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Clinical Trials

Integrating Clinical Data and Tear Proteomics to Assess Efficacy, Ocular Surface Status, and Biomarker Response After Orthokeratology Lens Wear

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Purpose: This study evaluated the efficacy and ocular surface status of Breath-O Correct, novel orthokeratology (OK) lenses, worn overnight for 3 months. Lens-induced changes in the tear proteome were evaluated.

Methods: Thirty-one subjects, aged 19 to 26 years with refractive error from −1.00 to −5.00 D, were randomly assigned 1:1 to the treatment or control group. Refraction, visual acuity, corneal integrity, biomechanics and endothelial health, ocular surface changes, and subjective symptoms were assessed at the baseline, one-month, and three-month visits. The tear proteome was characterized over time using sequential window acquisition of all theoretical ion spectra mass spectrometry.

Results: Lenses improved uncorrected visual acuity and reduced spherical powers with similar efficacy to other OK lenses. Significant reductions ($P < 0.05$) in corneal hysteresis (11.12 ± 1.12 to 10.38 ± 1.36 mm Hg) and corneal resistance factor (11.06 ± 1.32 to 9.90 ± 1.45 mm Hg) were observed in the treatment group after one month of lens wear, whereas other assessed factors remained unchanged. Thirteen and eight differentially expressed proteins were found after one month and three months of lens wear, respectively. Two proteins (proline-rich protein 27 and immunoglobulin V regions) were differentially expressed at both visits.

Conclusions: Over a three-month period, Breath-O Correct lenses were overall safe, well tolerated, efficacious in refractive power reduction, and comparable with other OK lenses. Furthermore, their use caused only minor noninflammatory protein expression changes in the tear proteome.

Translational Relevance: This study investigated the safety of orthokeratology contact lenses on the ocular surface in molecular aspects and standard clinical parameters.

Introduction

Orthokeratology (OK) is a popular and effective intervention for juvenile myopia control, typically resulting in a 33% to 46% reduction in axial elongation compared with untreated controls.[1–7] By use of a reverse geometry lens design, overnight OK creates hydraulic forces promoting redistribution of corneal tissues, flattening and steepening the central and peripheral cornea respectively.[8,9] Such corneal remolding improves unaided daytime vision[10,11] and significantly increases the myopic defocus projecting on the peripheral retina, which has been suggested to be a

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potential mechanism in myopia control.[12,13] However, overnight modality shares a similar risk of infection to other overnight soft lens modalities,[14] with the induction of corneal profile and cell density changes.[15–18] Therefore it is important to characterize the safety profile of new OK lenses.

Breath-O Correct lenses have been marketed in Hong Kong since 2018. They are ready-made OK lenses with an oxygen permeability of 78×10^{-9} dk/t and are characterized by high durability, elasticity, and flexural strength compared with traditional, rigid gas permeable lenses. Their physical properties have been characterized by the Contact Lens Impact Test (Toray method, referring JIS K7211-1), and the ISO-18369-4 standard for flexural strength.

Although clinical evaluation of the ocular surface has been commonly reported in various OK lens wear studies, molecular changes are rarely investigated. Because tear proteins play an important role in ocular defense,[19,20] altered tear protein composition may facilitate or reflect inflammation or compromise of the ocular surface.[21,22] Mass spectrometry-based proteomics approach studies have revealed expression changes in protein S100 A8, cystatin, lysozyme, and secretoglobin for daily rigid gas permeable and soft contact lenses.[23,24] For OK lens wear, selected tear proteins have been investigated and significant increases in albumin and lactate dehydrogenase concentration were noted in the first over-night OK lens wear.[25] A more recent long-term study further revealed a rise in tear inflammatory markers including IL6, IL8, and MMP9 after one year of OK lens wear.[26] Although these studies supported differentiated tear protein expression with short- and long-term OK lens wear, they were limited by the protein identities that could be resolved using conventional molecular techniques. A more comprehensive review and evaluation of the protein profile change is warranted.

The first-generation gel-based or shotgun proteomics is known to have suboptimal performance in quantification and reproducibility. Under-sampling and bias towards highly abundant proteins are also intrinsic technical limitations. Recent developments in next-generation proteomics using data-independent acquisition allow better-quality datasets in terms of reproducibility, sensitivity, and coverage and holds great potential for overcoming most constraints of traditional proteomic methods.[27] To the best of our knowledge, this study is the first to investigate ocular surface status together with changes in the global tear proteome after overnight OK lens wear, using a high-throughput next-generation proteomics platform.

Methods

Subjects

The study (clinicaltrials.gov Identifier NCT03616600) recruited subjects from the Optometry Clinic of The Hong Kong Polytechnic University. Subjects provided written informed consent before participation in the study. The subjects meeting the inclusion criteria in Table 1 were recruited. The study was approved by the Human Ethics Committee of the university and adhered to the tenets of the Declaration of Helsinki.

Study Treatment

Thirty-one eligible subjects were randomized 1:1 to the treatment group (Breath-O Correct lenses in one or both eyes) or the control group (single vision spectacles) in open-label fashion. The treatment group comprised 16 subjects who were required to wear their lenses for at least six hours per night. Of the 30 eyes initially fitted, data on only 28 were analyzed as one

Table 1. Inclusive Criteria for Subject Recruitment

Item	Criterion
Age (year)	18–30
Spherical refractive error (D)	−1.00 to 5.00
Cylindrical refractive error (D)	less than half of the spherical power (against-the-rule astigmatism ≤ 0.75D)
Best corrected Visual Acuity in ETDRS	0.00 or better
Ocular Health	No ocular disease
	No clinical signs of anterior infection or inflammation
	No contraindications or history of rigid gas permeable lens or overnight OK lens wear
	No refractive surgery
	Suspension of soft contact lens wear for at least one month before joining this study
General Health	No known systemic diseases

ETDRS, Early Treatment Diabetic Retinopathy Study.

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subject withdrew from the study. In the control group, 15 subjects met the inclusion criteria involving a total of 20 eyes. Test subjects had to follow a fixed cleansing regime, including rubbing with Biocleans cleaner (Ophtecs, Tokyo, Japan) after lens removal and soaking with Cleadew (Ophtecs) disinfection system. Aftercare visits were scheduled using a standard OK lens treatment protocol: first overnight, first week, first month, and third month).

Study Assessments

Figure 1a shows the schedule of visits and the assessments conducted for both groups. At each visit, other than the tests listed in Figure 1a, clinical parameters, including anterior corneal health and high- and low-contrast visual acuity (Early Treatment Diabetic Retinopathy Study) were assessed. Subjective spherical refractive errors (SER) were examined at the baseline visit in all subjects and at the one- and three-month visits in the treatment group. The ocular surface disease index questionnaire (OSDI) was used to assess dry eye severity (Supplementary Table S1). Refraction and uncorrected visual acuity (UVA) were assessed at the first and second week and the one- and three-month visits in the treatment group. A five-point scale evaluating lens comfort, ease of lens handling, and visual quality was also conducted at the one- and three-month visits for the treatment group. Tear fluid was collected from the eyes meeting the eligibility criteria at the one- and three-month visits in all subjects. Table 2 describes the equipment used in the study.

Analysis of Clinical Data

Normally distributed data were compared longitudinally and, if applicable, between treatment groups using repeated-measures analysis of variance, whereas Friedman test was used for non-normal distributed

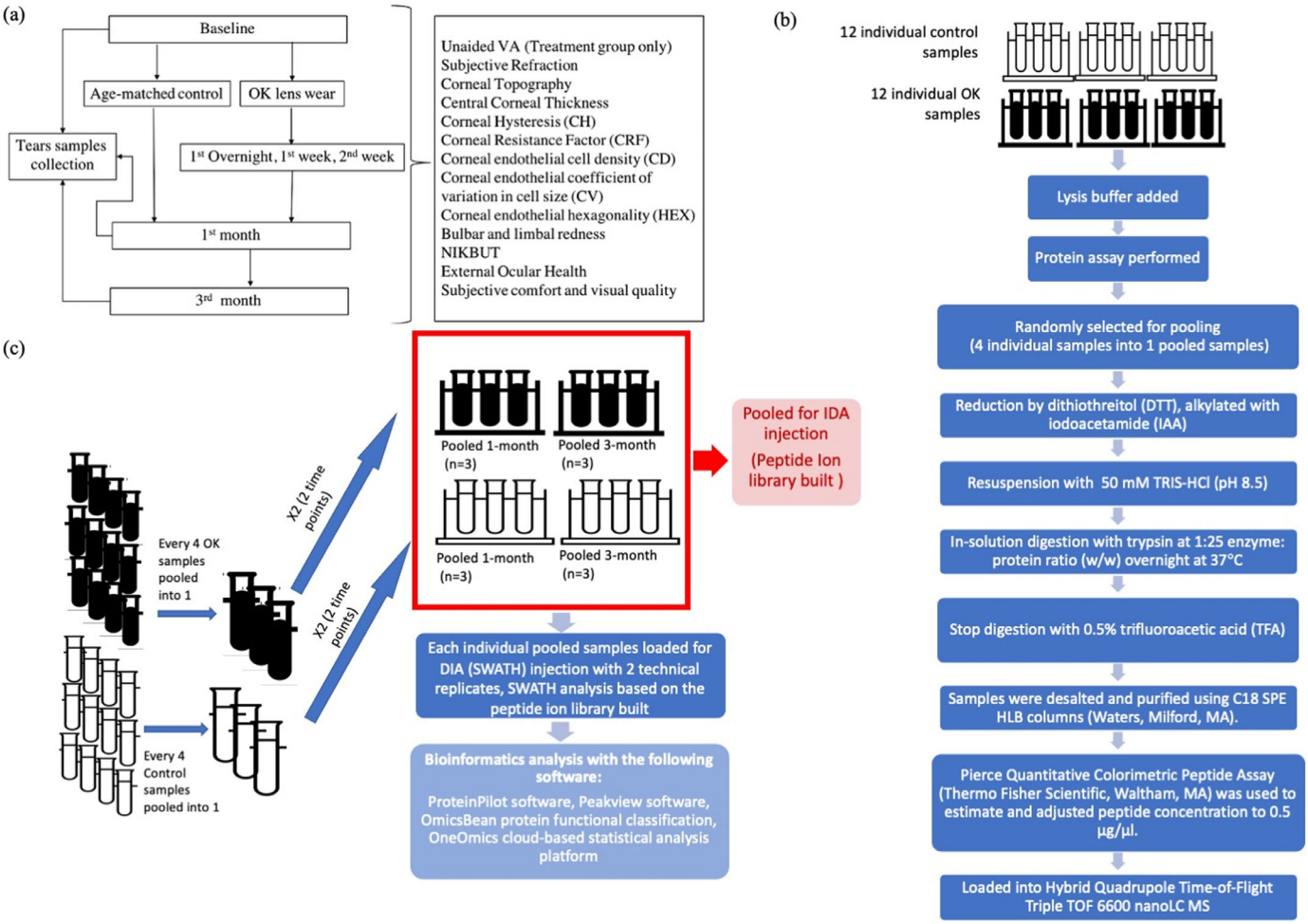

Figure 1. Study design. (a) Flow chart illustrating follow-up schedule and clinical tests performed at each visit. (b) Flow chart showing the overall proteomics workflow from sample collection to MS. (c) Flow chart illustrating samples pooling strategy for MS data acquisition and processing.

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Table 2. Equipment Used for the Measurement of Different Clinical Parameters in This Study

Equipment	Assessment
Ocular Response Analyzer (Reichert; AMETEK, Inc., USA)	Corneal topography
Ocular Response Analyzer(Reichert; AMETEK)	Corneal hysteresis
	Corneal resistance factor
Specular Microscope CEM-530 (Nidek Co. Ltd., Japan)	Central corneal thickness
	Corneal endothelial cell density
	Corneal endothelial coefficient of variation in cell size
	Corneal endothelial hexagonality
Keratograph 5M (Oculus, Wetzlar, Germany)	Bulbar and limbal redness
	First and average NIKBUT

data. The χ^2 test was used to assess the distribution of corneal staining.

Tear Sample Collection

Tear samples of approximately 10 µL were collected from the lower meniscus using a disposable MicroCap microcapillary tube (Drummond Scientific, Broomall, PA, USA) at the baseline, one- and three-month visits. The samples were immediately frozen at −20°C. One eye was randomly selected for sample collection at baseline and later visits if the subjects were eligible for bilateral OK lenses wear. Only subjects with sufficient tear fluid and protein concentration collected at all visits were included in the subsequent proteomics analysis (n = 24, 12 subjects each in the treatment and control groups).

Tear Protein Extraction, Sample Pooling, and LC-MS/MS

Sample preparation for proteomics has been described in detail previously.[28] A schematic workflow is shown in Figures 1b and 1c.

Two micrograms per pooled sample were loaded onto the MS for analysis. Both Information-Dependent Acquisition (IDA) MS and data-independent analysis (DIA) of sequential window acquisition of all theoretical ion spectra (SWATH) were obtained using a hybrid Quadrupole Time-of-Flight Triple TOF 6600 mass spectrometer (Sciex, Framingham, MA, USA). Liquid chromatography separation was performed under 350 µL min^{-1} using a C18 analytical column for a three-hour total gradient. For DIA, the instrument was tuned for a variable isolation window in a looped mode over the mass range of 100 m/z to 1800 m/z scan of 100 overlapping variable windows.

Ion Library Generation for SWATH Analysis

Before SWATH quantification, a master ion library was created by combining equal amounts of protein from all pooled samples per visit for IDA injections. The resulting MS data were searched against Homo Sapiens Uniprot database and protein identification was acquired using ProteinPilot (v5.0.1; Sciex), with the search criteria: trypsin as an enzyme, IAA for cysteine alkylation, thorough search effort, and biological modification. A 1% false discovery rate (FDR) was set as the filter.

After the generation of the master ion library, quantitative DIA (SWATH) analysis was performed. All 12 pooled samples from both treatment groups (one month and three months of lens wear) and time-matched controls were injected into LC/MS. For all samples, identical technical duplicates of the pooled samples were run for averaging. Protein spectra were extracted with PeakView (v2.2, Sciex) against the master ion library with retention time calibration of all 24 SWATH files. All data were uploaded to a novel OneOmics Cloud platform for data analysis and quantification. Only peptides achieving at least 75% confidence filter with 0.2 reproducibility were included for ratio calculation.[29] Normalization of all SWATH files was performed based on the most-likely-ratio algorithm before peptide quantitation. *P* value was determined by *t*-testing on the normalized weighted-average peptide areas for each protein across all samples in an experimental group.

Proteomic Bioinformatics Analysis

Gene ontology (GO) enrichment and protein pathway interaction network analysis of identified proteins (1% FDR) were performed using the OmicsBean online platform (http://www.omicsbean.cn) on the obtained proteome and differentially expressed

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Table 3. Baseline Demographics of the Subjects Who Completed the Three-Month Study

	Treatment ($n = 15$)	Control ($n = 15$)
Number of eyes studied	28	20
Gender		
Male	5	6
Female	10	9
Mean age (year)	19.8 ± 0.7	22.2 ± 2.4
Mean SER (D)	−3.47 ± 1.01	−3.71 ± 1.76

proteins (DEPs) at both time points where the top ten most significantly enriched GO terms with $P \leq 0.05$ were selected. DEPs at each time point were also loaded into Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway database for pathway analysis.

Results

Clinical Assessment

Thirty of the thirty-one subjects completed the three-month study. One subject in the treatment group withdrew after the 1-month visit due to mild visual quality disturbance, which resolved spontaneously after 1-week cessation of lens wear. No adverse events of ocular infection or inflammation were reported. Table 3 shows the demographics of subjects in both groups.

In the treatment group, the mean SER significantly reduced from −3.52 D at baseline to −0.17 D after one week ($P < 0.001$), and further reduced to −0.03 D at three months ($P < 0.001$ vs. baseline). Consistent with the mean SER reduction, the mean UVA improved over time. High- and low-contrast BCVA at one and three months were slightly worse than, but not significantly different from, baseline ($P > 0.05$). OSDI, comfort level, and other anterior ocular health assessments were conducted to evaluate any decompensation linked to a reduction in spherical power.

At baseline, there was no significant difference or indications of dry eye in the mean OSDI between groups. In the treatment group, the mean OSDI increased to 14.4 ± 10.4 at the one-month visit, falling to 12.4 ± 7.9 two months later, but it was not significantly different from the baseline ($P > 0.05$). In the one- to five-point comfort scale grading (higher score more favorable ratings), the mean score for each item increased after two months of wear and maintained a level higher than the median (Table 4). Staining gradings of all subjects at all visits were either negative or Grade 1 in Elfron scale. In the treatment group, the proportion of subjects with Grade 1 staining increased at the one-month visit, followed by a reduction at the three-month visit compared with baseline. The χ^2 test showed insignificance for interactions between staining frequency and OK lens wear ($P > 0.05$, Table 5). The results of ECD, CV, and HEX remained similar between groups and were essentially stable over time ($P > 0.05$, Fig. 2).

Table 4. Results of Vision Performance and Comfort in Treatment Groups in Different Visits

	Treatment				
	Baseline	1-Week	1-Month	3-Month	*P* Value
Mean SER (D)*	−3.52 ± 1.02	−0.17 ± 0.99†	−0.11 ± 0.92	−0.03 ± 0.82	<0.001† (baseline vs 1-week)
Mean UVA‡	N/A	0.41 ± 0.28	0.13 ± 0.16	0.14 ± 0.18	
High contrast BCVA‡	−0.10 ± 0.06	N/A	−0.07 ± 0.08	−0.08 ± 0.08	0.261
Low contrast BCVA‡	0.08 ± 0.08	N/A	0.22 ± 0.14	0.17 ± 0.13	0.085
Lens comfort§	N/A	N/A	3.7 ± 0.5	3.9 ± 0.5	0.317
Ease of lens handling§	N/A	N/A	3.9 ± 0.8	4.0 ± 0.5	0.705
Visual quality§	N/A	N/A	3.1 ± 0.8	3.4 ± 0.6	0.096

N/A, data not taken due to clinical insignificance of the parameter at that timepoint.
*Repeated-measures analysis of variance.
†Significant difference compared with baseline.
‡Friedman test.
§Wilcoxon signed rank test.

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Table 5. Results of Parameters Assessed in Both Groups at Various Visits

	Control				Treatment			
	Baseline	1-Month	3-Month	*P* Value	Baseline	1-Month	3-Month	*P* Value
OSDI Score*	9.5 ± 10.0	10.2 ± 9.86	7.6 ± 8.0	0.551	8.9 ± 7.4	14.4 ± 10.4	12.4 ± 7.9	0.089
CH (mm Hg)†	11.10 ± 1.36	11.20 ± 1.89	11.19 ± 1.66	0.88	11.12 ± 1.12	10.37 ± 1.36	10.14 ± 0.89	<0.05‡
CRF (mm Hg)†	11.14 ± 2.23	10.92 ± 2.45	10.79 ± 2.17	0.24	11.07 ± 1.32	9.87 ± 1.45	9.99 ± 1.37	<0.05‡
ECD cell/mm^2)*	2861 ± 181.05	2818 ± 182.65	2825 ± 177.80	0.29	2909 ± 145.31	2923 ± 123.01	2917 ± 157.34	0.89
CV*	29.35 ± 3.66	29.00 ± 3.58	30.01 ± 3.32	0.33	28.68 ± 3.78	28.47 ± 3.51	29.00 ± 6.53	0.97
HEX (%)†	63.65 ± 4.80	66.25 ± 3.31	63.50 ± 5.79	0.09	64.39 ± 5.02	66.64 ± 3.23	65.96 ± 4.49	0.63
Bulbar redness†	0.58 ± 0.28	0.52 ± 0.21	0.61 ± 0.27	0.84	0.57 ± 0.18	0.47 ± 0.19	0.54 ± 0.31	0.13
Limbal redness*	0.29 ± 0.26	0.31 ± 0.21	0.38 ± 0.28	0.06	0.26 ± 0.19	0.23 ± 0.15	0.27 ± 0.21	0.18
NIKBUT (s) (Average)*	13.24 ± 6.56	11.86 ± 5.99	9.57 ± 5.02	0.12	10.94 ± 4.15	12.42 ± 5.73	12.07 ± 6.76	0.94
NIKBUT (s) (first break)*	9.92 ± 6.76	9.17 ± 5.63	7.64 ± 4.97	0.82	6.72 ± 3.96	9.13 ± 5.45	9.83 ± 6.37	0.23
No corneal stain§	55%	35%	30%	0.132	32%	25%	49%	0.233
G1 corneal stain§	45%	65%	70%		68%	75%	51%	
≥G2 corneal stain	0%	0%	0%		0%	0%	0%	

N/A, data not taken because of clinical insignificance of the parameter at that timepoint.
*Friedman test.
†Repeated-measures analysis of variance.
‡Significant difference compared with baseline.
§χ^2 test.

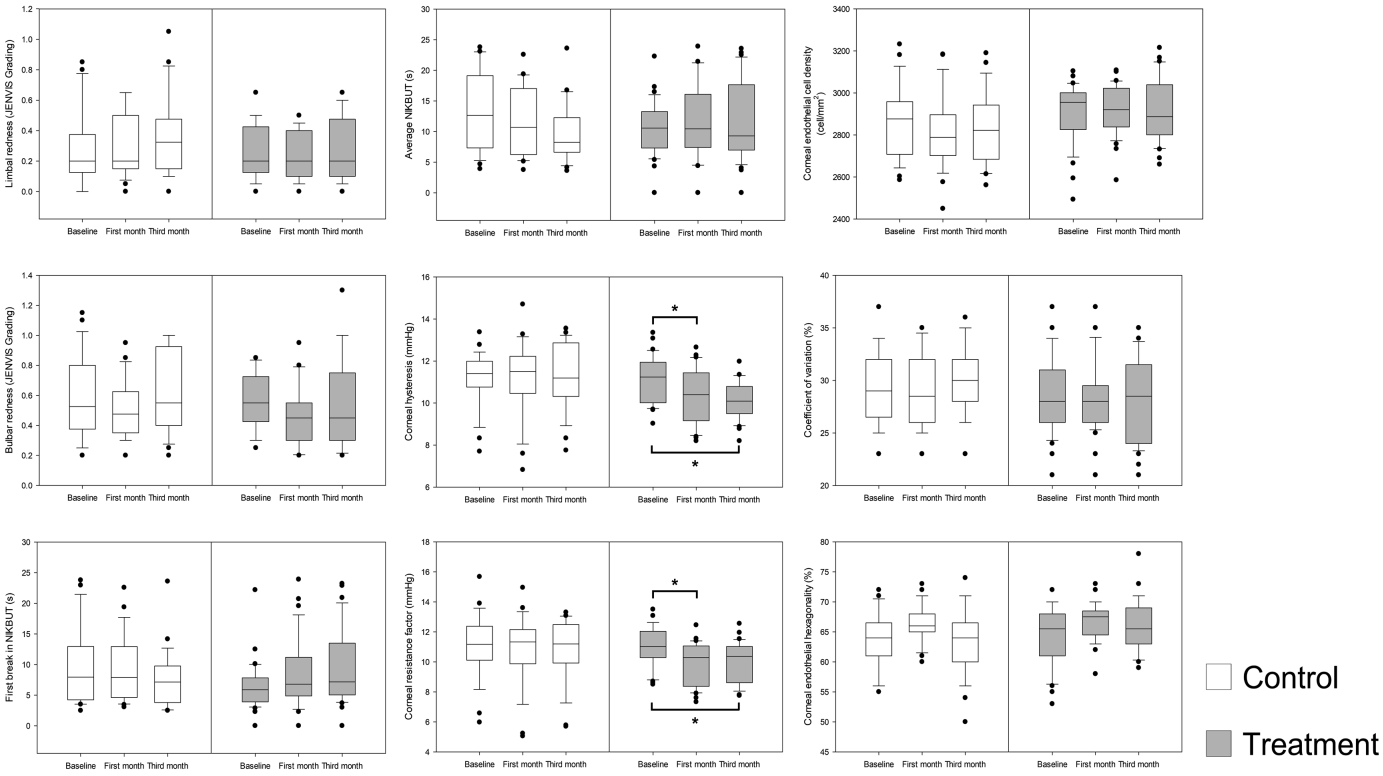

Figure 2. Clinical parameters (Control vs. Ortho-K) at baseline, one-month, and three-month visits. ° denotes outlying data points; * denotes significant difference of $P < 0.05$ between measurements after Bonferroni post-hoc adjustment.

The most noticeable changes were corneal biomechanics. Significant reductions in mean CH and CRF were observed in the treatment group as compared with baseline at one month ($P < 0.05$) and remained stable after two months (Fig. 2). CH and CRF did not change over time in the control group.

There were no significant physiological changes of the ocular surface in terms of bulbar and limbal

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redness over time or between groups ($P > 0.05$, Fig. 2). The noninvasive keratograph tear break up time (NIKBUT) results over time trended numerically higher in the treatment group and lower in the control group but without statistical significance ($P > 0.05$, Fig. 2).

Tear Proteome and Functional Classification

Sufficient tear fluid was collected from 12 subjects each in the treatment and control groups. The combined search of all the IDA injections identified a total of 519 unique tear proteins (6745 peptides) at 1% FD with a dynamic range covering approximately five orders of magnitude. The full list of protein names and identification numbers has been published[28] and all MS raw data generated from this study were peer-reviewed and released in the Peptide Atlas public repository for free access (Data ID PASS01367).

To characterize the obtained tear proteome, the online OmicsBean online platform (http://www.omicsbean.cn) was used for the classification of identified proteins based on GO-defined terms for biological process (BP), cell component (CC), and molecular function (MF) shown in Figure 3a. According to the classification for BP, 227 genes (∼50%) were related to the stress response, and ∼40% of genes were related to vesicle-mediated transport and response to external stimulus. In terms of CC, the majority of the proteins (∼80%) were located extracellularly. Further categorization for MF showed that ∼90% of the genes had a binding function, including protein, receptor, and antigen-binding. The remaining genes could be related to various activities, such as endopeptidase and serine hydrolase activities. The list of identified proteins was also loaded into the KEGG pathway database for pathway analysis (Fig. 3b). Pathways were categorized into six main groups by the pathway for which the most genes were identified: (A) metabolism–metabolic pathways (∼12%); (B) genetic information processing–protein processing in endoplasmic reticulum (∼3%); (C) environmental information processing–hypoxia-inducible factor-1–signaling pathway and ECM receptor interaction (∼2%); (D) cellular process–lysosome (∼5%); (E) organismal systems–estrogen signaling pathway (∼4%); (F) human diseases–*Staphylococcus aureus* infection (∼4%).

SWATH-MS Quantitation

Differential protein expressions between OK lens wear and control subjects were compared at baseline, one month, and three months. All 36 injections for SWATH-MS analysis passed quality control and achieved very good alignment in terms of normalization and retention time alignment over several days of continuous running.

At the one-month visit, 488 proteins were identified in both the treatment and control groups, of which 13 proteins were differentially expressed between the therapeutic groups as tabulated in Table 6 (≥1.5-fold change, $P < 0.05$), including 10 up-regulated and three down-regulated proteins. At the three-month visit, of the 491 proteins identified in both the treatment and control groups, eight were differentially expressed, including three up-regulated and five down-regulated proteins as tabulated in Table 7 (≥1.5-fold change, $P < 0.05$). The commonly DEPs in the one-month and three-month visits did not vary at baseline between the treatment and control groups.

Investigation of the total tear proteome indicated that OK lens wear led to expression change in fewer than 3% and 2% of all identified proteins after one and three months, respectively. Regarding the longitudinal protein changes, OK lens wear–induced up-regulation of proline-rich protein 27 and down-regulation of immunoglobulin V regions were the only expression changes that were consistently found at both visits. There was no change in expression of known inflammatory mediators (e.g., cytokines or pro-inflammatory interleukins [IL-1, IL-6], tumor necrosis factor-alpha, vascular endothelial growth factor, or matrix metalloproteinases) or major tear proteins (e.g., lactotransferrin, lysozyme C, and lipocalin-1).

DEPs at both time points were loaded for GO analysis individually shown in Figures 4a and 4b for one month and three months, respectively. According to the classification for BP, the top three processes were response to external stimulus, movement of a cell or subcellular component, and the regulation of cellular component movement for one-month study, whereas regulation of proteolysis, proteolysis, and the regulation of protein metabolic process for 3 months study. In terms of CC, the majority of the genes (>80%) for both time points were located extracellularly which was also in line with the obtained proteome (Shown in Figs. 4a and 4b). Further categorization for MF showed that binding functions (protein and receptor) and molecular function regulator were the top three functions for the one-month study, whereas inhibitor activities (enzyme, peptidase, and endopeptidase) were observed for the three-month study. Further pathway enrichment analysis on the DEPs for the one-month and three-month studies were showed in Figures 4c and 4d, respectively.

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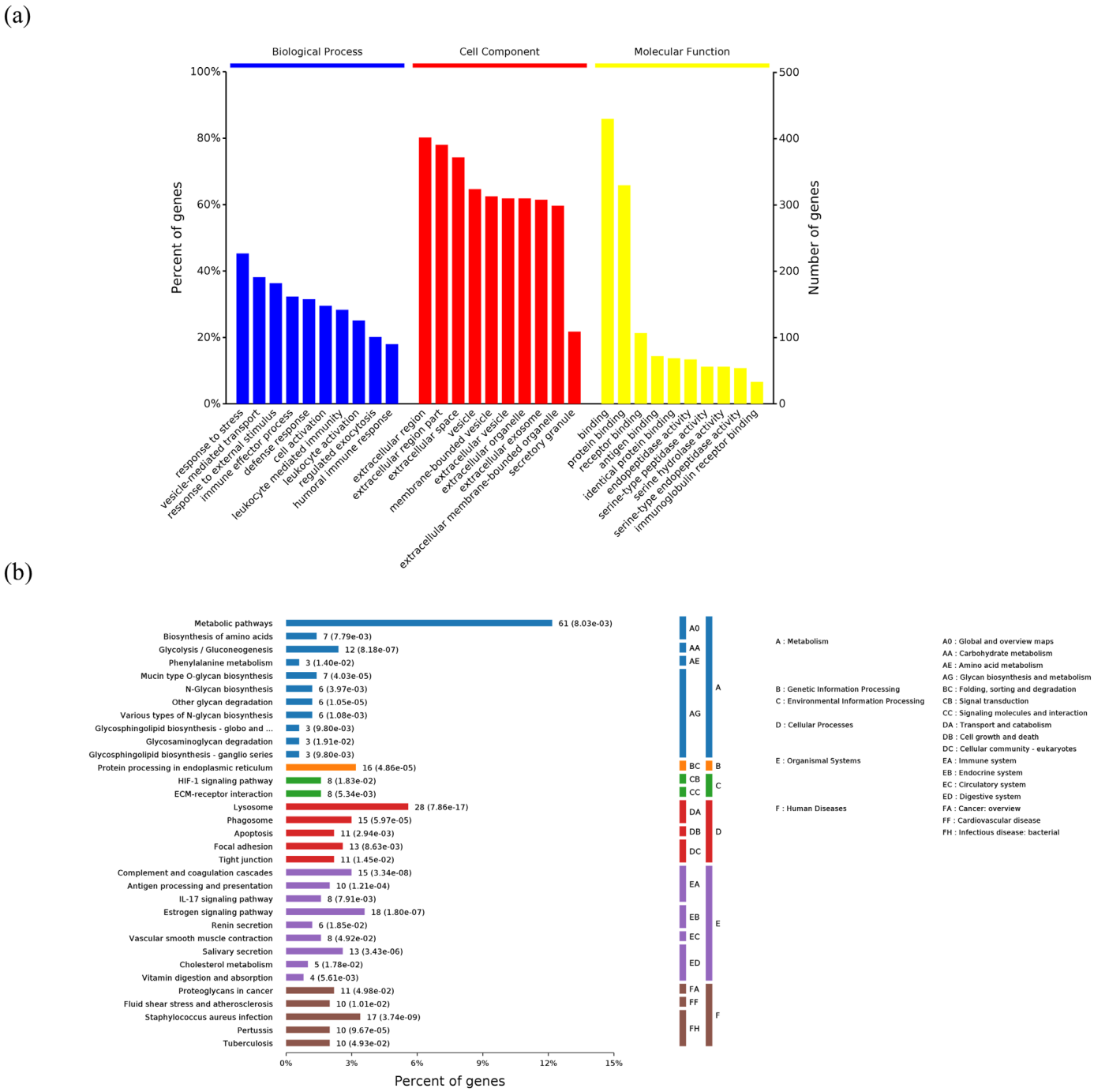

Figure 3. (a) An overview of GO analysis of the obtained tears proteome (1 % FDR) using the OmicsBean online platform of the top 10 most significant enriched terms of BP, MF, and CC which are represented by *blue*, *red*, and *yellow bars*, respectively. (b) Pathway enrichment analysis of the obtained tears proteome with KEGG pathway database.

Discussion

Clinical and Patient Reported Outcomes

Despite its well-established effectiveness in myopia control,[30] previous studies have shown various physiological changes in OK lens wear, including significantly decreased tear break up time (TBUT), aggravated corneal staining, increase in OSDI score, as well as a drop in CH and CRF.[31,32] The most significant changes in NIKBUT and bulbar redness were noticed in the first week and month, which then reverted to a level comparable to baseline after one year of wear,

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Table 6. Summary of the DEPs in Tears From Treatment Group Compared to Control at the One-Month Visit

Uniprot Name	Gene ID	Protein Name	Fold Change	*P* Value
Up-regulated proteins				
P21246|PTN_HUMAN	PTN	Pleiotrophin	3.27	<0.01
Q6MZM9|PRR27_HUMAN	PRR27	Proline-rich protein 27	1.91	<0.01
Q6UXB2|CXL17_HUMAN	CXCL17	C-X-C motif chemokine 17	1.67	<0.01
Q9UDW1|QCR9_HUMAN	UQCR10	Cytochrome b-c1 complex subunit 9	1.59	<0.01
Q9UBT3|DKK4_HUMAN	DKK4	Dickkopf-related protein 4	1.59	<0.01
P14138|EDN3_HUMAN	EDN3	Endothelin-3	1.58	<0.01
P07477|TRY1_HUMAN	PRSS1	Trypsin-1	1.53	0.03
Q14050|CO9A3_HUMAN	COL9A3	Collagen alpha-3 (IX) chain	1.52	<0.01
Q02487|DSC2_HUMAN	DSC2	Desmocollin-2	1.51	<0.01
Q9NRJ3|CCL28_HUMAN	CCL28	C-C motif chemokine 28	1.51	0.01
Down-regulated proteins				
A0A0C4DH31|HV118_HUMAN	IGHV1-18	Immunoglobulin heavy variable 1–18	−1.88	0.05
O14745|NHRF1_HUMAN	SLC9A3R1	Na (+)/H (+) exchange regulatory cofactor NHE-RF1	−2.05	<0.01
P59666|DEF3_HUMAN	DEFA3	Neutrophil defensin 3	−15.31	0.02

Table 7. Summary of DEPs in Tears From Treatment Group Compared to Control at the Three-Month Visit

Uniprot Name	Gene ID	Protein Name	Fold Change	*P* Value
Up-regulated proteins				
P28325|CYTD_HUMAN	CST5	Cystatin-D	3.61	<0.01
Q6MZM9|PRR27_HUMAN	PRR27	Proline-rich protein 27	1.60	0.02
Q96DA0|ZG16B_HUMAN	ZG16B	Zymogen granule protein 16 homolog B	1.55	<0.01
Down-regulated proteins				
P04430|KV116_HUMAN	IGKV1-16	Immunoglobulin kappa variable 1–16	−2.13	<0.01
P06702|S10A9_HUMAN	S100A9	Protein S100-A9	−2.45	<0.01
A0A075B6Q5|HV364_HUMAN	IGHV3-64	Immunoglobulin heavy variable 3–64	−3.00	0.02
P36952|SPB5_HUMAN	SERPINB5	Serpin B5	−4.84	<0.01
P31947|1433S_HUMAN	SFN	14-3-3 protein sigma	−5.61	0.03

Criteria for a protein to be considered as differentially expressed: ≥1.5-fold change with at least 2 quantifiable peptides per protein (ion score ≥ 99), FDR <1%, identified in all three biological samples and two technical replicates, $P < 0.05$, analyzed by *t*-test.

suggesting a physiological adaptation to lens wear.[33] In our study, staining occurrence insignificantly rose in the first month and then reduced to lower than baseline in the third month, being consistent with other studies,[11,34] which revealed a most noticeable increase in initial wear, which subsided over a longer treatment period. The OSDI score in the current study shares similar findings with previous reports.[31,35,36] However, the accuracy of the test would be limited by questions on vision, which may be affected by residual astigmatism or ocular aberration caused by OK lens wear. Compared with other studies, the insignificant improvement of first and average NIKBUT in the current treatment group compared with the control was unexpected. This may be attributable to the high variation potential of NIKBUT measurement depending on time and humidity. Considering the minimal impact from NIKBUT and bulbar redness, as well as insignificant changes in OSDI score, endothelial cells parameters, and corneal staining, a less irritative profile of the Breath-O Correct lenses may be suggested. The lenses' durability and flexibility may play a role in reducing irritation to the cornea and allowing easier adaptation.

Regarding the morphological and histological changes during remolding of corneal shape, many studies have explored the impacts of OK on corneal biomechanics. Sharing similar findings with other studies, the current study revealed a significant decline over three months in CH and CRF in the OK group, with the greatest changes in the first month.[37–39] In this study, the corneal biomechanics changed without accompanying impactful physiological consequences or increased inflammatory mediators in tears,[37–39] suggesting such an alteration could be physiologically associated with OK lens wear but not detrimental.

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Proteomic Analysis of OK Lens Tears

GO analysis on the DEPs from both studies indicated that while the CC did not alter much (>80% of genes were from the extracellular space/region), the high number of enriched genes from the one-month study had a BP for the response to external stimulus. This suggested that the introduction of OK-lens could trigger protein changes initially but will return to a normal state because these were not observed in the three-month study (Shown in Figs. 4a and 4b). Furthermore, GO analysis on the OK-lens tears proteome obtained from this study indicated a wide range of protein functions, including inflammatory pathways (shown in Figs. 3a and 3b). Although the DEPs (CXCL17 and CCL28) after one month of lens wear could play a role in immune signaling pathways,[40] such expression changes were not observed after three months of lens wear using pathway enrichment analysis (shown in Figs. 4c and 4d). Results of the tear-fluid proteomic analysis support the clinical findings, in that there were no increases in inflammation-associated proteins nor change in major tear-fluid components, and only minor changes in protein expression were observed. Overall, the tear proteome of lens wearers showed fewer changes after three months of OK lens wear than at one month.

Common differential expressions of protein after both one and three months included up-regulation of proline-rich protein (PRR) 27 and down-regulation of immunoglobulin. According to the ELICIR core database, the gene for PRR27 has the highest expression level in the minor salivary gland, and it was noted to be involved in tooth surface defense.[41] The tear proteomic profile in three distinct ocular surface diseases (keratoconus, pterygium, and dry eye related to graft-versus-host disease[42,43]) suggested that PRR27 in tears could be a possible biomarker for keratoconus, but the function of PRR27 in tears remains to be elucidated. Immunoglobulin is an antimicrobial protein that increases with immune response,[44] and the reasons for the specific Immunoglobulin V region down-regulation following OK lens wear remains to be determined.

Cystatin-D (CST5), PRR27, and Zymogen granule protein 16 homolog B (ZG16B) were found to be up-regulated after three months of OK lens wear.

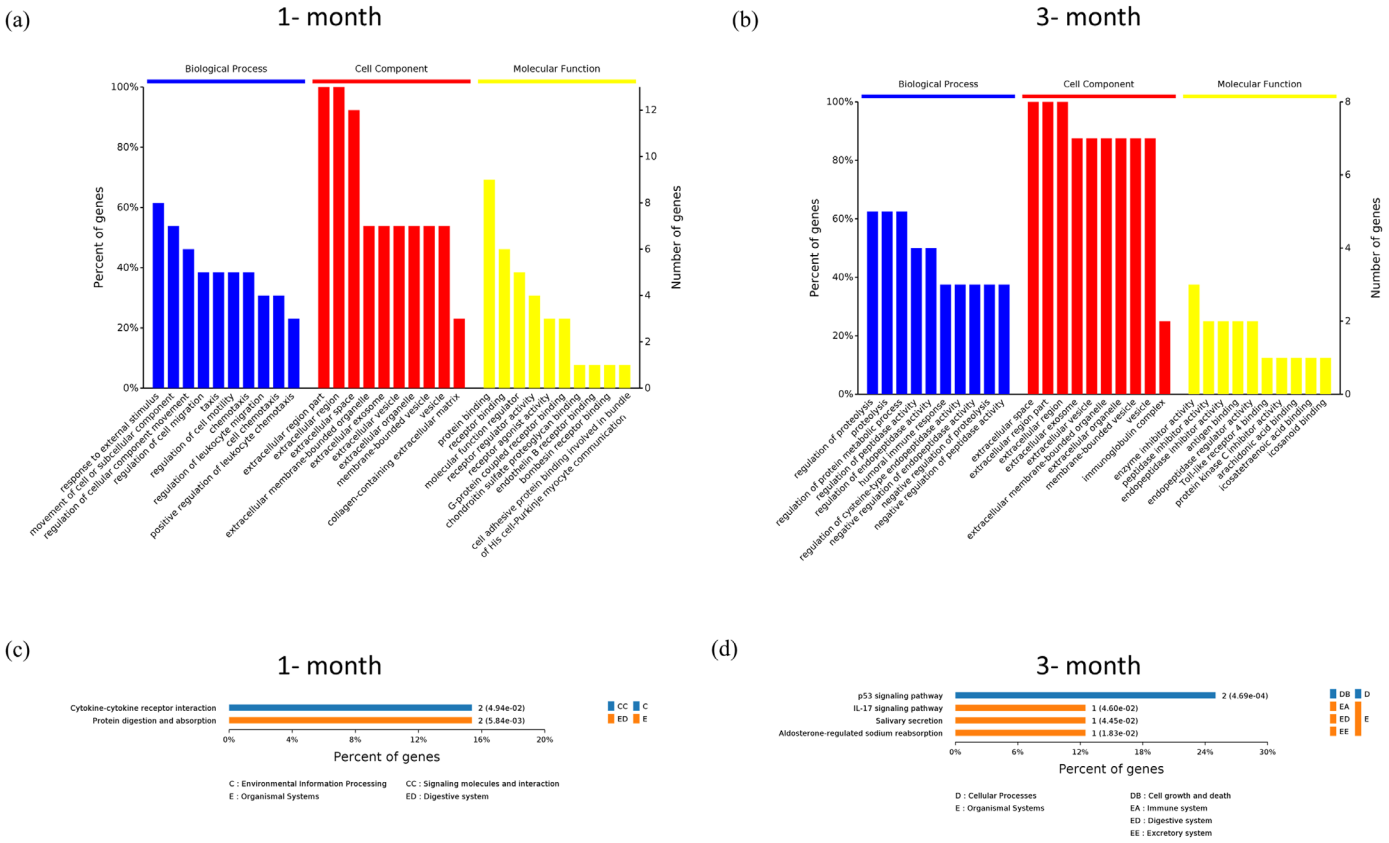

Figure 4. GO analysis on the DEPs from SWATH quantitation in (a) one-month and (b) three-month studies using the OmicsBean online platform. The top 10 most significant enriched terms of BP, MF, and CC are represented by *blue*, *red*, and *yellow bars*, respectively. Pathway enrichment analysis of the DEPs with KEGG pathway database in (c) one-month and (d) three-month studies.

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Cystatins are proteinase inhibitors playing a protective role against tissue damage and may be reduced in keratoconus and blepharitis patients.[45–47] Although functions of ZG16B alone in human tears are unclear, similar up-regulation of cystatin and ZG16B were noted in daily rigid contact lens wearers,[23,24] whereas up-regulation of PRR isoform (PRR4) and down-regulation of immunoglobulin were reported in human reflex tears.[48]

Therefore tear proteomic changes in this study may be caused by the rigid contact lens wear, the subsequence reflex responses, or the significant corneal biomechanical changes as mentioned before. Although further studies are needed to investigate the mechanism behind the protein changes associated with OK lens wear, the overall change in tear proteins did not indicate any inflammatory or infectious events and the results showed the materials of the new OK lens should be safe to wear from a global proteomic perspective.

Limitations of this Study

Limitations of the present study include a small sample size, relatively older subjects than those normally wearing OK lens, a short study period, and lack of comparison with other studies in terms of tears proteomics in OK as this approach is novel. Because of the higher operating cost with SWATH-MS (much higher count of individual sample injections) than typical shotgun MS, the sample pooling method was applied in this study to reduce cost as a discovery-based strategy, which has also been adopted in a number of our previous studies using SWATH-MS.[49–51] Although sample pooling is a common strategy, it may mask potential biomarkers and not be sensitive to detect an individual change; however, it should not artificially produce false results because the sample starting amount (concentration) was equalized. The trend of a particular protein expression after pooling should remain the same if the general trend of that protein is following the same direction in the majority of the subjects. Furthermore, the proteomics data obtained in this pilot study was not intended for a direct comparison between individual subjects with clinical data collected, but to provide an overview of the general differences between the two groups under the observation period (one month and three months) using SWATH-MS quantitation as an initial filtering method. Thus the screened targeted proteins using individual samples should be further validated using other methods such as Western blot or multiple-reaction monitoring in separate cohorts for orthogonal validation.

Overall, this study supports the efficacy of Breath-O Correct lenses in correcting spectral power in young subjects with myopia up to −4.00 D without undesirable effects on ocular health. The clinical acceptance, efficacy, and effects on corneal health appear to be similar to the characteristics of those reported for other OK lenses. This study for the first time demonstrates the feasibility of tear proteome characterization in clinical safety and efficacy studies. Because dynamic molecular alterations in the tears can be monitored using the sensitive SWATH-MS approach, this has opened an opportunity to explore the interaction between tear proteome regulation and ocular stimulation in various contact lens wear modalities, especially overnight OK wear.

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References

1. Efron N, Morgan PB, Woods CA, The International Contact Lens Prescribing Survey Consortium. Survey of contact lens prescribing to infants, children, and teenagers. *Optom Vis Sci*. 2011;88:461–468.
2. Cho P, Cheung SW. Retardation of myopia in orthokeratology (ROMIO) study: a 2-year randomized clinical trial. *Invest Ophthalmol Vis Sci*. 2012;53:7077–7085.
3. Kakita T, Hiraoka T, Oshika T. Influence of overnight orthokeratology on axial elongation in childhood myopia. *Invest Ophthalmol Vis Sci*. 2011;52:2170–2174.
4. Santodomingo-Rubido J, Villa-Collar C, Gilmartin B, Gutiérrez-Ortega R. Myopia

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control with orthokeratology contact lenses in Spain: refractive and biometric changes. *Invest Ophthalmol Vis Sci*. 2012;53:5060–5065.
5. Walline JJ, Jones LA, Sinnott LT. Corneal reshaping and myopia progression. *Br J Ophthalmol*. 2009;93:1181–1185.
6. Santodomingo-Rubido J, Villa-Collar C, Gilmartin B, Gutiérrez-Ortega R, Sugimoto K. Long-term efficacy of orthokeratology contact lens wear in controlling the progression of childhood myopia. *Curr Eye Res*. 2017;42:713–720.
7. Zhu M-J, Feng H-Y, He X-G, Zou H-D, Zhu J-F. The control effect of orthokeratology on axial length elongation in Chinese children with myopia. *BMC Ophthalmol*. 2014;14:1–9.
8. Caroline PJ. Contemporary orthokeratology. *Cont Lens Anterior Eye*. 2001;24:41–46.
9. Swarbrick HA, Wong G, O'Leary DJ. Corneal response to orthokeratology. *Optom Vis Sci*. 1998;75:791–799.
10. Chan B, Cho P, Mountford J. Relationship between corneal topographical changes and subjective myopic reduction in overnight orthokeratology: a retrospective study. *Clin Exp Optom*. 2010;93:237–242.
11. Lui WO, Edwards MH. Orthokeratology in low myopia. Part 1: efficacy and predictability. *Cont Lens Anterior Eye*. 2000;23:77–89.
12. Kang P, Swarbrick H. Peripheral refraction in myopic children wearing orthokeratology and gas-permeable lenses. *Optom Vis Sci*. 2011;88:476–482.
13. Kang P, Swarbrick H. New perspective on myopia control with orthokeratology. *Optom Vis Sci*. 2016;93:497–503.
14. Bullimore MA, Sinnott LT, Jones-Jordan LA. The risk of microbial keratitis with overnight corneal reshaping lenses. *Optom Vis Sci*. 2013;90:937–944.
15. Nieto-Bona A, González-Mesa A, Nieto-Bona MP, Villa-Collar C, Lorente-Velázquez A. Short-term effects of overnight orthokeratology on corneal cell morphology and corneal thickness. *Cornea*. 2011;30:646–654.
16. Nieto-Bona A, González-Mesa A, Nieto-Bona MP, Villa-Collar C, Lorente-Velázquez A. Long-term changes in corneal morphology induced by overnight orthokeratology. *Curr Eye Res*. 2011;36:895–904.
17. Stillitano IG, Chalita MR, Schor P, et al. Corneal changes and wavefront analysis after orthokeratology fitting test. *Am J Ophthalmol*. 2007;144:378–386.
18. Zhong X, Chen X, Xie RZ, et al. Differences between overnight and long-term wear of orthokeratology contact lenses in corneal contour, thickness, and cell density. *Cornea*. 2009;28:271–279.
19. McDermott AM. Antimicrobial compounds in tears. *Exp Eye Res*. 2013;117:53–61.
20. Davidson HJ, Kuonen VJ. The tear film and ocular mucins. *Vet Ophthalmol*. 2004;7:71–77.
21. Nichols JJ, Green-Church KB. Mass spectrometry-based proteomic analyses in contact lens-related dry eye. *Cornea*. 2009;28:1109–1117.
22. Zhou L, Zhao SZ, Koh SK, et al. In-depth analysis of the human tear proteome. *J Proteomics*. 2012;75:3877–3885.
23. Kramann C, Boehm N, Lorenz K, et al. Effect of contact lenses on the protein composition in tear film: a ProteinChip study. *Graefes Arch Clin Exp Ophthalmol*. 2011;249:233–243.
24. Manicam C, Perumal N, Wasielica-Poslednik J, et al. Proteomics unravels the regulatory mechanisms in human tears following acute renouncement of contact lens use: a comparison between hard and soft lenses. *Sci Rep*. 2018;8:1–15.
25. Choy CKM, Cho P, Benzie IFF, Ng V. Effect of one overnight wear of orthokeratology lenses on tear composition. *Optom Vis Sci*. 2004;81:414–420.
26. González-Pérez J, Villa-Collar C, Moreiras TS, et al. Tear film inflammatory mediators during continuous wear of contact lenses and corneal refractive therapy. *Br J Ophthalmol*. 2012;96:1092–1098.
27. Gillet LC, Navarro P, Tate S, et al. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. *Mol Cell Proteomics*. 2012;11:1–17.
28. Tse JSH, Lam TC, Cheung JKW, Sze YH, Wong TK, Chan HHL. Data on assessment of safety and tear proteome change in response to orthokeratology lens–Insight from integrating clinical data and next generation proteomics. *Data Brief*. 2020;29:105186.
29. Shan SW, Tse DYY, Zuo B, et al. Integrated SWATH-based and targeted-based proteomics provide insights into the retinal emmetropization process in guinea pig. *J Proteomics*. 2018;181:1–15.
30. Wen D, Huang J, Chen H, et al. Efficacy and acceptability of orthokeratology for slowing myopic progression in children: a systematic review and meta-analysis. *J Ophthalmol*. 2015;2015:360806.
31. Lee KW, Jung JW. Comparison of changes in ocular surface status after wearing orthokeratologic and rigid gas permeable lens. *J Korean Ophthalmol Soc*. 2016;57:546–554.

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32. Li J, Dong P, Liu H. Effect of overnight wear orthokeratology lenses on corneal shape and tears. *Eye Contact Lens*. 2018;44:304–307.
33. Xie W, Zhang X, Xu Y, Yao YF. Assessment of tear film and bulbar redness by keratograph 5M in pediatric patients after orthokeratology. *Eye Contact Lens*. 2018;44:S382–386.
34. Chan B, Cho P, Cheung SW. Orthokeratology practice in children in a university clinic in Hong Kong. *Clin Exp Optom*. 2008;91:453–460.
35. Yang L, Zhang L, Hu RJ, Yu PP, Jin X. The influence of overnight orthokeratology on ocular surface and dry eye-related cytokines IL-17A, IL-6, and PGE2 in children. *Cont Lens Anterior Eye*. 2021;44:81–88.
36. Liu X, Zhang L, Luo X. 角膜塑形镜对青少年眼表和泪膜的影响 [Influence of the orthokeratology on the ocular surface and tear film in adolescents]. *Int J Ophthalmol*. 2019;19:2170–2173.
37. Lam AK, Hon Y, Leung SY, Shu-Ho L, Chong J, Lam DC. Association between long-term orthokeratology responses and corneal biomechanics. *Sci Rep*. 2019;9:1–9.
38. González-Méijome JM, Villa-Collar C, Queirós A, Jorge J, Parafita MA. Pilot study on the influence of corneal biomechanical properties over the short term in response to corneal refractive therapy for myopia. *Cornea*. 2008;27:421–426.
39. Yeh TN, Green HM, Zhou Y, et al. Short-term effects of overnight orthokeratology on corneal epithelial permeability and biomechanical properties. *Invest Ophthalmol Vis Sci*. 2013;54:3902–3911.
40. Hernández-Ruiz M, Zlotnik A. Mucosal Chemokines. *J Interferon Cytokine Res*. 2017;37:62–70.
41. Hajishengallis G, Russell MW. Innate humoral defense factors. 4th ed. *Mucosal Immunol*. USA: Academic Press; 2015:251–270.
42. de Almeida Borges D, Alborghetti MR, Leme AFP, et al. Tear proteomic profile in three distinct ocular surface diseases: keratoconus, pterygium, and dry eye related to graft-versus-host disease. *Clin Proteomics*. 2020;17:1–16.
43. de Almeida Borges D, Alborghetti MR, Franco Paes Leme A, et al. Tear proteomic profile in three distinct ocular surface diseases: keratoconus, pterygium, and dry eye related to graft-versus-host disease. *Clinical Proteomics*. 2020;17:42.
44. Sen DK, Sarin GS. Immunoglobulin concentrations in human tears in ocular diseases. *Br J Ophthalmol*. 1979;63:297–300.
45. Acera A, Suárez T, Rodríguez-Agirretxe I, Vecino E, Durán JA. Changes in tear protein profile in patients with conjunctivochalasis. *Cornea*. 2011;30:42–49.
46. ter Rahe BS, van Haeringen NJ. Cystatins in tears of patients with different corneal conditions. *Ophthalmologica*. 1998;212:34–36.
47. Koo BS, Lee DY, Ha HS, Kim JC, Kim CW. Comparative analysis of the tear protein expression in blepharitis patients using two-dimensional electrophoresis. *J Proteome Res*. 2005;4:719–724.
48. Perumal N, Funke S, Wolters D, Pfeiffer N, Grus FH. Characterization of human reflex tear proteome reveals high expression of lacrimal proline-rich protein 4 (PRR4). *Proteomics*. 2015;15:3370–3381.
49. Cheung JK, Li KK, Zhou L, To CH, Lam TC. Data on protein changes of chick vitreous during normal eye growth using data-independent acquisition (SWATH-MS). *Data Brief*. 2020;30:105576.
50. Yu FJ, Lam TC, Sze AY, et al. Alteration of retinal metabolism and oxidative stress may implicate myopic eye growth: Evidence from discovery and targeted proteomics in an animal model. *Journal of Proteomics*. 2020;221:103684.
51. Kang BS, Lam TC, Cheung JK-w, Li KK, Kee C-s. Corneal proteome and differentially expressed corneal proteins in highly myopic chicks using a label-free SWATH-MS quantification approach. *Scientific Reports*. 2021;11:5495.

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