N. Assessment of stromal keratocytes and tear film inflammatory mediators during extended wear of contact lenses. *Cornea* 2006;25:1-10.
[10] Efron N. Contact lens-induced changes in the anterior eye as observed in vivo with the confocal microscope. *Prog Retin Eye Res* 2007;26:398-436.
[11] Ladage PM, Petroll WM, Jester JV, Fisher S, Bergmanson JP, Cavanagh HD. Spherical indentations of human and rabbit corneal epithelium following extended contact lens wear. *CLAO J* 2002;28:177-180.
[12] Gonzalez-Meijome JM. Objective analysis of properties and material degradation in contact lens polymers using different techniques. *PhD Thesis: University of Minho* 2007;Available at: http://hdl.handle.net/1822/6757.
[13] Gonzalez-Meijome J, Lopez-Alemany A, Jr., Almeida JB, Parafita MA. Consistency of Surface Analysis of Silicone-Hydrogel Contact Lens Polymers with Atomic Force Microscopy. *Invest Ophthalmol Vis Sci* 2006;47:2387.
[14] Tighe B. Silicone hydrogels: structure, properties and behaviour. In: Sweeney DF, ed. *Silicone hydrogels. Continuous-wear contact lenses*. 2nd Edition ed. London: Butterworth-Heinemann; 2004;1-27.
[15] O'Hare N, Stapleton F, Naduvilath T, Jalbert I, Sweeney DF, Holden BA. Interaction between the contact lens and the ocular surface in the etiology of superior epithelial arcuate lesions. *Adv Exp Med Biol* 2002;506:973-980.
[16] Craig JP, Sherwin T, Grupcheva CN, McGhee CN. An evaluation of mucin balls associated with high-DK silicone-hydrogel contact lens wear. *Adv Exp Med Biol* 2002;506:917-923.
[17] Morgan PB, Efron N. Hydrogel contact lens ageing. *CLAO J* 2000;26:85-90.
[18] Jones L, Senchyna M, Glasier MA et al. Lysozyme and lipid deposition on silicone hydrogel contact lens materials. *Eye Contact Lens* 2003;29:S75-S79.
[19] Zhivov A, Stave J, Vollmar B, Guthoff R. In vivo confocal microscopic evaluation of langerhans cell density and distribution in the corneal epithelium of healthy volunteers and contact lens wearers. *Cornea* 2007;26:47-54.
[20] Efron N, Perez-Gomez I, Morgan PB. Confocal microscopic observations of stromal keratocytes during extended contact lens wear. *Clin Exp Optom* 2002;85:156-160.
[21] Holden BA, Sweeney DF, Vannas A, Nilsson KT, Efron N. Effects of long-term extended contact lens wear on the human cornea. *Invest Ophthalmol Vis Sci* 1985;26:1489-1501.

[22] Liu Z, Pflugfelder SC. The effects of long-term contact lens wear on corneal thickness, curvature, and surface regularity. *Ophthalmology* 2000;107:105-111.
[23] Donshik PC. Contact lens chemistry and giant papillary conjunctivitis. *Eye Contact Lens* 2003;29:S37-S39.
[24] Robboy MW, Comstock TL, Kalsow CM. Contact lens-associated corneal infiltrates. *Eye Contact Lens* 2003;29:146-154.
[25] Bontempo AR, Rapp J. Protein and lipid deposition onto hydrophilic contact lenses in vivo. *CLAO J* 2001;27:75-80.
[26] Bohnert JL, Horbett TA, Ratner BD, Royce FH. Adsorption of proteins from artificial tear solutions to contact lens materials. *Invest Ophthalmol Vis Sci* 1988;29:362-373.
[27] Soltys-Robitaille CE, Ammon DM, Jr., Valint PL, Jr., Grobe GL, III. The relationship between contact lens surface charge and in-vitro protein deposition levels. *Biomaterials* 2001;22:3257-3260.
[28] Maissa C, Franklin V, Guillon M, Tighe B. Influence of contact lens material surface characteristics and replacement frequency on protein and lipid deposition. *Optom Vis Sci* 1998;75:697-705.
[29] Dumbleton K. Adverse events with silicone hydrogel continuous wear. *Cont Lens Anterior Eye* 2002;25:137-146.
[30] Maldonado-Codina C, Morgan PB, Schnider CM, Efron N. Short-term physiologic response in neophyte subjects fitted with hydrogel and silicone hydrogel contact lenses. *Optom Vis Sci* 2004;81:911-921.