Atomic force microscopy analysis of the effect of plasma treatment on gas permeable contact lens surface topography

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Abstract

Purpose: Using atomic force microscopy (AFM) to investigate anterior surface topography (AST) in worn and unworn, plasma surface-treated (PST) and untreated (UT) gas permeable (GP) lenses, and influence of surface topography on *in vivo* comfort.

Methods: GP lens AST evaluated with AFM in tapping mode, using an uncoated, 40nm symmetric tip (sampling frequency: 300kHz), at five randomised locations, over a 100μm^2 area, to produce mean average roughness (Ra) and root mean square (RMS) values for each sample. Four unworn lenses (two PST, two UT) were examined (Quasar/Boston EO material). Twenty worn lenses (ten PST, ten UT) of same design and material as unworn lenses collected after 3 months lens wear. General wearing comfort reported by visual analogue scale (VAS) at 3 months visit. For sample preparation, two worn UT GP lenses were divided into four segments; each segment underwent a different lens rinse and drying method.

Results: Unworn: UT lenses had significantly higher mean RMS and Ra values compared to PST (Mann-Whitney, $p<0.05$). Worn: UT Median RMS values were significantly higher than PST (Mann-Whitney, $p<0.05$). Comfort: no correlation found between general comfort and RMS or Ra scores. Sample preparation: Method 4 (purified, distilled water rinse/nitrogen gas dry) produced optimum median RMS and Ra values.

Conclusions: Unworn PST GP lenses had lower Ra and RMS values compared with unworn UT GP lenses. After 3 months wear, PST lenses had smoother surface topographies than UT lenses. No relationship was found between surface topography and lens wear comfort. Sample preparation protocol directly impacts AFM results.

Highlights:

- Plasma-surface treatment reduces roughness of unworn gas permeable contact lenses.
- Benefit of plasma treatment continues for at least 3 months of daily wear.
- No relationship was found between surface roughness and wear comfort.
- A sample preparation protocol was developed to produce repeatable results.

Keywords: gas permeable contact lenses, plasma-treatment, surface roughness, comfort

Introduction

The surface roughness of a device in contact with a living system will influence the biological reactivity of the device with the surface (Hosaka et al, 1983). So, for a contact lens placed on the ocular surface, the lens polymer should interfere as little as possible with the epithelial surface, cornea and conjunctiva (Efron et al, 2013). This is important for maintenance of ocular health and patient tolerance of the lens.

Gas permeable (GP) contact lenses are typically prescribed for full-time daily wear, often for many months. Planned replacement after 6 or 12 months wear is common, but sometimes lenses are worn until degradation of comfort or acuity necessitates replacement. Despite cleansing and disinfection procedures, organisms and deposits adhere to lens surfaces. Wear, handling and cleaning of GP contact lenses changes the physio-chemical properties (hydrophobicity, electrostatic charge and surface roughness) of the contact lens surface.

Plasma surface-treatment (PST) of GP lenses is proposed as a method for improving wear comfort and resistance to deposition, over that achieved with un-treated (UT) lenses, by altering the superficial polymer surface without significantly affecting the remaining underlying material (Chu et al., 2002). In this way, surface properties of the lens, including wettability, adhesion, adsorption, chemical reactivity and sensitivity to light, may be altered (Ru and Jie-rong, 2006). However, it may wear off over time (Valsesia et al., 2004).

In GP lenses, PST aims to remove residual spoilation from the lens manufacturing process and thereby reduce the contact angle to make the lens more wettable. It has been suggested that this may improve lens comfort and vision (Port and Loveridge, 1986; Schafer, 2006; Young and Tapper, 2007; Yin et al., 2008). Furthermore, it is thought that PST reduces surface roughness and binding of potentially sinister microbes, such as pseudomonas aeruginosa (Bruinsma et al., 2003). However, no research relating GP surface quality to the performance or comfort of the lens has been performed.

Atomic force microscopy (AFM) maps the topography of a polymer surface using a scanning probe to create a three-dimensional image (Meyer, 1992; Stuart, 2002). It is usually performed in ambient conditions and, because no electrical surface conductivity is required, many inorganic and polymer surfaces may be studied with minimal cost and relative ease, since little or no sample preparation is required (Munk and Aminabhavi, 2002).

AFM uses a fine-tipped probe which is positioned several angstroms above the surface of the sample. It measures the interaction force between the tip of the probe and the surface. The resultant force has two components: an attractive van der Waals component, typical for molecules in contact, and a repulsive component that does not allow the molecules to overlap (Munk and Aminabhavi, 2002). The probe is an insulator and is attached to a cantilever with a reflective surface, which is scanned in the x-y plane. A piezo-electric support is used to mount the sample and this moves in response to surface changes sensed by the probe. The deflections are monitored by a reflected laser beam. Measurements can be made either in contact (no oscillation of the cantilever), or by tapping (with oscillation of the cantilever) mode.

Atomic force microscopy (AFM) is a well-established technique in flatness analysis and imaging of polymer surfaces, including biopolymers (Merrett et al., 2002; Munk and Aminabhavi, 2002). AFM has been used to analyse the surface of both GP (Baguet et al, 1995; Bhatia et al, 1997; Bruinsma et al., 2002; Munk and Aminabhavi, 2002; Yin et al, 2008; Ren et al, 2009) and soft contact lenses (SCL) (Gonzalez-Meijome et al., 2006; Giraldez et al. 2010). In SCL studies, AFM has been described as a very powerful tool for high resolution examination of lens surface structure and identification of significant differences in worn and unworn lens morphology (Bhatia et al, 1997).

This study examined the surface topography of unworn PST and UT GP lenses, and of 3 months worn PST and UT GP lenses, using AFM, with the aim of investigated whether samples that have undergone surface modification have smoother topographies than UT samples, irrespective of wear, and whether there is any correlation between lens comfort and topography, i.e. the smoother the lens, the better the subjective comfort. An initial method development was required for optimising of lens sample preparation.

Materials and Methods

Atomic Force Microscope

The AFM (Nanoscope IIIa Dimension 3100, Digital Instruments, Santa Barbara, USA) was operated in tapping mode, at five locations, using an uncoated, symmetric tip of 40nm, at a sampling frequency of 300kHz. The five locations were selected randomly on each lens surface (Fig. 1). Root mean-square-roughness (RMS) and average surface roughness (Ra) were obtained from the roughness analysis program using the Nanoscope III software (Digital Instruments, Santa Barbara, USA). Both values were expressed in nanometres. These measures were selected because they have been widely used in other surface roughness studies, as they give the most meaningful and reliable statistical interpretation of the surface topography (González-Méijome et al., 2006). RMS represents the standard deviation for the mean surface plane, and Ra represents the average distance of the roughness profile to the centre plane of the surface profile. Some earlier studies also report maximum roughness values, however reporting the peak roughness value of an area does not reflect the topography of the lens and may be unreliably high due to local imperfection or sample contaminations (Bruinsma et al., 2003).

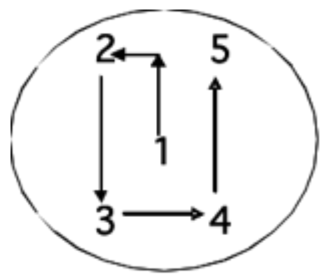


Fig. 1: Approximate position of the five surface locations on GP lens selected for AFM analysis.

Surface roughness images were also recorded at each location on each sample. This imaging technique was employed to visualise the local variation in topography within a sample. This technique was not evident in other published work (Bruinsma et al., 2003).

Comparison of GP lens sample preparation

In the following protocols, only dry sample preparation was investigated. Four different methods for GP lens sample preparation were examined. The methods employed to prepare GP samples for AFM were based on work which investigated multiple surface properties of worn GP lenses (Bruinsma et al., 2003).

Worn UT fluorosilicone acrylate GP lenses (Quasar, No7 Contact Lens Laboratory Ltd, Hastings, UK) were collected from both eyes of a single subject who had worn them on a standard, all-day protocol, for 3 months (giving two lenses in total for further study). The lenses were stored in a lens case filled with care solution (Menicare Plus, Menicon Co. Ltd, Japan), and transported to the laboratory. Each lens was removed from its transport container and transferred to fresh Menicare Plus solution in a sterile well, using sterile stainless-steel tweezers. The lens remained in solution for a minimum of 5 mins.

Each lens was then removed from its case and cut into four smaller segments using a sterile surgical knife. A single, worn lens was thus used to produce four samples in order to provide one sample for four sample preparation methods. Since both lenses of the subject were treated in this way, two lens surface samples were supplied for each method.

Following removal from the lens case, four sample preparation methods were used (Table 1). Method 1 matched the protocol of Baguet et al. (1993), with the lenses dipped five times in 0.9% saline (non-preserved), and excess saline removed by gently tapping the lens edge on a paper tissue. The lenses were allowed to air dry. In Method 2, the lenses were not rinsed, but were only dried using a nitrogen gas hose (pressure: 2 bar). In Method 3, the lenses were dipped five times in 0.9% saline (non-preserved), and excess saline removed by gently tapping the lens edge on a paper tissue. The lenses were then dried using the nitrogen gas hose. In Method 4, the lenses the lenses were dipped five times in purified, distilled water and excess water removed by gently tapping the lens edge on a paper tissue. The lenses were then dried using the nitrogen gas hose.

Finally, each lens section was mounted onto the AFM platform using adhesive tape.

Method	Storage	Lens rinse preparation			Lens drying	
	Menicare Plus	0.9% saline (unpreserved)	Not rinsed	Purified, distilled water	Air dried	Nitrogen hose
Method 1	✓	✓			✓	
Method 2	✓		✓			✓
Method 3	✓	✓				✓
Method 4	✓			✓		✓

Table 1: Overview of the sample preparation used in each Method (Method 1 is based on Bruinsma et al., 2003); (Menicare Plus, Menicon Co. Ltd, Japan).

Repeatability of GP surface AFM measurement

A single worn UT GP lens (Quasar, No7 Contact Lenses, Hastings, UK) was collected from a subject who had worn it on a standard, all-day protocol, for 3 months. The lens was stored in a lens case filled with care solution (Menicare Plus, Menicon Co. Ltd, Japan), and transported to the laboratory. The lens was removed from its transport container and transferred to fresh Menicare Plus solution in a sterile well, using sterile stainless-steel tweezers. The lens remained in solution for a minimum of 5 mins. The lens was then removed from its case and cut into two smaller segments using a sterile surgical knife. The two lens sections were prepared for AFM using the Method 4 protocol. Five 100μm^2 areas were scanned on each lens sample, referred to as Sample 1 and Sample 2.

Unworn lens samples

Four unworn GP lenses (Quasar, No7 Contact Lenses, Hastings, UK, with Boston EO material, Polymer Technologies, Boston, USA) were examined under AFM. Two lenses were PST and two were UT, but they were otherwise identical. The lenses were removed from their lens case and storage solution (Menicare Plus, Menicon Co. Ltd, Japan) in which they had been transported from the manufacturing laboratory and placed in a sterile vial filled with fresh Menicon Plus solution, with the aid of sterile metal tweezers. Using the tweezers to avoid contamination, the lenses were then cut into smaller segments using a sterile surgical knife and prepared using the Method 4 protocol.

Worn lens samples

Lens samples were collected from subjects recruited for a separate study investigating the clinical benefits of PST on the same type of GP lenses (Quasar, No7 Contact Lens Laboratory Ltd, Hastings, UK, with Boston EO material, Polymer Technologies, Boston, USA). Following 3 months of daily GP wear, twenty lenses were collected: ten PST and ten UT. These lenses were prepared for AFM using the Method 4 protocol. As an additional step, subject comfort with the lenses was measured using a visual analogue scale (VAS), rating comfort on a 10 cm scale between '0 = Not at all comfortable' and '100 = Very comfortable'.

Statistical analysis

Data was analysed using SPSS 16.0 (SPSS Inc., Chicago, USA) and examined for normality by the Shapiro-Wilk test. As the results were not normally distributed, the median and range values for root mean square (RMS) and surface roughness (Ra) were used to describe the results. Differences between groups were assessed by Mann-Whitney, Kruskal-Wallis and Wilcoxon Rank tests, and correlation by the Pearson test. A probability value of <0.05 was used for statistical significance.

Results

Sample preparation

As the results were not normally distributed, the median and range values of RMS and Ra for each preparation method (1-4) are shown in Fig. 2, and examples of the surface images produced in two and three dimensions are shown in Fig. 3.

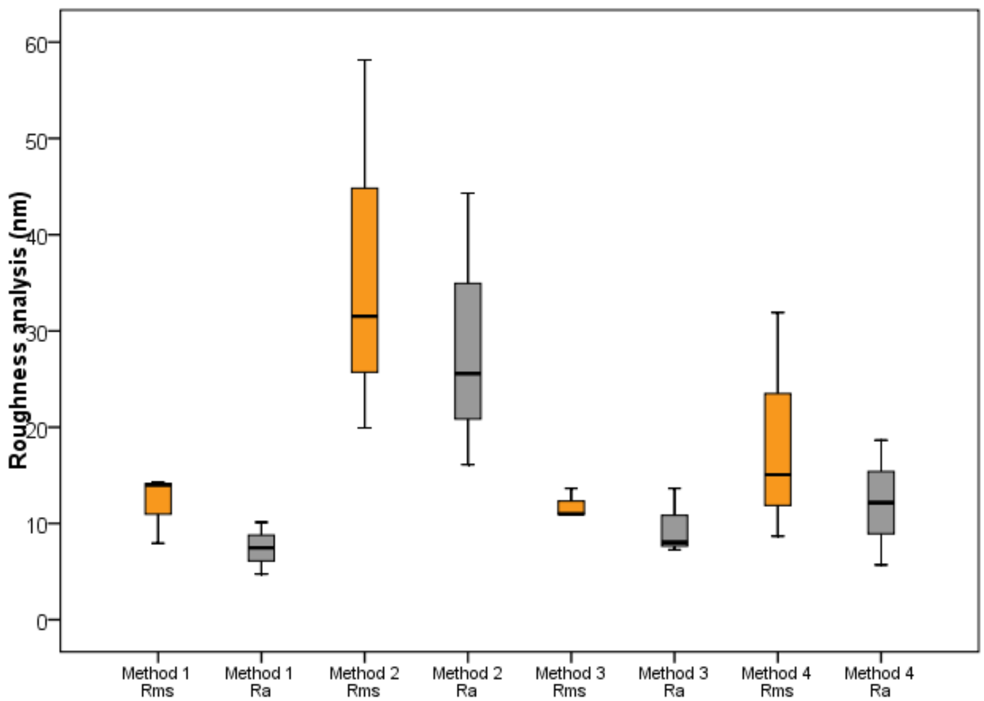


Fig. 2: Box-plots showing median and range values of the surface analysis results for each of the four sample preparation methods.

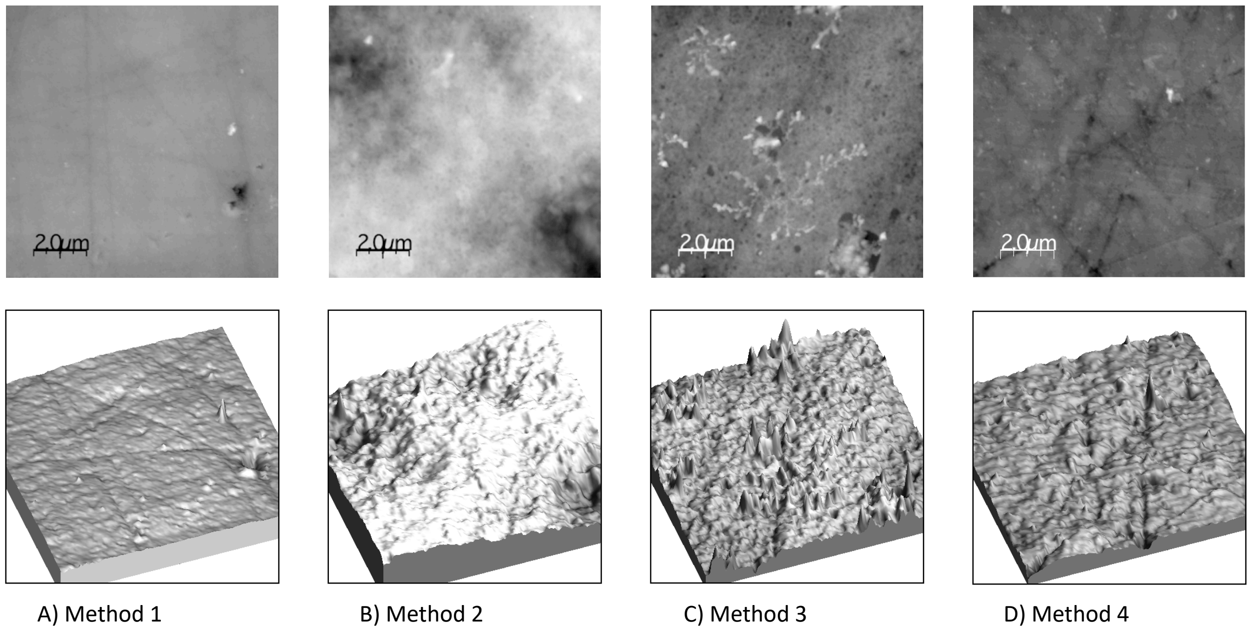


Fig. 3: Two (upper) and three-dimensional (lower) image examples for each sample preparation method.

Preparation Methods 1 and 3 (where samples were rinsed with saline prior to AFM) showed similar results, with the lowest median RMS and Ra values and the least variability (Mann-Whitney, RMS and Ra; $p=0.70$ and $p=0.70$). However, visual comparison revealed visible sodium crystals on the lens surface as the saline solution evaporated. Evidence of this is illustrated in Fig. 3C.

Method 2, where the Menicare Plus solution was not rinsed from the lens surface prior to AFM, gave higher RMS and Ra scores, and a wider range, compared with the other preparation methods. Method 4 produced median RMS and Ra values of 15.07nm and 12.16nm, respectively. These values were lower than Method 2 and marginally higher, with a wider range, than those produced by Methods 1 and 3. Statistically, results were not significantly different (Kruskal-Wallis, $p=0.25$ and $p=0.21$, for RMS and Ra) between Methods 1, 3 and 4.

Repeatability of AFM for measurement of GP surface topography

Considering the five measures on each sample, Sample 1 showed a larger range of results for RMS and Ra than Sample 2, but no statistically significant difference was found between results for either RMS or Ra in the two lens samples (Wilcoxon Rank, RMS and Ra; $p=0.35$ and $p=0.89$) (Fig. 4).

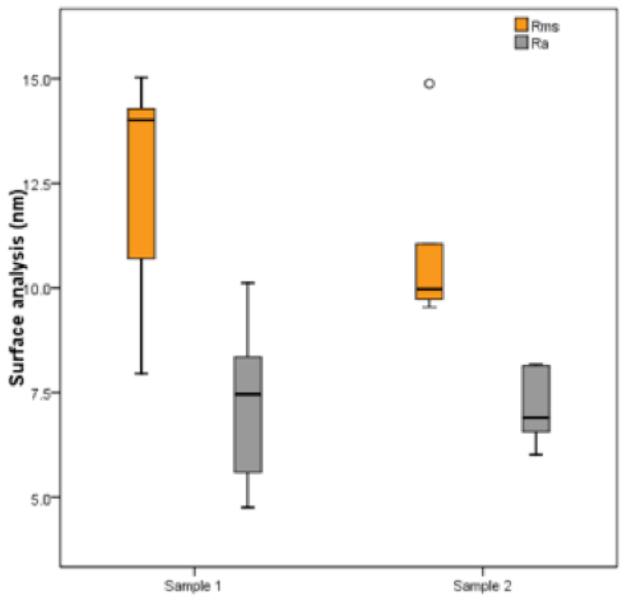


Fig. 4: Box-plot showing median, upper and lower quartiles and range AFM repeatability study results for the two samples taken from the same lens.

Unworn lens samples

The surface roughness analysis results for the two factory-new UT and the two factory-new, PST GP lenses are listed in Table 2 and displayed in Fig. 5, and a three-dimensional image example of the lenses is shown in Fig. 6. The results showed that the UT lenses had significantly higher mean RMS and Ra values compared with the PST samples (Mann-Whitney, $p<0.05$).

	Unworn				Worn			
	Ra		RMS		Ra		RMS	
	Median	Range	Median	Range	Median	Range	Median	Range
Untreated (UT)	12.37	11.10-17.80	17.63	15.00-27.03	12.92	11.34-26.59	18.70	15.01-32.94
Plasma-treated (PST)	11.53	7.46-15.76	14.92	11.10-20.93	11.18	7.68-15.97	14.82	11.24-20.99

Table 2: Ra and RMS median and range for unworn and worn untreated (UT) and plasma-treated (PST) GP lenses.

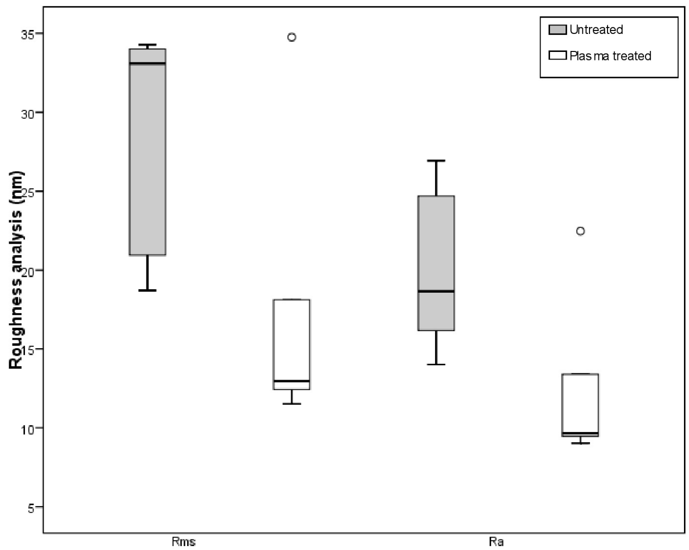


Fig. 5: Box-plot showing median and range values for roughness analysis of unworn PST and UT lenses (2 lenses, 2 samples from each lens, 5 readings per sample).

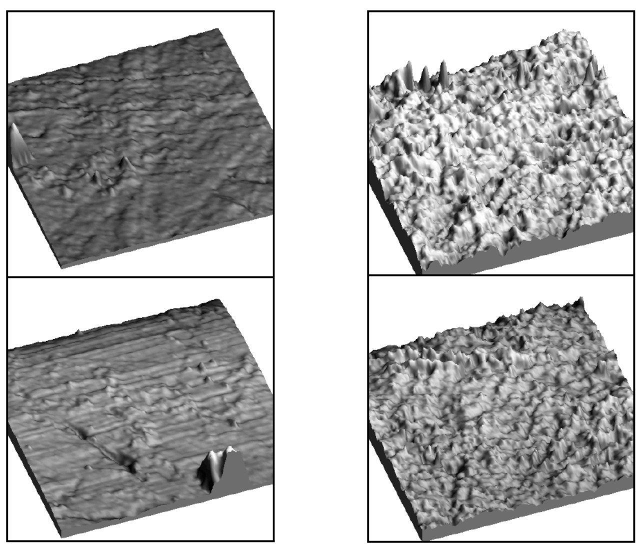


Fig. 6: Example surface appearances of unworn GP lenses; (left) PST, (right) UT.

Worn lens samples

Median Ra values were higher in the worn UT lenses [12.92nm (range 11.34-26.59)] than the worn PST lenses [11.18nm (range 7.68-15.97)], a difference which approached statistical significance (Mann Whitney, $p=0.06$). Median RMS scores were significantly higher in worn UT samples [18.70nm (15.01-32.94)] than the worn PST samples [14.82nm (11.24-20.99)] (Mann-Whitney, $p<0.05$) (Table 2 and Fig. 7).

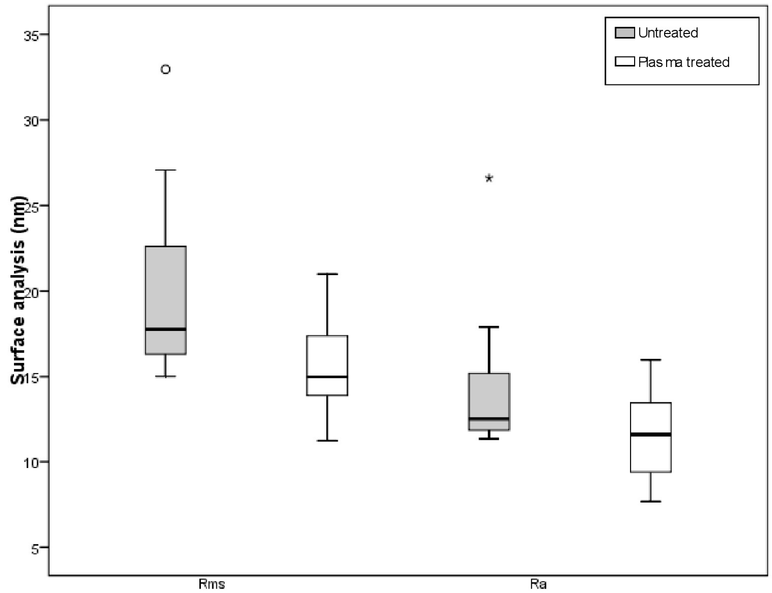
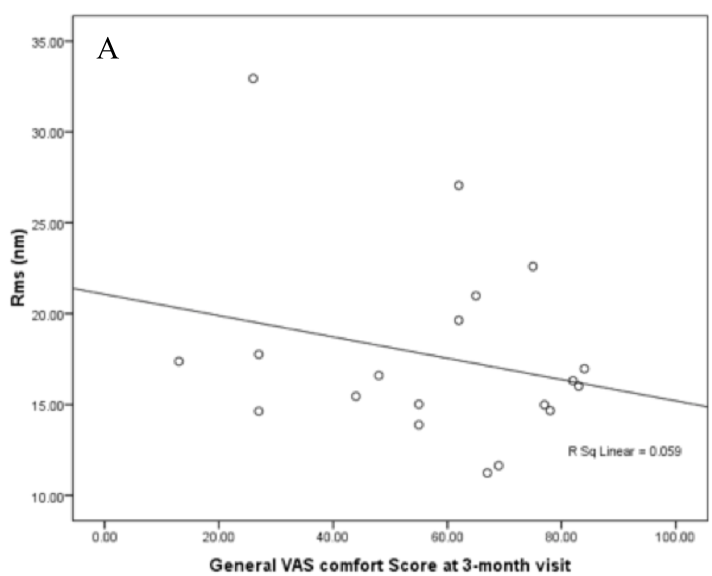


Fig. 7: Box-plot showing median and range values for surface analysis results for worn PST and worn UT samples.

No correlation was found between general comfort, reported by VAS at the 3 months visit, and RMS or Ra scores (Pearson, RMS and Ra; $p=0.73$, $R^2=0.059$, and $p=0.80$, $R^2=0.069$) (Fig. 8). No correlation was found between surface treatment and roughness (Pearson, $p=0.36$).



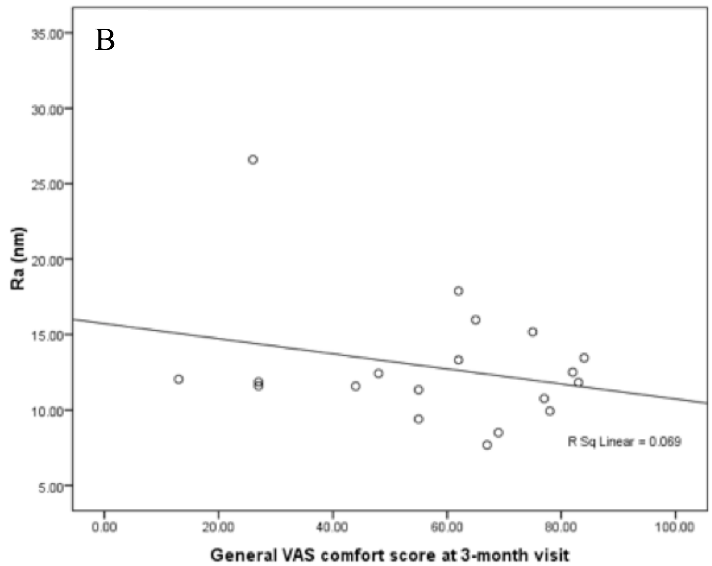


Fig. 8: Correlation between surface roughness measured by AFM and VAS comfort after 3 months of lens wear; A: Correlation between general comfort VAS scores and RMS, and B: Correlation between general comfort VAS and RA (0=Not at all comfortable, 100=Very comfortable).

Discussion

AFM has been used to analyse the surfaces of both GP and SCL lenses. In SCL studies, AFM has been described as a very powerful tool for high resolution examination of lens surface structure and identification of significant differences in worn and unworn lens morphology (Bhatia, Goldberg and Enns, 1997). González-Méijome et al. (2006) reported significant differences in AFM results when investigating surface topography of three different unworn soft lenses, with the highest roughness result observed in a PST modified lens. This finding may have implications regarding lens spoilation, resistance to bacterial adhesion or mechanical interaction with the ocular surface.

Bruinsma et al. (2003) examined worn GP lenses to explore the relationship between surface roughness and bacterial adhesion, and found that, within each individual, major changes in lens surface properties occur during wear. They found that variations in roughness from 4-14nm have little influence on bacterial deposition, while higher roughness levels increase bacterial adhesion. The study concluded that wearing GP lenses for longer periods (over 50 days) increased roughness and, therefore, GPs should be prescribed with a planned replacement strategy. While it is known that the risk of MK with GP lenses is already low, frequent replacement of GP lenses may help to reduce surface deposition, improve wetting and maintain an optimum visual performance, to ensure the risk of MK is kept at a minimum. For PST lenses, it has been reported that the treatment wears off over a period of months (Young and Tapper, 2007; Sanchis et al., 2008). This may cause an increase in surface roughness and physiological influence on wearing comfort. However, it has been hypothesised that patients and their tear physiology are adapted to the lens material by this point, so it is relatively unimportant (Young and Tapper, 2007).

Sample Preparation

When AFM is used to measure surface topography of worn lenses, it is important that the preparation of samples is consistent and avoids degradation or surface disruption to ensure accurate, reliable results. Sample contamination could lead to falsely high, surface roughness readings. SCL are generally examined under aqueous buffered conditions (González-Méijome et al., 2006). However, GP lenses may be examined either wet or dry. Published work investigating GP surfaces using AFM has described only one method of sample preparation (Baguet et al., 1993), but this may not be the best protocol for AFM. In particular, in this published method, the lens sample is dipped five times into non-preserved saline and the lens tapped on tissue paper before analysis, which may contaminate the sample surface.

This current study has demonstrated that the sample preparation protocol directly impacts AFM results. As such, it is critical that the sample is not contaminated prior to AFM, so that the results produced are consistent, accurate and meaningful. Avoiding contamination during sample preparation is critical in producing reliable surface analysis results with AFM.

Method 1 has been previously employed in AFM surface analysis of GP lenses (Bruinsma et al., 2003). The Ra values produced in this study are similar to those produced by Bruinsma et al. (2003); where Ra was found to be 9±4nm in worn lenses. Both studies investigated worn lens (ninety days in this study compared with fifty days in Bruinsma et al. (2003)), although the materials tested were different. However, this study found that it was not advisable to rinse the sample in saline prior to measurement because, when the lens dries, sodium crystals contaminate the lens surface. For this reason, Methods 1 and 3 should both be considered unsuitable.

In Method 2, AFM was performed on a lens coated with Menicare Plus solution. Menicare Plus is a multi-purpose cleaning and conditioning agent. It contains lubricating factors to coat the lens surface and so improve on-eye comfort and wetting. However, since AFM investigates only the most anterior layers of the sample, this may mean that any overlying dried lens solution masks the true lens surface, making this preparation method also unsuitable prior to AFM.

In Method 4, where the lens is stored in Menicare Plus solution, rinsed in ultra-purified, distilled water and then dried with a nitrogen hose, there is the least likelihood of contamination of the sample via care solution or air-borne contaminants. This methodology is similar to that used in sample preparation in other biological AFM research (Thundat et al., 1994). Air-drying the sample may permit air born particles to adhere to the lens surface, therefore drying with dry nitrogen after rinsing is a superior preparation technique (Thundat et al., 1994). Interestingly, when using Method 4, the RMS and Ra results were higher, though not significantly, than with Methods 1 and 3. This suggests that the Method enables the true surface roughness quality to be assessed. Further study using this method is needed to confirm this finding. It would appear that Method 4 preparation poses the least risk of lens contamination and should be used when preparing GP samples for AFM.

Measures of surface roughness using a standard protocol appear repeatable within a single sample, implying that any portion of the lens is representative of its surface topography. This is important because examination of an entire lens surface is impractical with this method of AFM. The results demonstrate that values for Ra and RMS vary both within-sample and between-sample, indicating that surface topography varies across the lens. This concurs with studies which have found that the manufacturing process is responsible for surface topography variations (Fourny et al., 1989; Merindano et al., 1998). All GP lenses are made by lathe-cut technology and this has been attributed to linear surface scratches detected on unworn GP lenses when examined by SEM (Merindano et al., 1998).

One limitation of this study arises from having investigated only one lens at two locations with five readings at each location, and reproducibility over time was not examined in this study. A further investigation of repeatability following prolonged storage and involving a larger sample would be interesting for future work.

Unworn/worn lenses

In this study, as anticipated, unworn PST GP lenses had lower Ra and RMS values compared with unworn UT GP lenses. This finding agrees with the findings of Valsesia et al. (2004) who investigated the surface topography and characterisation of PMMA co-polymer films with and without PST. Since surface roughness has been found to increase bacterial adhesion and may adversely affect contact lens comfort, the findings of this study suggest that there is a clinical benefit associated with PST of GP lenses.

Interestingly, unworn UT lenses in this investigation had the highest roughness scores, higher even than worn UT lenses, and they had a greater variability in the measurement. This may be because factory-new lenses have many surface contaminant residues from the manufacturing process, whereas worn lenses are ‘cleaned’ by wear and the daily cleaning regimen. However, this trend may be dampened by increasing the sample size.

PST lenses that had been worn for 3 months were also smoother than worn UT lenses. This confirms that PST of GP lenses can reduce surface roughness initially, and that the benefits of treatment, improved hydrophilicity and resistance to protein deposition, are maintained with lens wear. It has been suggested that contact lens PST ages and wear off over a period of months (Sanchis et al., 2008). In this study it was found that, after 3 months wear, PST was still evident, although surface roughness scores were lower than unworn PST.

The reduction in surface smoothness of the worn PST lenses may be due to several reasons, but the most obvious and logical one is that the PST has diminished over time and lost some of its smoothing properties. This idea is supported by Young et al. (2007), who suggested that PST wears off with cleaning and wear. In addition, the variability of results may be due to inter-subject differences such as variation in hygiene, differences in wear schedule, lifestyle and patients’ tear physiology. Where possible, these factors have been controlled; for example, patients were instructed to follow the same care procedure and use the same contact lens solutions, and all were advised to wear lenses on a full-time basis for 12 weeks. However, non-compliance issues are commonplace in contact lens patients (Polse et al., 1999). The random allocation of subjects should ensure that non-compliance with lens care had a similar influence on both lens groups, but it is possible that poor lens care had less influence on the PST lens surfaces than UT.

Any measured surface roughness of a brand-new lens has two possible origins: material properties or manufacturing method. Scanning electron microscopy (SEM) and interferential shifting phase microscopy (ISPM) results indicate that, in general, GP surface roughness values tend to increase with increasing Dk (Merindano et al., 1998). Using ISPM, Merindano et al. (1998) found linear marks on the anterior lens surface of factory-new GP lenses (González-Méijome et al., 2006), which may be explained by the lathe-cutting technology used to produce them. An AFM study of unworn SCLs found magnification also significantly affects roughness analysis values, noting that surface roughness increases as observation area is increased (Young and Tapper, 2007; Sanchis et al., 2008).

It should be noted that the samples used in this study will have varied in time since manufacture, as well as on which lathe the lens was made, since it has been found that exposure to atmospheric conditions may contaminate lens surface and impact on AFM results (Shakesheff, 1995). Another possible influence on the results could be that, following lens harvesting, the lenses were stored in Menicare Plus solution for varying periods (<3 weeks) before examination with AFM. Local variations in topography in single samples were found, as anticipated. However, by measuring surface roughness at five separate areas within each sample, the median values could be calculated, which improved repeatability.

To establish whether the results seen here are a direct result of lens aging, it would be interesting to investigate how PST lenses are affected over longer periods, e.g. 6 or 12 months. Also, it has been indicated that solutions play a pivotal role in contact lens comfort and lens hygiene, and some solutions, when digitally rubbed onto the lens surface, may scratch or alter the PST surfaces.

Comfort

This study also aimed to investigate whether the differences between surface topography in PST and UT lenses, both worn and unworn, had any influence on *in vivo* comfort. It was hypothesised that comfort would be improved with reduced surface roughness, as a result of PST. However, although surface roughness was reduced by PST, subjective comfort was not improved. This finding may be because the surface analysis results are at microscopic levels and therefore do not significantly impact on ocular comfort. Alternatively, the comfort responses may be affected by other factors such as edge finish, lens fit, tear stability, lens lid interaction or corneal sensitivity. These differences will vary between subjects, independently of surface roughness, and will impact on subjective comfort. The 3 months wearing period may also have been insufficient time for surface roughness to have changed significantly, and to start affecting lens wear comfort.

The measurement of surface roughness before and after wear would allow the measurement of change in roughness over time, but the preparation technique used involved cutting the lens into smaller pieces before mounting on the microscope stage. This destructive technique currently prevents AFM measurement prior to wear. However, if a curved body for mounting the lens were produced, it may be possible to mount the entire lens for investigation. Care would be needed in securing the lens to the mount, as use of an adhesive (as in this study) may leave residues on the back surface of the lens.

A limitation of AFM is that is does not investigate the surface chemistry. Future work might involve further analysis of the lens samples using X-ray photoelectron spectroscopy (XPS). This technique may lead to better surface characterisation and a clearer understanding of correlation between lens surface effects on lens performance following PST.

Conclusions

The work was successful in designing a sample preparation protocol capable of producing repeatable AFM results. It confirmed the initial hypothesis that sample preparation impacts the AFM results. Thus, it is critical to consistently use a specific preparation methodology to minimise surface damage or sample contamination and to produce accurate, repeatable AFM results.

The protocol recommended for GP lens preparation prior to AFM is as follows:
After harvesting, the lenses should be stored in a clean lens case filled with Menicare Plus solution and transported immediately to the laboratory. The lens should be transferred to fresh Menicare Plus solution in a sterile well, using sterile stainless-steel tweezers. The lens should be cut into smaller parts using a sterile surgical knife. The sample should then be dipped five times in, distilled, ultra-purified water and dried with a nitrogen hose. Finally, the lens is secured onto an adhesive mount for AFM measurement.

Unworn PST GP lenses had lower Ra and RMS values compared with unworn UT GP lenses. After 3 months wear, PST lenses have smoother surface topographies than UT lenses, suggesting a clinical benefit of coating, since increased surface roughness has been found to increase bacterial adhesion. However, no relationship was found between surface topography and lens wear comfort.

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