Dry eye is a multifactorial disease of the ocular surface that may represent a diagnostic challenge. In fact, the diagnosis is made difficult for three main reasons: firstly, dry eye is a spectrum of diseases of the ocular surface with multiple etiologies. Secondly, a comprehensive definition of all abnormalities associated with dry eye is not easy. Thirdly, the lacrimal tests in clinical use for dry eye have many limitations. Aspects regarding the tear film stability in vivo are of major interest, to observe fine details of the cornea and understand new markers,19 the sodium carboxymethylcellulose (NaCMC), to evaluate in vivo the adhesive properties of the anterior corneal surface in dry eye patients and in control group subjects. In addition, we evaluated the correlation between the corneal adhesiveness and classical tear tests, as well as the diagnostic validity of our new method of OCT imaging. 

Methods
The study complied with the guidelines in the Declaration of Helsinki for research involving human subjects. Informed consent to participate was obtained from all subjects, after ethics approval obtained from the Office of Research Ethics, University of Cagliari. We studied 36 dry eye patients (52.5 ± 17.5 years [mean ± SD], 75% female). A control group of 49 subjects of similar age and sex distribution (52.6 ± 20.1 years, 80% female) was recruited from students and staff members of the School of Ophthalmology, and patients of the Eye Clinic. They were included in this group if they had no history of any substance, NIBUT), despite its limitations, is considered to be a valid surrogate measure of tear film stability. Recently, optical coherence tomography (OCT) has been used to obtain detailed cross-sectional images of anterior tissues of the eye and to investigate the dynamic behavior of tears on the ocular surface, suggesting to represent a promising tool to observe fine details of the cornea and understand new aspects regarding the tear film stability in vivo.

PURPOSE. The purpose of this work was to gather preliminary data on tear film stability, and the diagnostic validity of our new method of OCT imaging.

METHODS. We screened 85 human subjects for dry eye and classified them in two groups, as dry eye or normal patients. Sodium carboxymethylcellulose (NaCMC) adhesiveness over the central cornea was measured using Fourier domain anterior segment OCT. The corneal adhesiveness for NaCMC was compared between the two groups, correlated with classical tests, and analyzed for diagnostic validity and repeatability.

RESULTS. The corneal adhesiveness for NaCMC median and mode values was fair (between 1 and 3 minutes) for dry eye subjects (n = 36) and borderline (between 3 and 5 minutes) for control group subjects (n = 49), and was significantly different between the two groups (P < 0.001). Significant correlations were observed between the corneal adhesiveness measures and dry eye patients’ symptoms (P < 0.001), Schirmer I test (P < 0.001), ocular surface staining (P < 0.001), and, particularly, fluorescein break-up time (P < 0.001). The area under the receiver operating characteristic curve was 0.94 (P < 0.001), suggesting reliable sensitivity and specificity of OCT imaging. A statistically significant intraclass correlation (ICC) value of 0.99 was found for measurements of corneal adhesiveness on two subsequent days at the same time (P < 0.001).

CONCLUSIONS. This minimally invasive, novel technique of OCT imaging of the corneal surface following NaCMC drop instillation provides a measure of corneal adhesiveness. This technique may improve the clinician’s ability in the understanding and diagnosis of the dry eye syndrome.

Keywords: OCT, dry eye, artificial tear, ocular surface, adhesiveness
ocular irritation, a Schirmer I test score $> 5$ mm/5 min, a fluorescein break-up time (FBUT) $\geq 10$ seconds, and had no significant vital staining of the ocular surface (Oxford scheme score $\leq$ panel A).\textsuperscript{6,19}

Subjects with meibomian gland dysfunction or other external ocular diseases in the previous 6 months, any evidence of abnormal blinking or lid abnormality, history of eye surgery or systemic diseases, topical or systemic medication, history of contact lenses wear, were excluded from both groups.

Patients presenting with complaints of ocular irritation were evaluated by three examiners (PEN, FC, GMS) at The Eye Clinic. Clinical and instrumental exams were performed from July 2012 to December 2013. On the day before OCT imaging, a standard clinical assessment of the dry eye symptoms and signs was performed on all subjects in the same sequence. It included clinical history, McMonnies questionnaire, FBUT, fluorescein staining of the cornea and conjunctiva graded according to the Oxford system, Schirmer I test, and a slit-lamp examination of the lid margins and meibomian glands.

To enhance observation of conjunctival staining and the break in the fluorescent tear film over the entire cornea, FBUT and ocular surface fluorescein staining were evaluated with a biomicroscope and the $\times$10 objective under cobalt blue light and Kodak Wratten 12 yellow filter.\textsuperscript{6,19–23} Three evaluations of FBUT were conducted, and the mean value was taken.

Dry eye was diagnosed if the subject exhibited all these characteristics of dry eye: significant subjective symptoms (McMonnies questionnaire score $> 10$, including the positive score obtained by questions about symptoms n.5 and n.6), objective signs of tear instability (FBUT < 10 seconds), objective sign of reduced tear production (Schirmer I test $\leq$ 5 mm/9 min), and ocular surface staining (Oxford scheme $\geq$ panel B).

All examinations were conducted in the same conditions of temperature (within a range of 15°C–25°C), humidity (within a range of 30%–50%), and time of the day (between 3 PM and 5 PM) in a dimly lit consulting room.

**OCT Measurements and Procedures**

The OCT scans were performed by using Cirrus HD-OCT 4000 (Carl Zeiss Meditec, Inc., Dublin, CA, USA). This system is a Fourier-domain OCT platform that works at a wavelength of 840 nm, takes 27,000 axial scans per second, and has a 5-μm axial resolution. The cross-sectional ocular surface images were acquired using the Anterior Segment 5 Line Raster scanning protocol. The mode acquires a set of five parallel lines of equal length at 3 mm. The lines are horizontal and separated by 250 μm. Each line is composed of 4096 A-scans and each 5 Line Raster scan was taken approximately 0.75 seconds. After image capture, the individual line with greatest clarity of detail was selected for analysis.

Patients were instructed to fixate on a central target in the OCT. The axial distance of patients was adjusted so that the ocular surface has been within the middle third of the scan. One randomly selected eye of each patient was imaged for session. The subject was asked to blink normally during the examination period and before each scan. Scans were acquired on the horizontal axis passing across the corneal apex. The OCT images of the ocular surface were obtained at baseline and after the instillation of the artificial tear in four serial scans: immediately (within 30 seconds), at the first, at the third, and at the fifth minute. The OCT imaging was performed on subsequent 2 days by two different examiners masked to the study to verify the reproducibility and the inter-rater reliability. To make an unbiased comparison between scans, best efforts were made by the operators to acquire the highest-quality images.

The evaluation of the adhesive properties of the ocular surface was performed by analyzing in vivo the dynamic behavior of the precorneal tear film after the instillation of an artificial tear (35 μL, sodium carboxymethylcellulose [NaCMC] 0.5%, Optive UD, preservative-free; Allergan, Inc., Irvine, CA, USA). The artificial tear is detected by OCT as a two-layered structure localized onto the epithelial surface of the cornea (Fig. 1), consisting of an outer band of high reflectivity and an inner band of low reflectivity. The residence time of the double-band structure onto the epithelial surface of the cornea;
that is, the velocity of its progressive depletion (thinning), was considered an index of the adhesive properties of the ocular surface (Fig. 2). Thus, we have classified the corneal adhesiveness into four categories: poor (between 0 and 1 minutes), fair (between 1 and 3 minutes), borderline (between 3 and 5 minutes), and excellent (greater than 5 minutes).

All information was recorded by the researchers so that subjects could not be identified, directly or through identifiers linked to the subjects.

**Statistical Analysis**

Statistical analysis was performed using Statistical Package for Social Science SPSS version 21.0 (SPSS, Inc., Chicago, IL, USA).

The Mann-Whitney $U$ test was performed for the comparison of corneal adhesiveness differences between the two groups. Correlation analysis between corneal adhesiveness and tear tests was performed calculating Spearman’s $\rho$ test. Diagnostic results for dry eye were analyzed by receiver
operating characteristics (ROC) curve, and the area under the ROC curve (AUC) was calculated. Data analysis was accompanied by scatter plots.

The corneal adhesiveness at the second OCT imaging was considered for the inter-rater reliability and reproducibility analysis. Thus, the correlation between individual measurements was calculated by the intraclass correlation coefficient (ICC). *P* values less than 0.05 were considered significant.

**RESULTS**

The results of clinical and instrumental exams obtained in the individuals included in this study are summarized in Tables 1 and 2.

Informative images of ocular surface were obtained easily in all patients. The corneal adhesiveness for NaCMC median and mode values was between 1 and 3 minutes (fair) for dry eye patients, and between 3 and 5 minutes (borderline) for control group subjects. At the first day of OCT imaging, an excellent, borderline, fair, and poor time of corneal adhesiveness was found, respectively, in 0%, 8.3%, 66.7%, and 25% of dry eye patients, and in 36.7%, 55.1%, 8.2%, and 0% of control group subjects. The following day, the OCT scans were repeated at the same time, obtaining equal values of corneal adhesiveness in dry eye patients, and percentages of 40.8%, 52%, 8.2%, and 0%, respectively, in control group subjects.

There was a significant difference in corneal adhesiveness between dry eye patients and control group subjects (*P* < 0.001, Mann-Whitney U test). Significant correlations were observed between the adhesiveness measures and dry eye patients’ symptoms, Schirmer I test, ocular surface staining, and, particularly, FBUT (Fig. 3). McMonnies score and OCT scheme grade were significantly negatively correlated with adhesiveness (Spearman’s *r* = -0.45, *P* < 0.001 and Spearman’s *r* = -0.52, *P* < 0.001, respectively). On the contrary, there were positive correlations with Schirmer I test and FBUT (Spearman’s *r* = 0.7, *P* < 0.001 and Spearman’s *r* = 0.85, *P* < 0.001, respectively).

The AUC calculated by the ROC technique was 0.94 (*P* < 0.001, Fig. 4). The cutoff value, derived from the ROC curve, is the point with an optimal relationship between sensitivity and specificity. The best cutoff point was calculated to be *t* = 3 minutes (the time point between fair and borderline values), with values less than 3 minutes suggesting abnormal corneal adhesiveness. Thus, a good diagnostic accuracy value of 91% was obtained. The sensitivity was 91% and the specificity was 92%. Positive predictive value (PPV) was 89% and negative predictive value (NPV) was 94%. A statistically significant ICC value of 0.99 (*P* < 0.001), indicating the excellent inter-rater reliability and reproducibility of the test, was found for measurements of corneal adhesiveness.

**DISCUSSION**

In this study, we evaluated in vivo the adhesive properties of the ocular surface by using a simple and minimally invasive test represented by the analysis of the precorneal residence time of an artificial tear, as detected by high-resolution Fourier-domain OCT imaging. We used NaCMC as an adhesion marker because of its good mucoadhesive capacities,24–26 and its unique properties to enhance detection of tear film dynamics by OCT. With this method, we found a significant difference in adhesiveness between dry eye patients and control group.

Under conditions of normal blinking, the precorneal residence time of the artificial tear detected by OCT is influenced mainly by the antagonistic action of gravitational force on the tear film.

**Table 1.** Patient Data

<table>
<thead>
<tr>
<th></th>
<th>Dry Eye Patients, <em>n</em> = 36</th>
<th>Control Group Subjects, <em>n</em> = 49</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Median (Mode)</td>
</tr>
<tr>
<td>McMonnies, values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTBUT, s</td>
<td>2.83</td>
<td>1.15</td>
</tr>
<tr>
<td>Schirmer, mm</td>
<td>2.25</td>
<td>1.15</td>
</tr>
<tr>
<td>Oxford scheme, score</td>
<td>2.58</td>
<td>0.64</td>
</tr>
<tr>
<td>Corneal adhesiveness, score on the first day‡</td>
<td>1.83</td>
<td>0.56</td>
</tr>
<tr>
<td>Corneal adhesiveness, min† on the first day‡</td>
<td>1.7</td>
<td>0.93</td>
</tr>
<tr>
<td>Corneal adhesiveness, score on the second day‡</td>
<td>1.83</td>
<td>0.56</td>
</tr>
<tr>
<td>Corneal adhesiveness, min† on the second day‡</td>
<td>1.7</td>
<td>0.93</td>
</tr>
</tbody>
</table>

McMonnies, McMonnies Questionnaire values; FTBUT, fluorescein tear break-up time; Schirmer, Schirmer I test; Oxford scheme, fluorescein staining of the cornea and conjunctiva graded according to the Oxford system: 1, panel A (score = 0); 2, panel B (score = 1); 3, panel C (score = 2); 4, panel D (score = 3); 5, panel E (score = 4); 6, panel > E (score = 5). Corneal adhesiveness score: 1, poor (between 0 and 1 minutes); 2, fair (between 1 and 3 minutes); 3, borderline (between 3 and 5 minutes); 4, excellent (greater than 5 minutes).

† Multiple modes exist. The smallest value is shown.
‡ The FD-OCT scans were repeated on subsequent two days to verify the repeatability of our method (there were only two different measurements).
§ The Class Interval Arithmetic Mean was obtained considering the mid value for each category: 0.5, 2, 4, 6 (minutes) for poor, fair, borderline, excellent (the largest values was 7 minutes) corneal adhesiveness grade, respectively.

**Table 2.** Corneal Adhesiveness Evaluated by FD-OCT: AUC, Sensitivity, Specificity, Accuracy, PPV, NPV, ICC, and Difference Between Control Group Subjects and Dry Eye Patients

<table>
<thead>
<tr>
<th>Corneal Adhesiveness, Precorneal Residence Time of Double Line Detected by FD-OCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
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<tr>
<td>Sensitivity, %</td>
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<tr>
<td>Specificity, %</td>
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<tr>
<td>Accuracy, %</td>
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<tr>
<td>PPV, %</td>
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<tr>
<td>NPV, %</td>
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<tr>
<td>ICC</td>
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<tr>
<td>Difference between control group subjects and dry eye patients</td>
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</tbody>
</table>

*P* < 0.001.
forces that bring the artificial tear down, and the strength of adherence forces that keep the artificial tear in contact with the ocular surface. Immediately after instillation, the artificial tear adheres completely to the cornea, but with time, blinking movements and gravitational forces induce a progressive washout of the tear from the corneal surface and a reduction of its thickness. Such thickness is only partially restored by the eyelids at every blink. The cycles of reduction and restore of NaCMC are easily observed on OCT, until the complete disappearance of the double line signal. While gravitational forces are presumed to be similar in all individuals, the adherence forces, represented by the interaction between the artificial tear polymer and tear film mucous layer, clearly differ according to the conditions of the individual ocular surface. In normal subjects, secretory mucins distributed in the tears, and transmembrane mucins that form the glycocalyx, contain polar regions that promote chemical interactions with NaCMC polymer and water, and sustain its corneal adhesiveness. 27–32 In dry eye, an alteration of mucin distribution and chemical state (glycosylation), and a dehydration of mucin with loss of its polar properties, induce a reduction of adhesive properties of the ocular surface, and a loss of tear film stability. 33,34

Corneal adhesiveness and tear film break-up time are clearly two different concepts, even if both may be considered an index of tear film stability. In fact, in the present study, we have demonstrated a statistically significant correlation between the time-length of NaCMC-corneal adhesiveness and the tear tests used, in particular with the FBUT. Nevertheless, corneal adhesiveness evaluated with NaCMC-enhanced corneal OCT imaging is a direct measure of the corneal mucin-polymer chemical interactions, while FBUT merely reveals fluorescein tear film disruption between blinks.

For all these reasons, we believe that the measure of NaCMC-corneal adhesiveness as detected by our method may indirectly represent a measure of the health status of the ocular surface, especially of tear film stability. However, unlike FBUT,
fluorometry, has several advantages: it allows a morphologic evaluation of the dynamic distribution of tear film; it does not require the F, which can penetrate through the cornea, and may create staining and pseudostaining of ocular surface (creating a bias in the measurements); it provides final results that are not influenced by the autofluorescence of the cornea; and, moreover, it may be useful in providing a permanent record, which permits masking of scoring and, therefore, provides greater objectivity. A further advantage is represented by the possibility that such records can be handled at a reading center, to provide improved standardization in clinical trials.

The data of our study clearly demonstrated that dry eye patients have a faster precorneal tear film turnover of NaCMC and, therefore, a reduced ability in retaining the tears in front of their central corneal surface. An increased tear film turnover could lead to a reduced time of contact between the epithelium of the central cornea and toxic factors, such as the proinflammatory cytokines, proteolytic enzymes, and cytotoxic agents, which are in increased concentrations in altered hyperosmotic tears of dry eye patients. Therefore, further studies should investigate the exact role of corneal adhesiveness in ocular surface physiology, and in different pathological conditions; for example, in patients with meibomian gland dysfunction.

Our study has some limitations. Although minimally invasive, it introduces the use of an artificial drop, the sodium carboxymethylcellulose (NaCMC). Moreover, it does not evaluate directly either the tear film thickness or the dynamic behavior of the precorneal tear film. In our research, we did not assess the relationship between corneal adhesiveness and the two main subtypes of dry eye (i.e., aqueous tear deficiency and evaporative dry eye). A more comprehensive database of normal individuals must be created to analyze possible age and sex-dependent variations.

In conclusion, in our study we have been able to quantify in vivo the adhesive properties of the ocular surface by using a simple and minimally invasive new test. On the basis of the clinical results and statistical significance of reliability, specificity, sensitivity, PPV, NPV, and accuracy, we believe that the novel method of OCT imaging described in this work may represent a useful, all comprehensive, single-test for the diagnosis and follow-up of dry eye.

### Acknowledgments

Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Seattle, Washington, United States, May 9, 2013 (as part of the work entitled "Anterior segment OCT study of the precorneal film and its relationship with dry-eye patients' symptoms and quantitative tests of the tear film").

**Disclosure:** P.E. Napoli, None; F. Coronella, None; G.M. Satta, None; I.A. Zucca, None; M. Fossarello, None

### References


In Vivo Measurements of Corneal Adhesiveness With OCT


