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Microstructural evaluation of the mucin balls and their relations to the corneal surface—Insights by in vivo confocal microscopy

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A B S T R A C T

Purpose: The purpose of the current study was to observe and correlate the characteristics of mucin balls to the ocular surface properties, and furthermore, to report the effect of different mucin balls size and number on structural alteration of the anterior cornea.

Methods: The study included, two groups of patients fitted with one-month continuous, extended wear lenses for therapeutic (group 1) and optical (group 2) purposes; the later serving as a control group. Group 1 was comprised of patients with recurrent erosion syndrome, while group 2 included subjects with mild myopia and voluntary use of continuous wear lenses. The examination was performed when mucin balls were encountered during a routine visit. Clinical examination was reinforced with laser scanning in vivo confocal microscopy, which provided microstructural observations. The appearance and size of the mucin balls were described and measured at two independent time points. Qualitative analysis included shape (round, elliptical and irregular) and reflectivity (bright, homogenous and dark, heterogonous).

Results: Clinically 1460 mucin balls were encountered (822 in group 1 and 638 in group 2). The number of mucin balls analyzed by in vivo confocal microscopy was 820. Diversity was higher in group 1. The mucin balls of group 2, were more uniform – rounded in shape 81,2% and regular in reflectivity 98%. Qualitative analysis revealed a negative correlation between the size of the balls and impact on the basal epithelium morphology and also “activation” of the anterior stroma in adjacent areas.

Conclusions: Mucin balls affect corneal surface including both epithelia disintegration as well as keratocyte “activation”. The main predisposing factor for mucin ball formation appear to be the corneal surface irregularity. As structural alterations of the cornea are transient, mucin balls might be beneficial for corneal restoration due to mechanical and/or biochemical stimulation. In vivo, confocal microscopy is an innovative tool for evaluating mucin balls in their diversity and dynamics.

1. Introduction

Mucin balls are translucent spherical bodies sized 20–200 μm, but usually around 50 μm, derived from the mucin layer of the tear film [1]. Their origin had been proved by histochemical and electron-microscopical techniques but the exact mechanism of their formation still remains unclear [2]. Most likely excessive tear film mucin is amalgamated into spherical particles due to the friction between the lens and the ocular surface. Furthermore, Miller et al. hypothesized that lens which is more incongruent to the ocular surface, has greater potential for mucin balls formation [2].

The presence, characteristics and roles of the mucin balls for the anterior ocular surface in contact lens wearers is debatable and somehow obscure. It has been proven that, the presence of mucin balls is associated with less infiltrative events. Furthermore, Szczotka-Flynn et al. reported that mucin has antibacterial properties [3]. However, those are mainly highlights on bio-chemical consequences of mucin accumulation in the tear film. Formation of the mucin balls, their size and shape and mechanical effects has been incidentally reported during the last decade. Interestingly, mucin balls are observed in rigid gas permeable and silicone-hydrogel wearers and are more universal in the case of continuous wear [4]. However, whether the formation is related to the modulus or to the “mucinophobic” properties of the material is, again subject to scientific speculation. Recently, the silicone-hydrogel lenses are featuring significantly lower modulus, but mucin balls are still observed even in those more flexible lenses. Therefore, the

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hypothesis that the "non-mucin absorbing" (mucinophobic) property of the material is a key factor for mucin balls formation, should be revived [5].

Nevertheless, the mucin balls are a frequent clinical observation in continuous wear and also in various therapeutic uses of silicone hydrogel contact lenses. Practitioners might suggest to a patient wearing contact lenses for optical indications to reduce the time of the continuous wear, but this is not possible for therapeutic use. Furthermore, there is lack of knowledge as to how exactly mucin balls affect the ocular surface. Papas suggested that the effect of the mucin balls is somehow similar to the effect of orthokertology lenses, with mean structural alterations described as "epithelial compacting" [6]. However, in the published literature micro-structural studies using in vivo confocal microscopy are very limited. Therefore, the current study was designed to improve the knowledge about mucin balls as a part of the ocular surface in silicone-hydrogel contact lens application (for optical or therapeutic purpose), utilizing the up-to date equipment for in vivo microstructural analysis at cellular level.

The purpose of the current study is to describe and correlate microstructural characteristics of the mucin balls and underlying alterations of the anterior cornea utilizing the laser scanning in vivo confocal microscopy. The study was designed as prospective, comparative observation and analysis of two groups of patients, continuously wearing silicone-hydrogel lenses for therapeutic or optical purposes.

2. Materials and methods

This prospective study was designed to clinically identify mucin balls according to the definition (translucent, opalescent round bodies between the surface epithelium and the contact lens, sized 20-200 μm). Subsequently, using in vivo confocal microscopy to image them, describe their microstructural characteristics, measure relevant morphometric parameters and attempt classification. All related observation at the level of corneal epithelium were also noted.

The study recruited a total of 42 eyes from 42 subjects, all fitted prior to inclusion with silicone-hydrogel lens. All lenses were made of balafilcone A and had a radius of curvature of 8.6 mm, diameter of 14.2 mm and dioptric power ranging from 0 to −3.5 D. The eyes were divided into 2 groups. Group 1 (therapeutic group) included 20 eyes (10 right and 10 left eyes) fitted with lenses for therapeutic purpose following recurrent erosion syndrome. The mean age of the subjects in this group was 45 ± 8 years. Group 2 (control group) was comprised of 22 subjects (11 right and 11 left eyes) with mild myopia (up to 3.75 D), with a mean age of 26 ± 3 years who were extended wearers (28–30 consecutive days) of their own volition. On the day of their routine visit (when mucin balls were encountered) all subjects had consented to in vivo confocal microscopy. All subjects were using only sodium hyaluronate based artificial tears. Non of the subjects used any topical medications, such as antibiotics and/or corticosteroids. The study was approved by the local ethics committee.

Clinical and microstructural examination was performed following the approved study protocol. Slit-lamp photography was taken with diffuse illumination and 12× magnification in a completely dark room, in order to visualize the mucin balls and provide image for multiple counting. Laser scanning in vivo confocal microscopy HRTIII-RCM (Heidelberg Retina Tomograph III – Rostock Cornea Module, Heidelberg Engineering GmbH, Germany, based on a diode laser with a 670 nm wavelength) utilizing the Rostock Corneal Software Version 1.2 was performed on all eyes after detailed clinical examination. As a coupling agent between applanating lens cap and the objective lens Corneregel (Bausch & Lomb GmbH, Berlin, Germany) was used. All eyes were anaesthetized, with topical Alcaine (0,5% collyre, Alcon). After explanation of the examination process the individual tomocap was approximated to the cornea, over the contact lens and all zones populated with mucin balls were imaged. The captured images also included adjacent zones clear of mucin balls and optical dissection of underlying corneal stroma. All images were stored on the secure server for further analysis. After examination a fresh lens was inserted and subjects were invited for follow up visit in 28 ± 2 days.

The mucin balls were counted on diffuse illumination by two independent examiners, and the mean number was taken for analysis. Subsequently, in vivo confocal, images were evaluated qualitatively and quantitatively. The in vivo confocal images of mucin balls were blindly classified quantitatively (two independent examiners) depending on the shape as: round, elliptical and irregular, depending on the reflectivity as: bright (homogenous) and with dark areas (heterogenous). All encountered mucin balls were precisely measured (the longest and perpendicular diameter) utilizing planimetric tool of "KLONK Image Measurement" software management tool by two examiners, in a masked fashion. Epithelial changes were observed and described in verbal manner. Notable variations in cell size and reflectivity was accepted as significant. Frames demonstrating changes in the anterior stroma were also collected and described.

The results from the counting and measurements, as a part of the prospective study, were analyzed using SPSS v.21 package and presented as bar chart, comprise of mean ± SD. Statistical significance were calculated by unpaired Student's *t*-test. A p-value < 0.05 was considered statistically significant (* $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$)

3. Results

All subjects (N = 42, 42 eyes), prospectively included in the study had complete clinical and in vivo confocal examination on both visits (84 examinations). The mean post-lens-insertion time was 23 ± 4 days.

3.1. Clinical observation

Total of 1460 mucin balls were counted clinically. During the baseline session 408 mucin balls were counted for the first therapeutic, group and 318 for the second optical, group. The results were similar for the follow up session 414 versus 320 mucin balls for groups 1 and 2 respectively. Results are presented in Table 1. On qualitative clinical examination none of the subjects presented any other pathological findings related to the anterior ocular surface, other than mucin balls (Fig. 1).

3.2. In vivo confocal analysis

Total of 820 mucin balls were selected from in vivo confocal sequences and evaluated qualitatively and measured at microstructural level (mean of 9 per eye (range 2–13). Qualitative analysis included classification of the mucin balls as: round, elliptical and irregular, and also depending on the reflectivity as: bright (homogenous) and with dark areas (heterogenous) (Fig. 2). Qualitative results are presented in

Table 1
Total number of mucin balls counted clinically for all included subjects and average number per eye.

	Total number of mucin balls	Average number of mucin balls per eye
Group 1 at base line (n = 20 eyes)	408	20.40
Group 2 at base line (n = 22 eyes)	318	14.55
Group 1 at follow up (n = 20 eyes)	414	20.79
Group 2 at follow up (n = 22 eyes)	320	14.40
Total (n = 42 eyes examined twice)	1460	N.A.

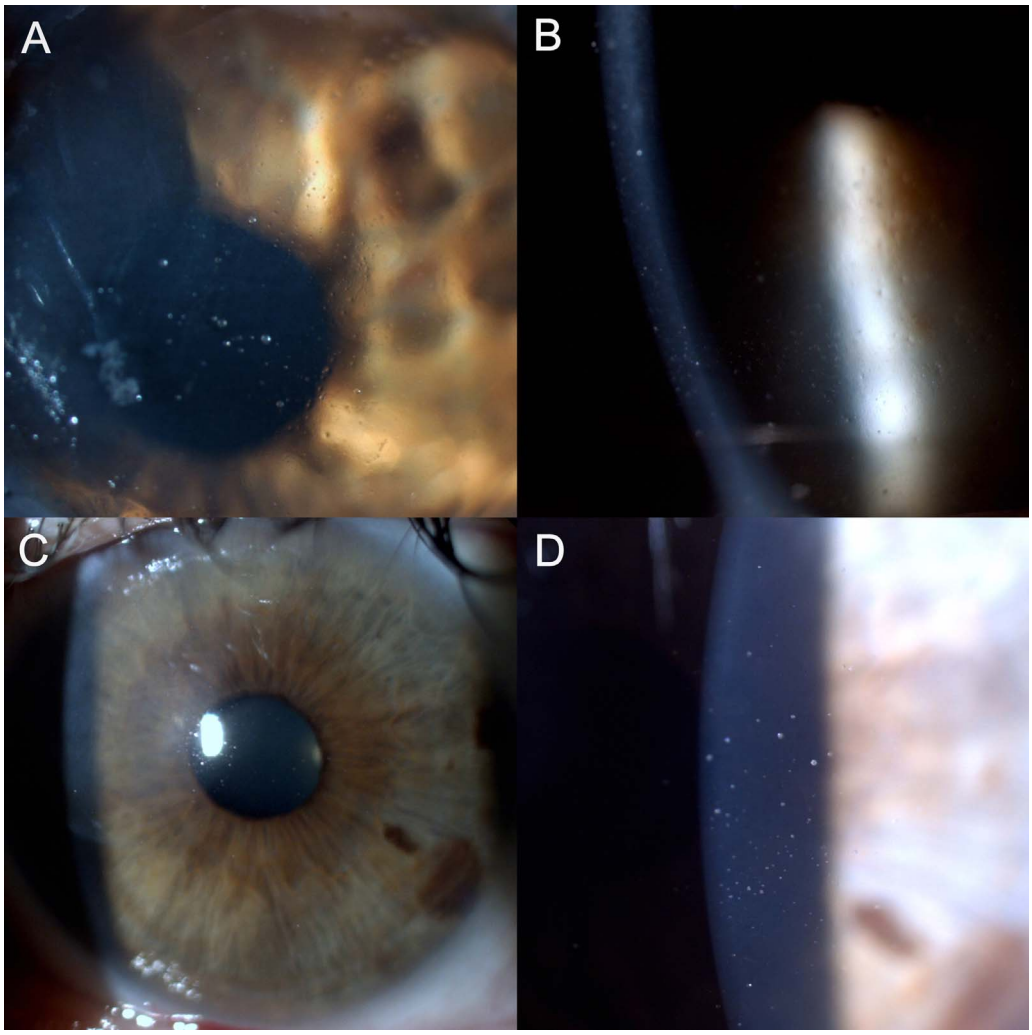

Fig. 1. Clinical slit-lamp photography demonstrating mucin balls of individuals fitted with 1 month of continuous wear silicone-hydrogel lenses for therapeutic purposes (A and B) and for refractive correction (C and D).

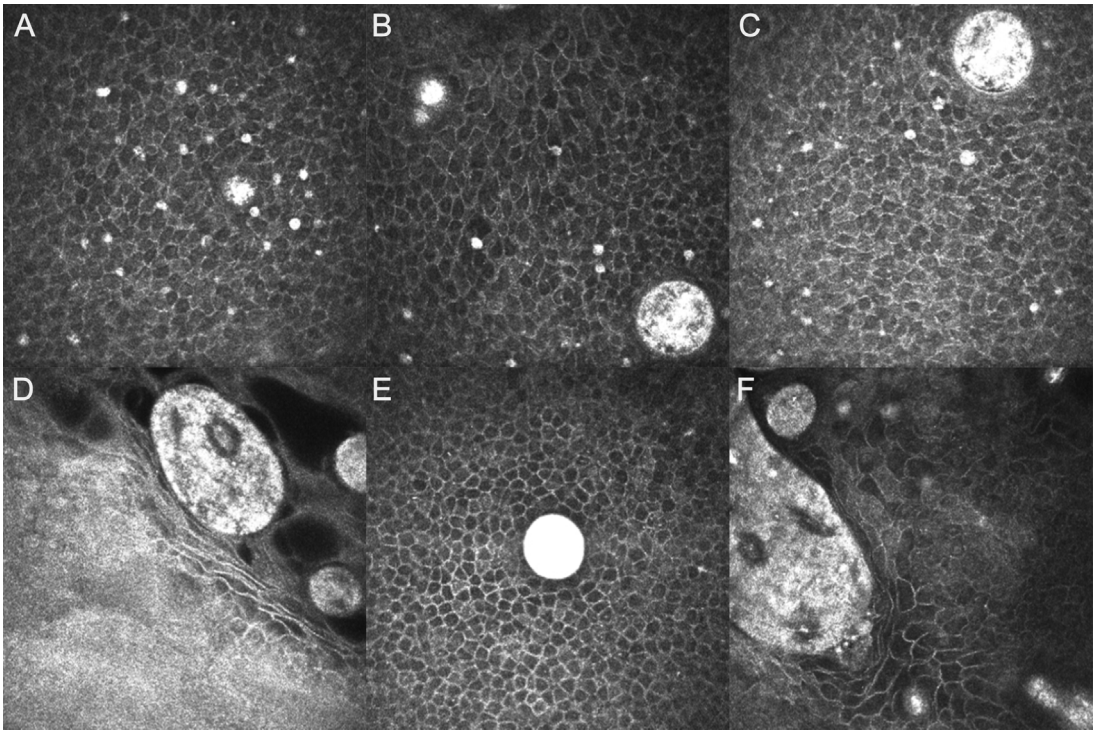

Fig. 2. In vivo confocal examination demonstrating small (A), large (B), round (C), elliptical (D), homogenous (E) and heterogenous (F) mucin balls.

Table 2
Total of 820 mucin balls were evaluated qualitatively and measured at microstructural level (mean of 5 per eye (range 2–13). The mucin balls were classified quantitatively depending on shape as: round, elliptical and irregular, depending on the reflectivity as: bright (homogenous) and with dark areas (heterogenous).

	Total number of mucin balls	Divided by shape			Divided by reflectivity	
		round	Oval	irregular	homogenous	heterogenous
Group 1 at base line	230	130	87	13	197	33
Group 2 at base line	193	155	36	2	187	6
Group 1 at follow up	208	132	71	5	185	23
Group 2 at follow up	189	155	34	0	187	2
Total	820	572	228	20	756	64

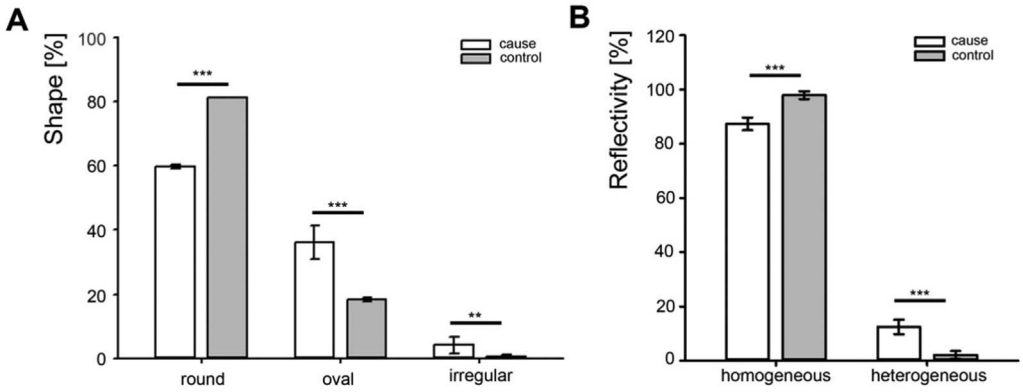

Fig. 3. Shifting in shape and reflectivity in therapeutic group in comparison to the control. Confocal microscopy observation demonstrated reduced round and increased oval and irregular shape (A), and significantly higher heterogeneous mucin balls (B), in the therapeutic group (group 1). Statistical significance consists of two independent measurements of 800 mucin balls each (* $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$).

Table 2. The control group presented more round in shape 81,2% and regular in reflectivity 98% mucin balls in contrast to group 1 where variation in shape and reflectivity were significantly higher. In this group round shape and regular reflectivity were respectively at 40,2% and 12,8%. Analysis is presented in Fig. 3. At the two time points similar characteristics for both groups were highlighted for the analyzed parameters, as presented in Table 2.

The adjacent epithelium and stroma were also observed for changes. Interestingly, smaller balls were associated with more epithelial distorsion and irregularity, than larger balls as seen on Fig. 4. Moreover, the surrounding epithelium of the areas without mucin balls presented normal characteristics for all examined corneas, regardless of the size and density as demonstrated in Fig. 4 c. Qualitative changes in the anterior stroma adjacent to the epithelial deformity were also encountered. Those changes were increased in number and "networking" of keratocytes, as demonstrated on Fig. 4 d. In current study, keratocyte alteration was only observed when the epithelium was demonstrating structural changes and/or activity.

All mucin balls imaged at microstructural level, were also precisely measured (the longest and perpendicular diameter) and the results are presented in Table 3. Statistical analysis of the two-time-points examinations is presented in Table 4. Significant differences were found in the number of the mucin balls ($t = -3{,}98$; $p < 0{,}0001$) and the size of the mucin balls ($t = -5{,}45$; $p < 0{,}0001$) between the cases and the controls.

4. Discussion

Mucin balls are incidental observation related to RGP or silicone-hydrogel lenses. They are not very well understood, neither evaluated deeply probably because they are not associated with symptoms and recognized as clinically significant findings [4]. Presumably, mucin balls are observed routinely in eyes fitted with therapeutic lenses, however, due to other clinical problems these findings are accepted as innocuous. In presented study, basal epithelial deformity was observed, which was not exclusively associated with larger mucin balls. Therefore epithelial damage it is most likely to be transient, as no permanent structural change in regions free of mucin balls was observer. Nevertheless, these microstructural alterations might be the reason for corneal "activation" in the case of mucin balls presence. In the elegant study by Szczotka-Flynn et all, using analysis with the Kaplan-Mayer curve, it was concluded that mucin balls are having a protective effect against corneal infiltrative events [3]. In another interesting microstructural study Ladage et al., highlighted the mechanical effect of mucin balls on corneal epithelium in human and rabbit corneas. [7] Based on presented by this study observation that mucin balls cause structural epithelial change, one might hypothesize that the "protective" effect of mucin formations might be bio-chemical and mechanical at the same time. Perhaps the deformed or even damaged epithelium triggers protective mechanisms on the anterior ocular surface and triggers regeneration. Furthermore, mucin balls are only localized, and therefore might have a stimulating, "puncture-like" effect on the epithelium.

Obviously, mucin balls are affecting the ocular surface regardless of the precise mechanism and positive or negative consequences, however, statistical analysis of the current study proved that ocular surface has a key role for the number and size of the mucin ball formations. Dumbleton et all highlighted that the "steep K reading" is the predisposing factor for mucin balls formation [8]. This might be explained by the aforementioned observation that the less congruent ocular surface in recurrent erosion syndrome is the main driving force. That highlights a lot of potential problems when silicone-hydrogel lenses are used for therapeutic purposes, as the irregularity of the surface is almost universal in those cases. However, it might also be an explanation for the beneficial effect of the therapeutic lenses. To this end, the mucin ball formation might have a protective, antibacterial effect together with stimulation of epithelial growth, leading to faster corneal epithelialization and restoration. This observation is very important in the context of corneal regeneration and transplantation. It remains to be understood what the actual effects of mucin balls on homologous, cultured corneal epithelium would be. It could be the case that the cultured cell lines react differently than the self-regenerating epithelium on the ocular surface.

Nevertheless, mucin balls are causing structural alteration of the

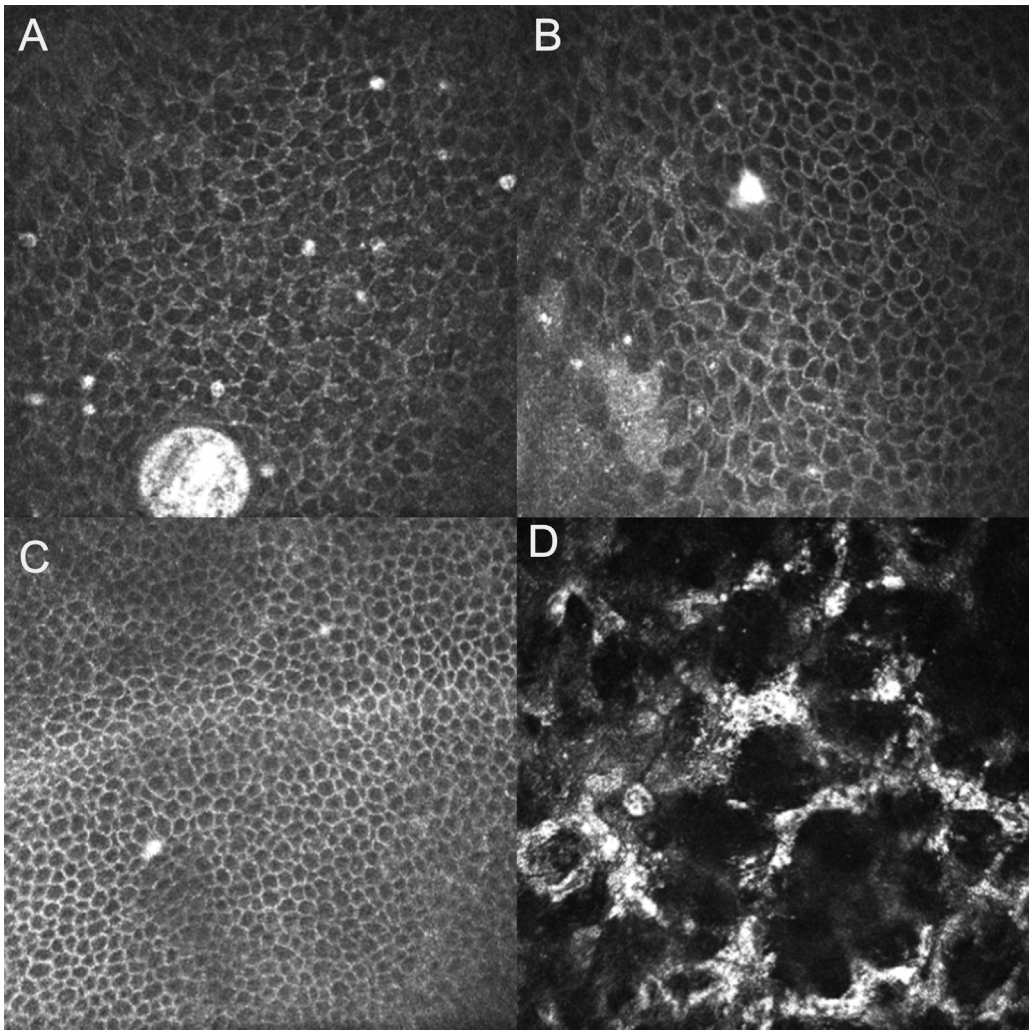

Fig. 4. In vivo confocal microscopy demonstrating microstrucrural alterations at the level of the epithelium. Normal looking epithelium surrounding the mucin ball measured 90 μm (A). Significant structural alterations associated with a small mucin ball measured 27 μm in diameter(B). The surrounding epithelium adjacent to a mucin ball area image demonstrating normal structure (C). Underlying keratocytes demonstrating "activation" and "networking" adjacent to the mucin balls related to areas of epithelial alterations (D).

anterior stroma. Prior to this study Efron et all demonstrated epithelial and stromal "activation" in the presence of mucin balls. [9] Current study confirmed this but also highlighted that structural changes are independent of the mucin ball size. Interestingly, keratocyte activation was linked to the epithelial disintegration and/or deformity. This study was unable to identify the precise correlation between the possible factors for the encountered structural misalignment, but it might be mostly biochemically driven.

The structure of mucin balls had been evaluated by electron microscopy [2]. In presented study assisted by in vivo confocal microscopy, authors also proved that mucin balls have different morphology, including size and reflective properties. That is consistent with electron microscopy findings of Millar et all, that the surface of a mucin ball has "variation density", which optically is seen as irregularity [2]. In the current study morphology of the mucin balls was strictly correlated with the properties of the ocular surface. In the two time points the therapeutic lens group demonstrated not only larger and more numerous but also more irregular in shape and reflectivity mucin balls. As selected patients were using only artificial tears and had no inflammatory events, it appears that mucin ball characteristics are dependent on the "contact lens–ocular surface" congruity. Therefore, diversity, size and number of the mucin balls depends on the mechanics between the ocular surface and back side of the contact lens. Another consideration should also be the mechanics and related co-exiting pathology of the lids.

Interestingly, mucin balls are not commented in the literature in association with the ortho-keratology. One might assume that sleeping in RGP lenses (with curvature design to mold ocular surface), should be a potent factor for mucin balls formation. On the other hand, that might be an explanation why inflammatory events in ortho-keratology

Table 3
The measured size of the mucin balls (the longest and perpendicular diameter) utilizing "KLONK Image Measurement" software management tool and relevant statistical analysis.

	Total number of measured mucin balls	Diameters (μm)		Mean diameter(μm)
		Longest meridian	the perpendicular meridian	
Group 1 at base line	230	90.22	64.22	77.72
Group 2 at base line	193	48.13	42.27	45.25
Group 1 at follow up	208	88.78	65.24	77.01
Group 2 at follow up	189	47.35	45.17	46.26
Total group 1	438	89.50	64.73	77.37
Total for group 2	382	47.74	43.72	45.81

Table 4
Relation between the diameter and the number of the mucin balls (Student's *t*-test).

	Groups	N	Mean	SD (±)	SE Mean	t	df	p	Mean Difference	95% CI of the Difference	
										Lower	Upper
Diameter	cases	40	77.36	30.69	4.85	−5.45	82	< 0.0001	−31.61	−43.14	−20.07
	controls	44	45.75	22.11	3.33						
Numbers	cases	40	20,55	7971	1,26	−3.98	69.3	< 0.0001	−6.05	−9.02	−3.08
	controls	44	14.50	5.614	0.86						

patients are not more frequently reported than in common contact lens wearers [2].

In conclusion, mucin balls are often ignored as an innocuous observation, however they do affect corneal surface at microstructural level including transient epithelial alteration and keratocyte "activation". The main predisposing factor for mucin balls formation appear to be irregular ocular surface, therefore in therapeutic application of silicone-hydrogel lenses, both a greater in number and diversity mucin balls must be expected. As structural alterations of the cornea associated with mucin balls are transient their presence might be beneficial for corneal restoration due to mechanical and/or biochemical stimulation. Last but not least in vivo confocal microscopy is a perfect tool for evaluating mucin balls in their morphology and dynamics. Future evaluation of more controlled prospectively recruited cohorts will provide further in depth knowledge about this transient but potentially significant to the ocular surface observation.

Conflict of interest

There is no conflict to disclose.

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