**Response to Editor and Reviewers**

**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.*

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| --- | --- | --- |
| *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)*  *5 min to mount each new sample* | *In plane drying displacements*  *4 hr min. (6 samples simultaneously)*  *Thickness measurements*  *2 hr min (6 samples)*  *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 cm2*  *Individualsample: 2.5 cm2 (Reference 9 in article)* | *6 samples: 1.68 cm2*  *Individual sample: 0.28 cm2* |

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