Journal of Visualized Experiments

# Measuring and modeling contractile drying in human stratum corneum

--Manuscript Draft--

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| **Manuscript Number:** | JoVE55336R2 |
| **Full Title:** | Measuring and modeling contractile drying in human stratum corneum |
| **Article Type:** | Invited Methods Article - JoVE Produced Video |
| **Keywords:** | Stratum corneum; Skin mechanics; Elastic modulus; Drying stress; Contractile; drying; Cosmetics |
| **Manuscript Classifications:** | 1.10.272.497: Epidermis; 1.17.815: Skin; 10.1.516.213: Cosmetics; 4.27.720.269: Cosmetics; 5.2.218: Cosmetic Techniques; 7.1.154.90: Biomechanics; 7.1.374.89: Biomechanics; 93.31: Engineering (General); 93.35.10: biomedical instruments (theory and techniques); 93.37: Mechanical Engineering |
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| **Abstract:** | Stratum corneum (SC) is the most superficial skin layer. Its contact with the external environment means that this tissue layer is subjected to both cleansing agents and daily variations in ambient moisture; both of which can alter the water content of the tissue. Reductions in water content from severe barrier dysfunction or low humidity environments can alter SC stiffness and cause a build-up of drying stresses. In extreme conditions, these factors can cause mechanical rupture of the tissue. We have established a high throughput method of quantifying dynamic changes in the mechanical properties of SC upon drying. This technique can be employed to quantify changes in the drying behavior and mechanical properties of SC with cosmetic cleanser and moisturizer treatments. This is achieved by measuring dynamic variations in spatially resolved in-plane drying displacements of circular tissue samples adhered to an elastomer substrate. In-plane radial displacements acquired during drying are azimuthally averaged and fitted with a profile based on a linear elastic contractility model. Dynamic changes in drying stress and SC elastic modulus can then be extracted from the fitted model profiles. |
| **Author Comments:** | |
| **Additional Information:** | |
| **Question** | **Response** |
| If this article needs to be "in-press" by a certain date, please indicate the date below and explain in your cover letter. | |

**TITLE:**
**Measuring and modeling contractile drying in human stratum corneum**

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**KEYWORDS:**
Stratum corneum, Skin mechanics, Elastic modulus, Drying stress, Contractile, Drying, Cosmetics.

**SHORT ABSTRACT:**
This article describes a method of quantifying the dynamic drying behavior and mechanical properties of *stratum corneum* by measuring spatially resolved in-plane drying displacements of circular tissue samples adhered to an elastomer substrate. This technique can be used to measure how different chemical treatments alter drying and tissue mechanical properties.

**LONG ABSTRACT**
Stratum corneum (SC) is the most superficial skin layer. Its contact with the external environment means that this tissue layer is subjected to both cleansing agents and daily variations in ambient moisture; both of which can alter the water content of the tissue. Reductions in water content from severe barrier dysfunction or low humidity environments can alter SC stiffness and cause a build-up of drying stresses. In extreme conditions, these factors can cause mechanical rupture of the tissue. We have established a high throughput method of quantifying dynamic changes in the mechanical properties of SC upon drying. This technique can be employed to quantify changes in the drying behavior and mechanical properties of SC with cosmetic cleanser and moisturizer treatments. This is achieved by measuring dynamic variations in spatially resolved in-plane drying displacements of circular tissue samples adhered to an elastomer substrate. In-plane radial displacements acquired during drying are azimuthally averaged and fitted with a profile based on a linear elastic contractility model. Dynamic changes in drying stress and SC elastic modulus can then be extracted from the fitted model profiles.

## INTRODUCTION

The outer most layer of the epidermis, or *stratum corneum (*SC) consists of cohesive corneocyte cells surrounded by a lipid rich matrix[1,2]. The composition and structural integrity of SC is essential for maintaining correct barrier functionality[3], which prevents invasion from microorganisms and resists both mechanical forces and excessive water loss[4]. The capacity of personal care products to maintain or degrade skin barrier function is of great interest to skin healthcare and the cosmetic industry[5]. The daily application of personal care products is known to alter the mechanical properties of the SC[6–8]. For example, surfactants contained in cosmetic cleansers can cause significant increases in the elastic modulus and a build-up of drying stresses in SC, increasing the tissue's propensity to crack[7,9]. Glycerol contained in nearly all cosmetic moisturizers can soften SC and decrease the build-up of drying stresses[8,10,11], reducing the likelihood of tissue rupture.

The method detailed in this article is capable of quantifying the dynamic drying behavior and mechanical properties of SC drying in controlled environments[7,8]. Previously, this technique has been demonstrated to be capable of elucidating the effect of different cosmetic products on changes in the dynamic drying behavior and mechanical properties of SC tissue. This is achieved by quantifying drying-induced shrinkage of human SC tissue adhered to a soft elastomer substrate, fitting drying displacements with a simple contractility model, and then extracting the elastic modulus and drying stress from the fitted profile. When testing of multiple SC samples is required, this method offers a more rapid alternative to uniaxial tensometry, utilizes significantly less tissue and provides more physiologically relevant drying by preventing evaporation from the sample underside.

## PROTOCOL

An exempt approval (3002-13) to carry out research using de-identified tissue samples pursuant to the Department of Health and Human Services regulations, 45 CFR 46.101(b)(4) was granted. Full thickness skin is received from elective surgery. In this article, the tissue source is 66-year-old Caucasian female breast.

### 1. Preparation of Elastomer Coated Coverslips

1.1. In a 20 mL glass vial, mix 0.107 g of Sylgard 184 curing agent with 5.893 g base. The total mixture mass is 6 g with a base to curing agent ratio of 55:1.

1.2. After mixing with a glass rod to ensure homogeneity, place the glass vial in a vacuum chamber and degas to remove all bubbles.

1.3. Place a glass cover-slip (55 mm x 25 mm) in the center of a spin coater. Add ~1 mL of the mixture onto the center of the cover-slip. Use a 5000 µL pipette with the end cut off with scissors. Spin coat the cover-slip at 2000 rpm for 60 s.

1.3.1. Repeat this process to create 5-6 substrates.

1.4. Cure the cover-slips in an oven for 12 h at 60 °C.

1.5. Use a razor blade to partially remove the elastomer film from a sacrificial substrate. Use an indelible marker to mark the topside of the elastomer film and the exposed glass.

1.6. Mount the sample on an inverted microscope and use a remote focus accessory to record the difference in z-height between the focal planes of the two marks. This corresponds to the elastomer substrate thickness, h.

**2. Preparation of the Stratum Corneum**

2.1. Use a water bath or heated stir plate to heat a glass beaker half filled with deionized water (DW) to 60 °C. Inside a biological safety cabinet, immerse the full thickness human skin in the water for 4 min.

2.2. Immediately transfer the skin sample into a beaker containing DW cooled to <10 °C for 4 min. Half filling the beaker minimizes splashing of biohazardous material.

2.3. Remove the skin from the beaker, place in a petri dish, and gently isolate the epidermis using a pair of bent nosed tissue tweezers.

2.4. Place the isolated epidermis basal side down in a petri dish lined with gauze. Ensure the basal layer is fully in contact with the gauze.

2.4.1. Soak the gauze in a 0.25 % (wt/vol) type IX-S porcine pancreas trypsin solution dissolved in 0.1 M phosphate buffered saline for 6-8 h at room temperature. Add only enough trypsin in the container to wet the gauze.

2.5. Lift the gauze with tissue tweezers and float it in a container partially filled with DW. Gently pull the SC to separate it from the gauze.

2.6. Wash the stratum corneum 3-4 times in DW to remove residual epidermal tissue that remains attached to the SC.

2.7. Float the isolated SC in a solution of 0.4 % glycine max(soybean) trypsin inhibitor in DW. Use a plate shaker to agitate the tissue for 10 minutes.

2.8. Float SC in a petri dish partially filled with DW. Use a plate shaker to agitate the tissue for 10 minutes.

2.9. Dry the isolated SC sheet on an ultra-fine plastic mesh for 48 h at room temperature (25 °C, 40% relative humidity (R.H.)).

2.10. Separate the SC from the mesh and cut out individual circular R=3 mm radius samples using a circular hole punch. Mark the center of the outmost face with a small spiral mark using an indelible marker. This provides a visual cue for recognizing the topside of the SC.

Note: The indelible mark should be applied in the center of the sample, where the drying deformations will be smallest. This will minimize the impact of the marker on recorded drying displacement profiles.

## 3. Sample Treatment and Deposition

3.1. Agitate SC samples for 30 min in 15 mL DW containing 90 µL fluorescent marker beads (505/515 nm, 1 µm diameter, carboxylate-modified). This deposits beads onto the SC surface

Note: While deposition of large numbers of beads on the SC may marginally slow drying relative to samples without beads present[12], it will maximize the spatial resolution of the in-plane deformation fields that can be subsequently obtained. The choice of bead volume added should therefore be made ad hoc.

3.2. Remove SC samples and place them in a petri dish partially filled with DW.

3.3. Partially immerse a substrate in the DW at a shallow angle of 15-30°.

3.4. Pin an edge of the floating SC sample at the contact line between the substrate and water interface. Vertically withdrawing the substrate from the water will smoothly laminate the SC sample to the substrate without wrinkles or entrapped air bubbles.

3.5. Repeat step 3.4 to place up to 6 SC samples onto each substrate. Leave at least a 2-3 mm gap between samples and avoid sample lamination close to the substrate edge. This prevents drying of one sample influencing drying displacements in another.

3.6. Dry the mounted SC samples in laboratory conditions for 60 min. This allows residual water between the SC and substrate to evaporate and ensures complete tissue adhesion.

Note: At this juncture, SC samples can be treated with a chemical or cosmetic formulations[7,8] by placing the substrates upside down in a desired solution for a requisite period of time. Repeat step 3.6 once the treatment step is performed. After drying, incomplete adhesion of SC samples to the substrate can be verified using transmitted light microscopy. Trapped bubbles under the SC sample or delaminated edges will form clear contrast variations in the sample with well defined edges.

3.7. Create a humidity chamber by placing a petri dish partially filled with water into a hermetically sealed container.

3.8. Place substrates into the chamber for 24 h to equilibrate to a relative humidity of 99%. Do not place the substrates into the petri dish.

## 4. Microscope Environmental Control

4.1. Achieve control of environmental conditions through a humidity control system connected to a microscope mountable perfusion chamber. Details of the humidity control system are provided in German et al (2013)[7] and Liu and German (2015)[8].

4.2. Mount the substrate on the microscope, place the perfusion chamber over the substrate and seal edges of perfusion chamber to the elastomer using vacuum grease.

4.3. Once mounted, equilibrate internal air to 99% R.H. prior to experimentation. This prevents evaporation of water prior to experimentation. Once imaging in sections 5 or 7 has begun, reduce internal air humidity to desired value.

Note: In this article, SC samples are dried to 25% R.H.

**5. Imaging in Plane Drying Displacements**

5.1. Acquire images of SC samples using an inverted microscope with 1X objective lens. Excite fluorescent beads using a light engine with FITC filter (503–530 nm emission bandpass). Multiple samples can be imaged sequentially throughout drying using an automated x-y stage.

5.2. Record fluorescent and transmitted light images using a digital CCD camera at a resolution of 1392 x 1040 pixels. The field of view of each image is 8.98 x 6.71 mm, allowing a single image to capture a full SC sample. Take images with a frequency of 10 min for 16 h.

**6. Substrate Preparation for Thickness Measurement**

6.1. In a chemical fume hood, place 1 mL silane (3-aminopropyltriethoxysilane, ≥ 98%) in some small plastic cap. Place elastomer substrates from section 1 and the cap in a sealed container for 5 h. Do not allow substrates to come directly in contact with the silane.

6.2. Add 5 mg of EDC (N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride ≥ 99%) in a 1.5 mL tube. Add 500 µL of DW to the EDC. Agitate the solution for 10 s with a vortex mixer.

6.3. Add 0.076 g sodium tetraborate and 0.1 g boric acid to 20 mL DW. Mix using a magnetic stirrer at 70 °C (1 h). Add boric acid until the pH is 7.4.

6.4. Add 20 mL of borate buffer to a 50 mL centrifuge tube. Add 60 µL of 1 µm beads (535/575 nm, carboxylate-modified) to the borate buffer. Finally, add 200 µL of EDC solution to bottle. Shake the tube to mix bead solution and then pour into a 10 cm diameter petri dish.

6.5. Remove the silanated substrates from the container and place them elastomer film-side down into the bead solution. Do so slowly to prevent bubbles from becoming trapped. Two substrates will fit in each petri dish.

6.6. Leave substrates to float in the bead solution for 45 minutes.

6.7. Use tweezers to remove the substrates from the bead solution, then rinse in DW to remove unbound beads.

6.8. Air dry the substrates. Blowing compressed air over the elastomer film surface reduces the formation of water spots.

6.9. Seal the substrates in an opaque box to prevent photo-bleaching of the beads until SC sample deposition.

7. **Imaging Thickness of SC**

7.1. Deposit SC samples on to a substrate using section 3. However, perform step 3.1 without adding fluorescent beads to the DW. Additionally, apply a 5 μL drop of undiluted fluorescent marker bead solution (505/515 nm, 0.1 μm diameter) to the surface of each deposited SC sample with a pipette before completing step 3.6.

7.2. Establish measurements of SC thickness using the microscope with 40X objective lens. Measure the thickness of SC samples over time using a remote focus accessory to record the difference in z-height between the two bead layer focal planes located at the SC-substrate interface and the topside of the SC.

7.3. Measure the thickness of 3 regions of each SC sample over a 3 h drying period. The thickness of SC samples reaches a steady state value within this time frame[8].

**8. Quantifying and Modeling Tissue Deformation.**

8.1. Use particle image velocimetry[13] to obtain spatially resolved in-plane drying displacements from the fluorescent images at each recorded time step.

8.2. Use MATLAB to obtain azimuthally averaged radial and azimuthal displacement profiles from the displacement field of each radially symmetric SC sample.

Note: An example dataset (entitled ‘d.mat’) and MATLAB code (entitled ‘PIV-processing.m’) that performs both this step and step 8.3 has been provided in the supplemental information.

8.3. Fit radial displacement profiles to a model[7,8,14–16] describing drying SC as a shrinking linear elastic circular disk of time varying thickness, $h_{SC}$, radius, R, and elastic modulus, $E_{SC}$, adhered to a deformable elastic substrate with elastic modulus, E. Assume SC has a well-defined and constant Poisson's ratio, $\nu_{SC} = 0.4$.[7,8]. Obtain best fits using a minimum least squares approach.

Note: The model used for fitting describes radial displacements in terms of modified Bessel functions are:

$$u_r(r,h) = -\alpha R I_1(\beta r/R) A(\beta) \qquad (1)$$

with $\alpha = P_{SC}(1+\nu_{SC})(1-2\nu_{SC})/E_{SC}(1-\nu_{SC})$, $\beta = R/l_p$ and

$$A(\beta)^{-1} = \beta I_0(\beta) - \left(\frac{1-2\nu_{SC}}{1-\nu_{SC}}\right) I_1(\beta).$$

The term $l_p$ corresponds to a penetration depth given by,

$$l_p^2 = \frac{E_{SC}(1-\nu_{SC})h_{SC}}{Y(1+\nu_{SC})(1-2\nu_{SC})}.$$

$Y = E/2(1+\nu)h$ denotes a substrate rigidity parameter; valid when sample sizes are much greater than the substrate thickness. Here, the parameters, $h$ and $\nu$ respectively denote the substrate thickness Poisson's ratio. The Poisson's ratio of the silicone elastomer substrate[17] is ν = 0.5.

8.4. Obtain model parameters α and β at each time step from the least squares fit of Equation (1) to the radial displacement profile.

8.4.1. Employ the fitting parameter β to obtain SC elastic modulus, $E_{SC}$, using the expression,

$$E_{SC} = \frac{R^2 E(1+\nu_{SC})(1-2\nu_{SC})}{2\beta^2(1-\nu_{SC})(1+\nu)h_{SC}h}$$

8.4.2. Use fitting parameter α to obtain the time varying contractile drying stress, $P_{SC}$, using the expression,

$$P_{SC} = \frac{\alpha E_{SC}(1-\nu_{SC})}{(1+\nu_{SC})(1-2\nu_{SC})}$$

**REPRESENTATIVE RESULT**

Figure 1(a) shows a representative fluorescent image of an SC sample coated with fluorescent beads (section 3). The corresponding transmitted light image of the sample is shown in Figure 1(b) overlaid with a quiver plot of spatially resolved drying displacements that form after 16 h drying at 25% R.H. Due to the circular symmetry of the samples, these displacements can be azimuthally averaged. Figure 1(c) shows radial ($u_r$, solid red line) and azimuthal ($u_\theta$, dashed blue line) displacement profiles plotted against the dimensionless radial position, r/R. Here, R denotes the mean SC sample radius, r/R = 0 denotes the sample center and r/R = 1 denotes the edge. Standard deviations at each radial position are denoted by the shaded regions around the mean. These variations are primarily caused by the structural heterogeneity of the SC[3,7,12]. Throughout drying, azimuthal displacements remain small. Radial displacement profiles however increase monotonically from center to edge and grow in magnitude until an equilibrium is reached.

*[Place Figure 1 here]*

Profiles recorded at 30 min intervals are plotted in Figure 2(a) and show the time evolution of in-plane displacements. The average SC thickness, $h_{SC}$, is plotted in Figure 2(b). Decreases of SC during drying primarily occur over the first 2 h.

*[Place Figure 2 here]*

Fitting displacement profiles with the linear elastic contractility model described by Equation (1) provides further insight into the mechanical properties of drying SC. Displacement profiles at each time step are fitted with the model using a minimum least squares approach, as shown in Figure 2(c). The contractile drying stress, $P_{SC}$, and elastic modulus, $E_{SC}$, are subsequently extracted from the model at each time step. Average changes in these parameters (based on 3

individual SC samples) are shown respectively in Figures 3(a) and 3(b). Both parameters increase rapidly over the first 2 h drying period and reach a plateau within 5 h.

*[Place Figure 3 here]*

**Figure. 1**: Circular SC sample (6.2 mm diameter) adhered to an elastomer substrate with elastic modulus E = 16 ± 1kPa after drying for 15 h in a 25 ± 1% R.H. environment
(a) Fluorescent image of the SC sample highlighting the deposited fluorescent marker beads used for tracking spatially resolved in-plane drying displacements. (b) Quiver plot of spatially resolved in-plane drying displacements overlaid on a transmitted light image of the SC sample. (c) Azimuthally averaged radial ($u_r$, solid red line) and azimuthal ($u_\theta$, blue dashed line) displacements of the sample plotted against dimensionless radial position, r/R. Positive values of $u_r$ correspond to contractile displacements. Shaded regions surrounding the lines indicate the standard deviation about the mean at each radial position.

**Figure. 2** (a) Overlay of radial displacement profiles ($u_r$, solid red lines) at 30 min intervals over a 15 h drying period in 25% R.H. conditions plotted against dimensionless radial position r/R for a typical SC sample. Positive values of $u_r$ correspond to contractile displacements. (b) Average SC sample thickness, ($h_{SC}$, n=3), plotted against drying time (c) Radial displacement profiles from (a) overlaid with minimum least squares fits (blue dashed line) of Equation (1) for the first and last recorded radial displacement profile.

**Figure. 3** (a) Averaged SC elastic modulus, $E_{SC}$, plotted against drying time over a 15 h period. (b) Average contractile drying stress, $P_{SC}$, plotted against drying time over a 15 h period.

## DISCUSSION

In this article, we describe a technique that can be used to measure the dynamic drying behavior and mechanical properties of human SC. Previous studies have demonstrated that this technique can be used to quantify the effects of environmental conditions and chemical products commonly used in cosmetic cleansers and moisturizers on the dynamic drying behavior of SC[7,8]. There are a number of key steps in the protocol. Firstly, SC swells notably with water content; therefore, measurements of SC thickness as well as in-plane displacements are essential for accurately predicting the elastic modulus and drying stress magnitude. Secondly, samples need to be fully adhered to the substrate. Incomplete adhesion, non-radially symmetric samples or samples with small tears or holes should be avoided because they will significantly impact the distribution of drying deformations and the radial displacement profiles used for model fitting.

The technique can be used if a humidity control system is unavailable. Without environmental control, tissue samples will dry in laboratory conditions[12]. As such, the laboratory environment should be continuously monitored and maintained, as drying behavior and the repeatability of results will be impacted by both diurnal and seasonal variations in temperature and humidity.

Currently, the technique is limited only to samples that can adhere to the substrate and induce deformations within the elastomer film. While the technique can be readily adapted to test samples that undergo smaller in-plane displacements, by reducing the substrate elastic modulus[12], results from samples that simply slip over the substrate will lack meaning.

Numerous *in-vivo* and *ex-vivo* techniques that can assess the drying behavior and mechanical properties of SC have been reported[3,8–10,18–21]. However, *in-vivo* techniques cannot fully distinguish mechanical changes in SC from the underlying epidermal and dermal layers. Moreover, *ex-vivo* techniques can typically only assess one sample per experiment. The method we report in this article allows up to 6 SC samples to be assessed per experiment. The size of the substrate and environmental chamber however could be scaled up to allow more samples to be assessed simultaneously. We estimate for n=6 SC samples, a timescale of ~13 h is required for preparation and testing, excluding substrate curing and tissue equilibration. In comparison, we estimate uniaxial tensometry testing would require more than twice this period. Significantly less SC tissue is also required per individual sample (0.28 $cm^2$) in comparison with those required for tensometry[9] (2.5 $cm^2$). This technique further enables more physiologically relevant drying by preventing evaporation from the underside of the SC tissue. In addition to assessing drying behavior and mechanics in SC, we believe this technique could also be applied to studies of polymeric or colloidal systems that form a cohesive film upon drying.

**ACKNOWLEDGEMENTS:**
The authors have no acknowledgements.

**DISCLOSURES:**
The authors have nothing to disclose.

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Figure 1

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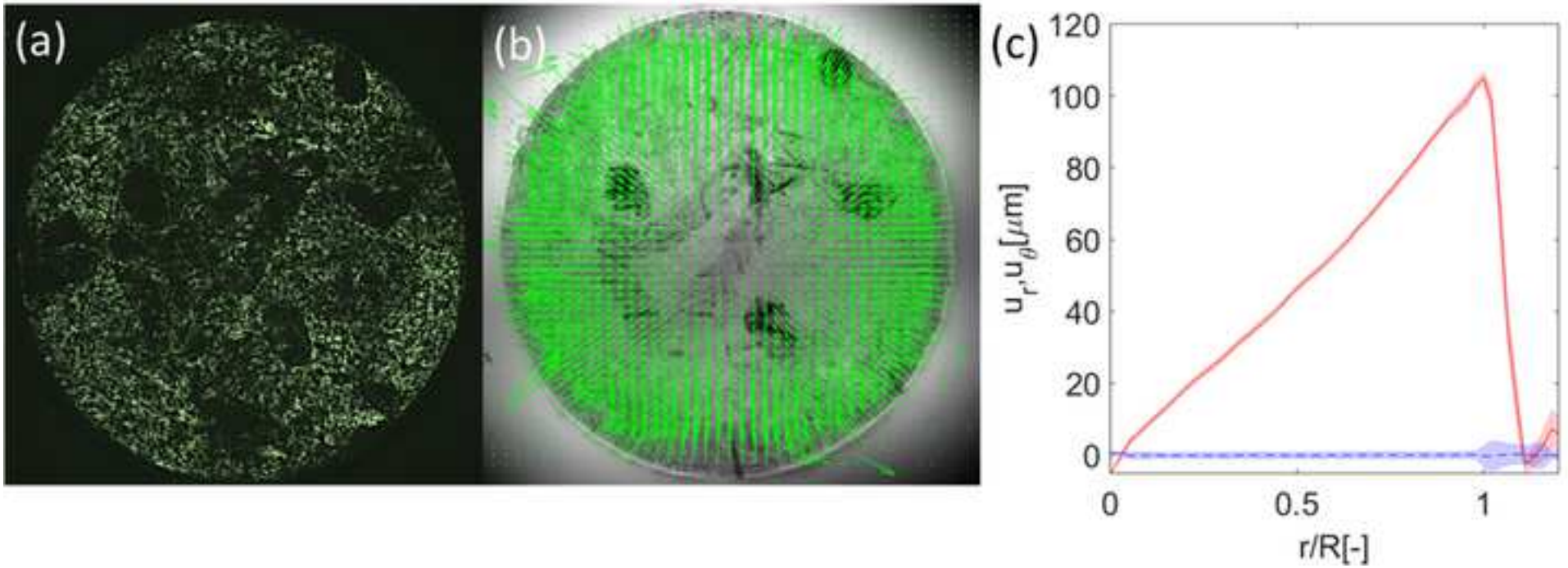

Figure 2

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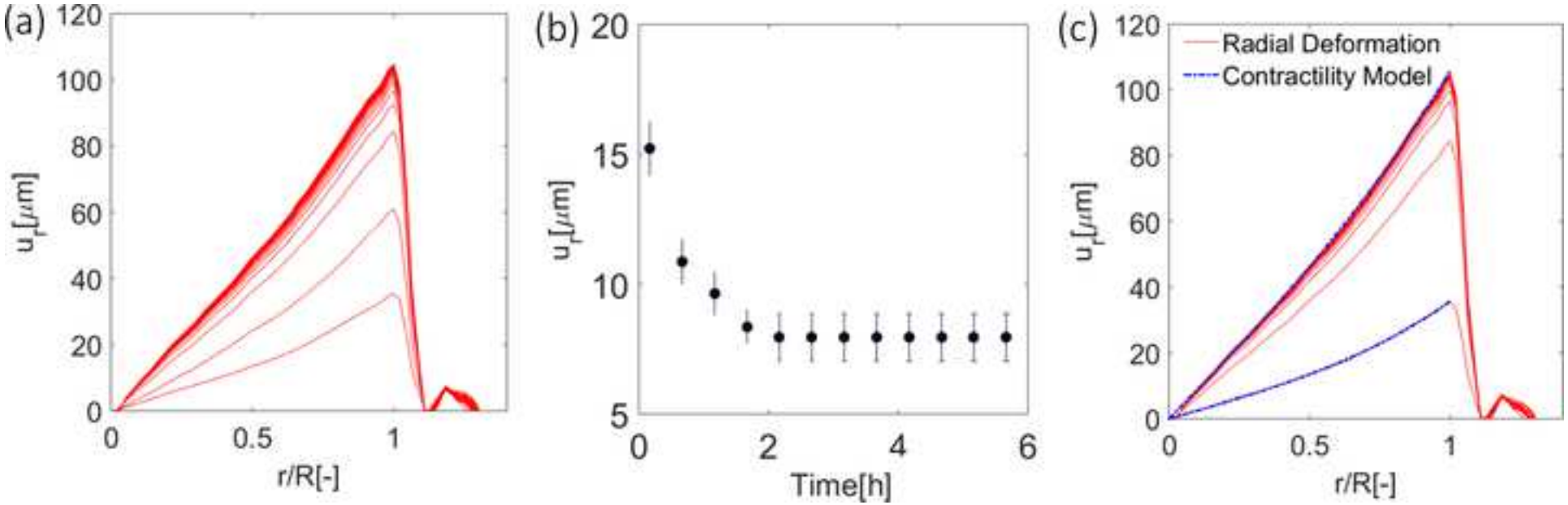

Figure 3

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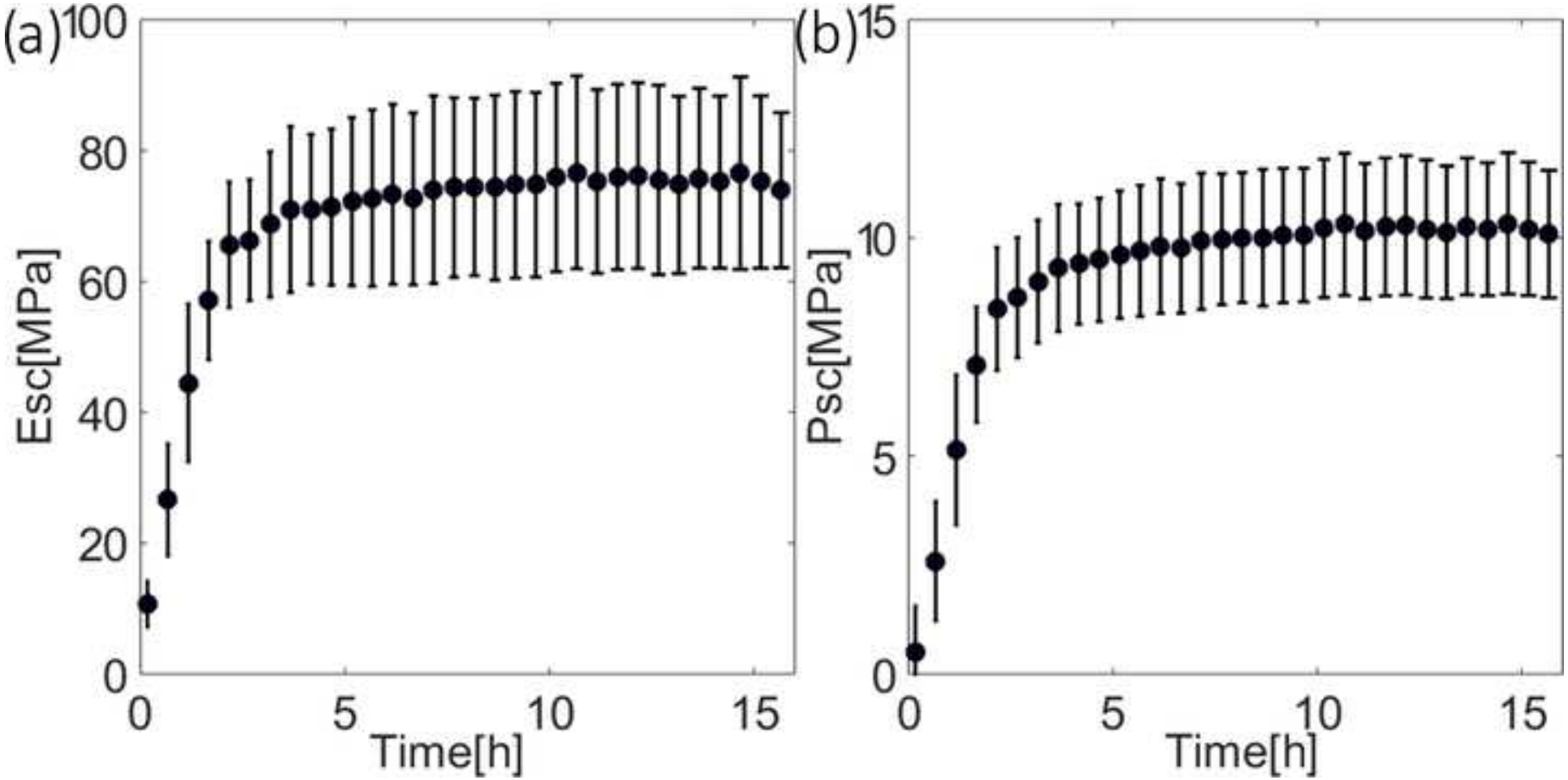

Excel Spreadsheet- Table of Materials/Equipment

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| Name of Material/ Equipment | Company | Catalog Number | Comments/Description |
|---|---|---|---|
| Silicone elastomer base | Dow-Corning | 1064291 | |
| Silicone elastomer Curing Agent | Dow-Corning | 1015311 | |
| FluoSpheres Carboxylate 0.1 µm yellow green fluorescent 505/515 | Thermo Fisher | F8803 | |
| FluoSpheres Carboxylate 1 µm yellow green fluorescent 505/515 | Thermo Fisher | F8823 | |
| FluoSpheres Carboxylate 1 µm nile red fluorescent 535/575 | Thermo Fisher | F8819 | |
| Trypsin from porcine pancreas | Sigma-Aldrich | T6567 | |
| Trypsin inhibitor type II-s | Sigma-Aldrich | T9128 | |
| (3-aminopropyl)triethoxysilane | Sigma-Aldrich | 440140 | |
| Sodium tetraborate | Sigma-Aldrich | 221732 | |
| Boric acid | Sigma-Aldrich | B0294 | |
| Phosphate buffered saline | Sigma-Aldrich | P7059 | |
| N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride | Sigma-Aldrich | E7750 | |
| Vortexer mixer | VWR | 58816-123 | |
| 6mm diameter hole punch | Sigma-Aldrich | Z708860 | |
| SOLA 6-LCR-SB | Lummencor light engine | No.3526 | |
| Cfi Plan Achro Uw 1x Objective | Nikon Plan UW | MRL00012 | |
| CFI Plan Fluor 40x Oil Objective 1.3 na - 0.20mm wd | Nikon Plan Fluor | MRH01401 | |
| Nikon Eclipse Ti-U inverted microscope | Nikon | MEA53200 | |
| Clara-E Camera | Andor | DR-328G-C02-SIL | |
| Remote Focus Attachment E-RFA Ergo Design | Nikon | 99888 | |
| Ti-S-E Motorized Stage | Nikon | MEC56110 | |

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Author(s): Xue Liu and G.K. German

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Article Title: Measuring and modeling contractile drying in human stratum corneum

Signature: [signature] Date: 8/2/16

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**Editorial comments:**
The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (55336_R1_081816.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.

**1. Please ensure that hours is abbreviated as h in the Figures.**
*Hours have now been correctly abbreviated in Figures 2 and 3.*

**2. Please copyedit for scattered grammar/formatting errors. A few examples:**
**-Line 197, temperature units are incorrectly spaced.**
*This has now been corrected.*

**-Punctuation in step 6.7 is awkward.**
*The text of this step has now been reworded to improve clarity*

**-The way step 7.1 is written is slightly confusing and may be difficult for some non-native English speakers to parse.**
*We have now rephrased the text of step 7.1*

**Reviewers' comments:**

**Reviewer #1:**

**Manuscript Summary:**

**Mechanical measurements of stratum corneum are important to several industries concerned with the topical application of formulations to skin as well as dermal patches. Changes in drying stresses are important to skin health and as noted by the authors can be positively and negatively influenced by environment, skin health, and topical treatments. The method described in this paper is a useful "high throughput" approach to screen for changes in SC drying stresses and as such is a very useful contribution to the methods literature.**

**I have no suggested revisions or concerns with this paper. The experimental method is described in good detail as are the reasons to be interested in the type of data generated and the appropriate cautions as to when the method is not appropriate.**

*Thank you*

**Reviewer #2:**
**Manuscript Summary:**
**The method and associate protocol are well presented and could be easily reproduced.**
*Thank you*

**As requested by the guidelines of this journal, some potential artefacts/limitation require to be more clearly mentioned and discussed.**

**Potential artifacts induced by the indelible marker (step 2.11) and more important beads deposit onto the SC surface. Some authors have reported about the importance to avoid such marks. Please comment in your manuscript. To balance this drawback of beads deposit don't you have an advantage based on the quality of local map deformations ? If YES please comment in your manuscript.**

*Thank you for highlighting that we have not addressed these aspects in the manuscript. We have now added additional text that relates to both the influence of the indelible marker and the beads.*

*Firstly, additional text highlighting that the indelible mark should be made at the center of each SC sample has been incorporated as a note in step 2.11. The center is chosen as this corresponds to where drying displacements are smallest. As such, the presence of the indelible mark imparts the least influence on the measured radial displacement profiles.*

*Secondly, drying timescales when beads are located on top of the drying SC sample (shown in Figure 3), and when beads are instead located below the sample (within the deformable silicone elastomer substrate to which the SC sample is adherent (German et al, Heterogeneous Drying Stresses in Stratum Corneum, Biophysical Journal 2013)) are similar for equivalent environmental conditions (25% relative humidity), albeit a little slower. The timescale required for the drying stress to plateau for beads within the substrate was ~ 100 min, whereas it is ~120 min when beads are placed on the SC surface. This difference is similar in magnitude to sample to sample variations in drying timescales. However, we understand the reviewers concern and have highlighted this difference as a note in step 3.1. We have also highlighted that increasing the number of beads on the SC sample will maximize the spatial resolution of recorded in-plane drying displacements, as the reviewer highlights.*

**You mention that "samples need to be fully adhered to the substrate. You have to detail in the protocol how you check that.**
*We have now included additional text in the step 3.6 note detailing how to check for full adhesion.*

**You declare in your introduction about " a high-throughput method...". Not convinced just by your argument that 6 samples can be measured. Please detail time of preparation of the samples which is manual, control of the deposit on the elastomer, and at least quantify how faster it is compared to a non high-throughput method like uniaxial tension test.**
*We have provided a table below detailing an estimate of the manual time scale required to complete 6 sample tests for both this technique and for uniaxial tensometry. This includes manual preparation work and timescales required to perform drying tests, but ignores passive substrate curing and tissue equilibration/isolation timescales, which do not require monitoring. Our estimate suggests the method we describe in this article is significantly faster for multiple sample (n=6) testing, however this is primarily influenced by the timescales required to perform the actual drying experiments. Based on this assessment, we have removed the high-throughput text in the introduction. However, we have also quantified the total amount of stratum corneum tissue required to perform the tests and discovered that the technique described in this article requires an order of magnitude less tissue per sample compared with standard uniaxial tensile testing. Moreover, this technique is more physiologically relevant than*

*uniaxial tensometry, which cannot prevent drying from the underside of the tissue. We have revised the introduction and discussion to address these aspects.*

| *Protocol or Parameter* | *Manual work required for Uniaxial Tensometry* | *Manual work required for Contractile Drying method (This article)* |
|---|---|---|
| *Stratum Corneum Isolation* | *~3 hr* | *~3 hr* |
| *Individual sample cutting* | *~15 min (6 samples)* | *~2 min (6 samples)* |
| *Substrate preparation* | *Not required* | *~ 30 min (6 samples)* |
| *Bead deposition* | *Not required* | *~ 30 min (6 samples)* |
| *Sample deposition to substrate* | *Not required* | *30 min (6 samples)* |
| *Drying adhesion to substrate* | *Not required* | *60 min (6 samples)* |
| *Humidity chamber preparation and sample placement (for equilibration to 99% R.H.)* | *~ 15 min (6 samples)* | *~ 15 min (6 samples)* |
| *Sample drying measurements* | *24 hr (4 hr min. for each sample)*<br>*5 min to mount each new sample* | *In plane drying displacements*<br>*4 hr min. (6 samples simultaneously)*<br>*Thickness measurements*<br>*2 hr min (6 samples)*<br>*20 min to mount each substrate and set up microscope imaging* |
| *Total Manual work Time* | *28 hr* | *12.5 hr* |
| *Tissue required* | *6 samples:15 $cm^2$*<br>*Individual sample: 2.5 $cm^2$ (Reference 9 in article)* | *6 samples: 1.68 $cm^2$*<br>*Individual sample: 0.28 $cm^2$* |

Table comparing the Manual work timescale required to perform testing of n=6 SC samples using uniaxial tensometry and the contractile drying method described in the article

Main PIV data processing MATLAB code

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MATLAB function file

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