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## The use of environmental dynamic mechanical analysis to predict the softening behavior of neural implants --Manuscript Draft--

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October 5, 2018

Dear Dr. Singh,

Thanks for inviting us to submit an article to JoVE. On behalf of my coauthors, I am pleased to submit the attached manuscript entitled "*The use of environmental dynamic mechanical analysis to predict the softening behavior of neural implants*" which we ask you to consider for publication as methods article with JoVE in the section Bioengineering.

This manuscript describes a method that allows predicting the *in vivo* mechanical behavior materials used for neural implants utilizing environmental dynamic mechanical analysis (DMA). Measuring DMA in solution gives an insight into the softening behavior of polymeric materials and allows quantifying the plasticization of those materials. It also allows for detailed studies of materials durability, and behavior after various treatments such as sterilization.

Beyond the appeal for bioelectronic medicines, we believe that understanding the behavior of polymers under body conditions would be of interest for a variety of biomedical applications. Overall, we think that our method improves the ability to characterize materials behavior *in vitro* and can therefore reduce the need of *in vivo* studies

To our knowledge, this method represents a significant advancement for future applications of responsive substrates for biomedical devices and is an important step towards chronic *in vivo* studies where reliable devices are a key step.

Please do not hesitate to contact me with any questions. Thank you for your consideration.

Sincerely,

A handwritten signature in blue ink that reads "Melanie Ecker".

Melanie Ecker, PhD

**TITLE:**

Environmental Dynamic Mechanical Analysis to Predict the Softening Behavior of Neural Implants

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**KEYWORDS:**

Dynamic mechanical analysis, softening polymer, plasticization, neural implants, immersion, environmental testing, thermomechanical measurements

**SUMMARY:**

To allow reliable predictions of the softening of polymeric substrates for neural implants in an *in vivo* environment, it is important to have a reliable *in vitro* method. Here, the use of dynamic mechanical analysis in phosphate buffered saline at body temperature is presented.

**ABSTRACT:**

When using dynamically softening substrates for neural implants, it is important to have a reliable *in vitro* method to characterize the softening behavior of these materials. In the past, it has not been possible to satisfactorily measure the softening of thin films under conditions mimicking body environment without substantial effort. This publication presents a new and simple method that allows dynamic mechanical analysis (DMA) of polymers in solutions, such as phosphate buffered saline (PBS), at relevant temperatures. The use of environmental DMA allows measurement of the softening effects of polymers due to plasticization in various media and temperatures, which therefore allows a prediction of the materials behavior under *in vivo* conditions.

**INTRODUCTION:**

A new generation of materials used as substrates for neural implants comprises softening shape memory polymers<sup>1-9</sup>. These materials are stiff enough during implantation to overcome critical buckling forces, but they become up to three orders of magnitude softer after implantation in a

body environment. It is predicted that these materials show a better device-tissue interaction due to the reduced mismatch in modulus as compared to traditional materials used in neural implants, such as tungsten or silicon. Traditional, stiff devices show inflammatory response after implantation, followed by tissue encapsulation and astroglial scarring which often results in device failure<sup>10,11</sup>. It is a common assumption that less stiff devices minimize the foreign body response<sup>12-14</sup>. The stiffness of a device is dictated by its cross-sectional area and modulus. Therefore, it is important to reduce both factors to improve the device compliance and, ultimately, the device tissue interaction.

The work on softening polymers was inspired by the work of Nguyen et al.<sup>15</sup>, who demonstrated that mechanically-compliant intracortical implants reduce the neuroinflammatory response. They have previously used mechanically-adaptive poly(vinyl acetate)/tunicate cellulose nanocrystal (tCNC) nanocomposites (NC), which become compliant after implantation.

The Voit lab, on the other hand, uses the highly tunable system of thiol-ene and thiol-ene/acrylate polymers. These materials are advantageous in that the degree of softening after exposure to *in vivo* conditions can easily be tuned by the polymer design. By choosing the right polymer composition and crosslink density, the glass transition temperature and Young's modulus of the polymer can be modified<sup>2,4-6,8</sup>. The underlying effect of the softening is the plasticization of the polymer in an aqueous environment. By having a polymer with a glass transition temperature ( $T_g$ ) above body temperature when dry (the state during implantation), but below body temperature after being immersed in water or PBS, the resulting stiffness/modulus of the polymer can shift from glassy (stiff) when dry to rubbery (soft) when implanted<sup>16</sup>.

However, exact and reliable measurements of the softening due to plasticization and the shift of  $T_g$  from the dry to wet states have not been able to be measured in the past. Traditional dynamic mechanical analysis is performed in air or inert gases and does not allow for measuring of the thermomechanical properties of polymers inside a solution. In previous studies, the polymers have been immersed in PBS for various amounts of time. Swollen samples were then used to perform dynamic mechanical analysis (DMA)<sup>6-8</sup>. However, since the procedure involves a temperature ramp, samples start to dry during the measurement and do not yield representative data. This is especially true if the sample size becomes smaller. In order to predict the softening of neural probes, it would be necessary to test 5 to 50  $\mu\text{m}$ -thin polymer films, which is not possible with traditional DMA due to the abovementioned drying of the samples during the measurement.

Hess et al.<sup>17</sup> have designed a custom-built microtensile testing machine to assess the mechanical properties of mechanically adaptive materials using an environmentally controlled method. They have previously used an airbrush system to spray water on samples during the measurement to prevent them from drying out.

The use of environmental DMA (**Figure 1**), however, allows for measurement of polymer films

in solutions, such as water and PBS, at various temperatures. This allows not only measurement of the polymer's thermomechanical properties in the soaked/softened state but also measurement of its softening kinetics. Even tensile tests and swelling measurements are possible inside the immersion bath of this machine. This allows for exact studies of the plasticization-induced softening of polymer substrates to predict *in vivo* behaviors.

## PROTOCOL:

### 1. Preparation of polymer samples for testing

1.1. Synthesize the softening thiol-ene polymer according to previous protocols inside a fume hood.<sup>1,2,4,18</sup> Briefly, mix quantitative amounts of thiol to alkene monomers with a total of 0.1 wt% photo initiator.

1.1.1. Prepare a 20 mL glass vial for polymer mixing. Cover the vial in aluminum foil to prevent incident light from contacting the monomer solution and keep at room temperature (RT). Use all chemicals as received without further purification.

1.1.2. For fully softening polymer, add 0.5 mol% 1,3,5-triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (TATATO), 45 mol% trimethylolpropane tris(3-mercaptopropionate) (TMTMP), and 5 mol% Tris[2-(3-mercaptopropionyloxy)ethyl] isocyanurate (TMICN) to the covered vial using a disposable plastic pipette.

1.1.3. Add 0.1 wt% of the photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DMPA) to the polymer solution.

1.1.4. Mix the contents inside the vial thoroughly by planetary speed mixing without exposing the solution to light.

NOTE: The polymer solution is sensitive to light and will start to polymerize after 45 to 60 min, even if covered with foil. Therefore, use the polymer solution as quickly as possible after mixing.

1.2. Spin coat the polymer solution prepared in section 1.1 as thin films between 5 to 50  $\mu\text{m}$  in thickness on microscopic glass slides or silicon wafers as a carrier substrate according to the spin curve (**Figure 2**). For 30  $\mu\text{m}$  film thickness, spin at 600 rpm for 30 s.

NOTE: When using a different SMP formulation, the spin speed and time may vary depending on the viscosity of the polymer solution.

1.3. Transfer the polymer films onto the carrier substrate immediately after spinning to the crosslinking chamber. Photo-polymerize the films for 60 min under 365 nm UV bulbs and post-cure for 24 h in a vacuum oven at 120 °C to further complete the conversion.

1.4. Cut the cured polymer films into rectangular samples with widths of 4.5 mm and lengths of 50 mm for the DMA testing. Thicknesses may vary from 5 to 50  $\mu\text{m}$ . The samples can be brought into measuring geometry applying two different methods (choose step 1.4.1 or 1.4.2).

1.4.1. Cut the cured polymer films into rectangles using a  $\text{CO}_2$  laser. Set the  $\text{CO}_2$  laser micromachining parameters to 5.0% power (2.0 W) and 10.0% speed (0.254 m/s) (**Figure 3A**).

1.4.2. Define the DMA samples using photolithography in a Class 10000 cleanroom facility (**Figure 3B**). Use the SMP-on-glass or wafer substrates as the starting substrates in the cleanroom.

1.4.2.1. Deposit low temperature silicon nitride to act as a hard mask for the following plasma etching processes. Pattern the device outline/shape using standard lithography techniques. Use a plasma etcher with  $\text{SF}_6$  and  $\text{O}_2$  plasma to remove the hard mask and SMP layer, respectively.

1.4.2.2. After the SMP layer is plasma etched down to the glass slide/wafer, etch the remaining silicon nitride hard mask away in diluted 10:1 HF dip.

1.5. Delaminate the test devices from the glass slide/wafer by soaking in deionized water as the last step.

## 2. Machine setup

2.1. Use a dynamic mechanical analyzer (DMA) with an immersion system. Equip the machine with the immersion fixture in tension mode (**Figure 1**). Connect the liquid nitrogen to the machine and enable LN2/air as a gas source for the furnace.

2.2. Write the method for dry measurements with the machine software, including the following three steps: conditioning, oscillation temperature ramp, and conditioning end of test, then set up the parameters as follows:

2.2.1. Set the following parameters for the conditioning options: mode = active, select "tension", axial force = 0.05 N, set initial value to "on", sensitivity = 0.0 N, proportional force mode = force tracking, compensate for modulus = on, select "axial force" then set dynamic force to 25.0%, minimum axial force = 0.05 N, programmed extension below 0.0 Pa, mode enabled, strain adjust = 0.05%, minimum strain = 0.1%, maximum strain = 0.5%, minimum force = 0.05 N, maximum force = 0.2 N.

2.2.2. Set the following parameters for the oscillation temperature ramp: start temperature = 10  $^{\circ}\text{C}$ , inherit set point = off, soak time = 0.0 s, wait for temperature = on, ramp rate = 2.0  $^{\circ}\text{C}/\text{min}$ , end temperature = 100  $^{\circ}\text{C}$ , soak time after ramp = 0.0 s, sampling rate = 1 pts/s, strain % = 0.275%, single point, frequency = 1 Hz.

2.2.3. Set the following parameters for the conditioning end of test: environmental control =

off, axial force adjustment = on, mode disabled, transducer/motor = off.

2.3. Write the method for the immersion testing with the machine software including the following four steps: conditioning, oscillation-time, oscillation-temperature ramp, and conditioning-end of test, then set up the parameters as follows:

2.3.1. Set the following parameters for the conditioning options: mode = active, select "tension", axial force = 0.05 N, set initial value to "on", sensitivity = 0.0 N, proportional force mode = force tracking, compensate for modulus = on, select "axial force" and set dynamic force to 25.0%, minimum axial force = 0.05 N, programmed extension below 0.0 Pa, mode enabled, strain adjust = 0.05%, minimum strain = 0.1%, maximum strain = 0.5%, minimum force = 0.05 N, maximum force = 0.2 N.

2.3.2. Set the following parameters for the oscillation time: temperature = 39.5 °C, inherit set point = off, soak time = 0.0 s, wait for temperature = off, duration = 3600.0 s, sampling rate = 1 pts/s, strain % = 0.275%, single point, frequency = 1 Hz.

2.3.3. Set the following parameters for the oscillation temperature ramp: start temperature = 10 °C, inherit set point = off, soak time = 300.0 s, wait for temperature = off, ramp rate = 2.0 °C/min, end temperature = 85 °C, soak time after ramp = 300.0 s, sampling rate = 1 pts/s, strain % = 0.275%, single point, frequency = 1 Hz.

2.3.4. Set the following parameters for the conditioning end of test: environmental control = off, axial force adjustment = on, mode disabled, transducer/motor = off.

### 3. Sample loading and unloading for dry measurements

3.1. Measure the actual thickness of the polymer specimen for dry (in air) testing with Caliper with 0.001 mm precision.

3.2. Enter the sample name, description, and sample geometry into the software.

3.3. Set the loading gap to 15 mm and load the sample. Make sure to center and align specimen before the clamps are screwed hand tight or use a torque wrench with 0.1 N (Figure 3C).

3.4. Close the furnace and start the measurement using the methods described in section 2.2.

3.6. Wait until the measurement is over. Open the furnace and remove the polymer sample from the machine.

### 4. Sample loading and unloading for immersion testing

4.1. Measure the actual thickness of the polymer specimen for immersion testing in PBS with Caliper with 0.001 mm precision.

4.2. Enter the sample name, description, and sample geometry into the software.

4.3. Prepare the setup with the immersion beaker fixed with a clamp at the upper grip (**Figure 4A,B**).

4.4. Set the loading gap to 15 mm and load the sample (**Figure 4C**). Make sure to center and align the specimen (**Figure 5**) before the clamps are screwed hand tight or use a torque wrench with 0.1 N.

4.5. Place the immersion bath on the bottom fixture and screw it tightly (**Figure 4D**). Fill the bath with RT PBS (**Figure 4E**), place the lid on top (**Figure 4F**), close the furnace (**Figure 4G**), and start the measurement immediately using the methods described in section 2.3. Ensure that the drain is closed (**Figure 4H**).

4.6. Wait until the measurement is over. Remove the PBS from the immersion baths using the drain. Open the furnace, remove the lid from the beaker, unscrew the immersion beaker, lift it, and remove the polymer sample from the machine.

4.7. Clean the clamps and immersion beaker with de-ironed water to remove any remaining salt from the PBS.

## 5. Measurements

5.1. Measure the polymer in air without the immersion beaker. Follow the instructions for sample loading and unloading as described in section 3. Repeat this measurement at least 3x to gather results with statistical relevance.

5.2. Measure the polymer inside the immersion bath following the steps described in section 4. Repeat the measurement at least 3x to gather results with statistical relevance.

## 6. Data interpretation

6.1. Open the **results** tab in the machine software, where the raw data can be viewed in a table format or plotted as a graph.

6.2. Plot the first part of the immersion measurement, the oscillation-time measurement, as storage modulus over time to evaluate the softening kinetics. The curve displays how fast the modulus of the polymer decreases over time while immersed in PBS.

6.3. Note the time at which the modulus levels out. This represents the time for softening under physiological conditions.

6.4. If the polymer is not fully softened after the set immersion time of 1 h, repeat the



measurement with increased immersion time.

6.5. Display the oscillation-temperature ramps of the measurements in air and PBS as storage modulus on the left axis and tan delta on the right axis over temperature to display the thermomechanical properties of the polymer before (dry) and after (in PBS) plasticization.

6.6. Plot the data for the dry (air) and PBS measurements together to better display the changes in thermomechanical properties due to plasticization.

6.7. Note the storage modulus of the dry material at 25 °C and of the soaked sample at 37 °C, as these are relevant numbers for evaluating how much the polymer will soften during implantation.

6.8. Note the changes in tan delta peak between the dry and soaked samples.

6.9. Export the data as a .txt or .csv file for further data interpretation and plotting with other software.

#### **REPRESENTATIVE RESULTS:**

The use of environmental DMA allows the analysis of softening kinetics and overall softening capabilities of polymers. By using the temperature-time measuring mode of the protocol, the softening profiles of different polymer formulations can be compared to each other (**Figure 6**). This method can also be used to quantify softening and swelling rates of polymers. It can be seen in **Figure 4** that different polymer formulations may undergo different degrees of softening while being immersed in the 37 °C PBS. The non-softening version remains in the GPa range, whereas the semi-softening polymer softens from 1700 MPa to 370 MPa, and the fully softening polymer to 40 MPa. The softening of all three polymer formulations takes place within 10 to 15 min.

The use of the combination of dry DMA measurements and measurements in PBS allows the assessment of water-induced plasticization of different polymer formulations, which is shown by depression of the  $T_g$  and overall downshift of the modulus curves (**Figure 7**). The softening of the polymers is working most effectively when the dry polymer has a  $T_g$  above body temperature but below that in the wet state. Thus, the modulus of the polymer drops from the glassy to rubbery modulus upon immersion under physiological conditions (**Figure 7A**). When the  $T_g$  of both the dry and wet states of the polymer are well above body temperature, the polymer will not soften under physiological conditions (**Figure 7B**).

#### **FIGURE LEGENDS:**

**Figure 1: Environmental DMA with immersion system.** (A) A more detailed view of the fixture for dry (B) and wet (C) measuring conditions. (B) and (C) are previously published by Ecker et al.<sup>2</sup>.

**Figure 2: Spin curves for fully softening thiol-ene polymer.** Spin curves for fully softening thiol-ene polymer showing the relationship between spin speed and time and the resulting film thickness.

**Figure 3: Fabrication of DMA test stripes on microscopic glass slides.** Fabrication of DMA test stripes on microscopic glass slides (A) or silicon wafers (B) using photolithography.

**Figure 4: Sample loading for measurement with immersion bath.** A (A) DMA equipped with immersion fixture, (B) immersion beaker temporarily fixed with clamps around upper grip, (C) loading of polymer sample at a clamp distance of 15 mm, (D) lowering of immersion beaker to lower fixture and fixation with screws, (E) filling the immersion beaker with PBS, (F) closing the lid, (G) closing the furnace, and (H) ensuring that drain is closed.

**Figure 5: Alignment of sample.** (A) The sample must be straight and centered between the top and bottom clamps. Samples should not be diagonal (B), too high or too low (C), or too much toward the edges (D). Sample should also not be buckled (E) but should be straight (F) to ensure reliable measurements.

**Figure 6: Softening kinetics of three different thiol-ene polymers.** Softening kinetics of three different thiol-ene polymers as measured with the oscillation-time protocol inside PBS at 37 °C for 1 h.

**Figure 7: Displays DMA measurements of two different SMP formulations.** Displays DMA measurements of two different SMP formulations before (orange) and after (blue) soaking in PBS, respectively. (A) A fully-softening (FS) version and (B) slightly-softening version (SS) of SMP. This figure has been modified from Ecker et al.<sup>2</sup>.

## DISCUSSION:

The use of environmental DMA allows the study of the behavior of various polymers used as substrates for neural implants<sup>19</sup> or other biomedical devices in solution and to mimic *in vivo* conditions. This includes, but is not limited to, polyimide, parylene-C, PDMS, and SU-8. Hydrogels and extracellular matrix (ECM) materials can also be investigated using this method. The differences of overall softening of the polymer as well as its softening kinetics can be easily compared between different solutions, including water, heavy water, and PBS. It is also possible to test the influence of different immersion temperatures or differences resulting from varying polymer thicknesses and compositions.

This method also allows studying of the influence of various treatments on softening behaviors of polymers and hydrogels. Treatments include application of various sterilization methods, accelerated aging in various media, and surface modification. This *in vitro* method will help researchers learn about the behavior and durability of these materials, obtain reliable *in vitro* measurements, and avoid unnecessary animal experiments. However, measuring in PBS is just one approach to mimic the biological environments. *In vivo* conditions may vary in many aspects, such as ion concentration and the availability of antibodies, proteins, and other species

inside biological media/tissues. Depending on the targeted area, experimenters may also consider using different media for environmental measurements, such as tris-buffered saline (TBS), TBS-T (TBS with polysorbate 20), bovine serum albumin (BSA), cerebrospinal fluid (CSF), and other body fluids.

In addition, it is possible to characterize the mechanical properties of probes after explantation from an animal after an *in vivo* study is completed. This will allow the investigation of probe behavior after softening in a body environment and comparison to *in vitro* data.

It should be noted that there is an offset between the temperature set for the solution bath and the actual temperature. This is due to the fact that two different temperature controllers are being used: one for temperature control (outside the immersion bath) and another for measuring the temperature (inside the immersion bath). We found that when the outside temperature is set to 39.5 °C, the temperature inside the bath stabilized at 37 °C.

The temperature range for measurements inside solutions are naturally limited by their crystallization and boiling temperatures. It is recommended to remain at least 10 K above and below these temperatures, respectively.

It is debated whether the starting temperature of the immersion solution used for soaking/softening measurements should be room temperature or pre-warmed to body temperature to best mimic the conditions during probe implantation. The use of RT PBS takes into account the fact that the probe is kept at RT before implantation and that it is usually kept in close proximity to the implantation side while it is aligned to the right position. At this stage, the probe may already start to soften due to the moist milieu. Starting with 37 °C PBS will better mimic a shotgun approach for insertion.

The described results were measured on polymer films in tension mode; however, the environmental DMA is also capable of measurements in compression and in shear when using the respective fixture. Therefore, this also allows for the measurement of other sample geometries. It should be noted that the available space inside the immersion beaker is limited and thus the samples used for measurements inside this beaker are restricted by their sizes.

Another limitation of this method is the load cell, which is used to detect the forces generated by the samples during the measurement (in dry and wet conditions). The load cell can only measure forces up to 35 N, which therefore limits the sample size/geometry.

#### **ACKNOWLEDGMENTS:**

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## DISCLOSURES:

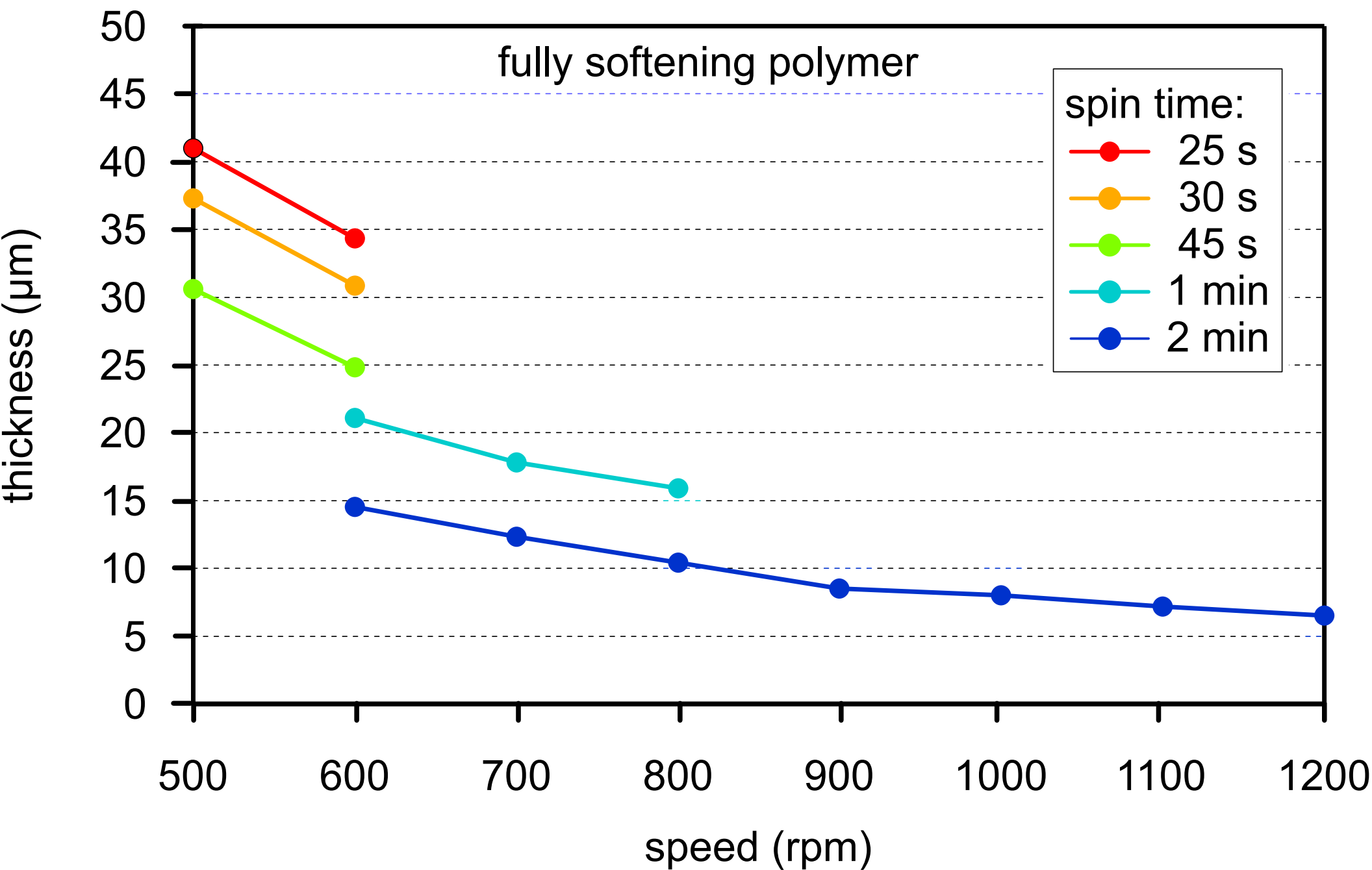
The authors declare that they have no competing financial interests.

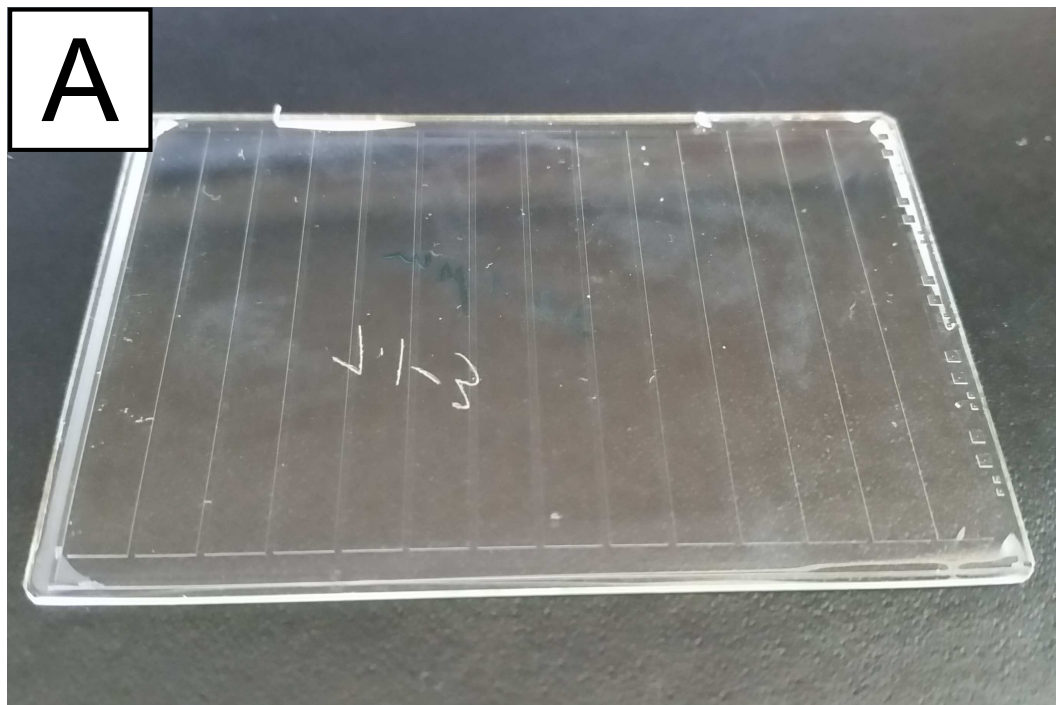
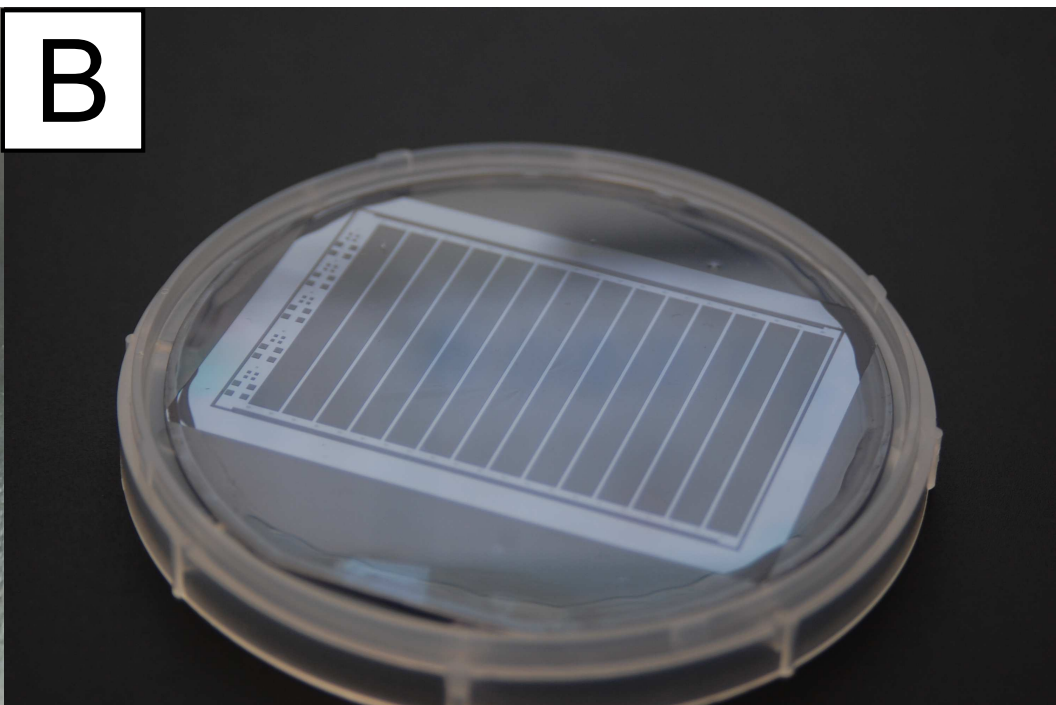
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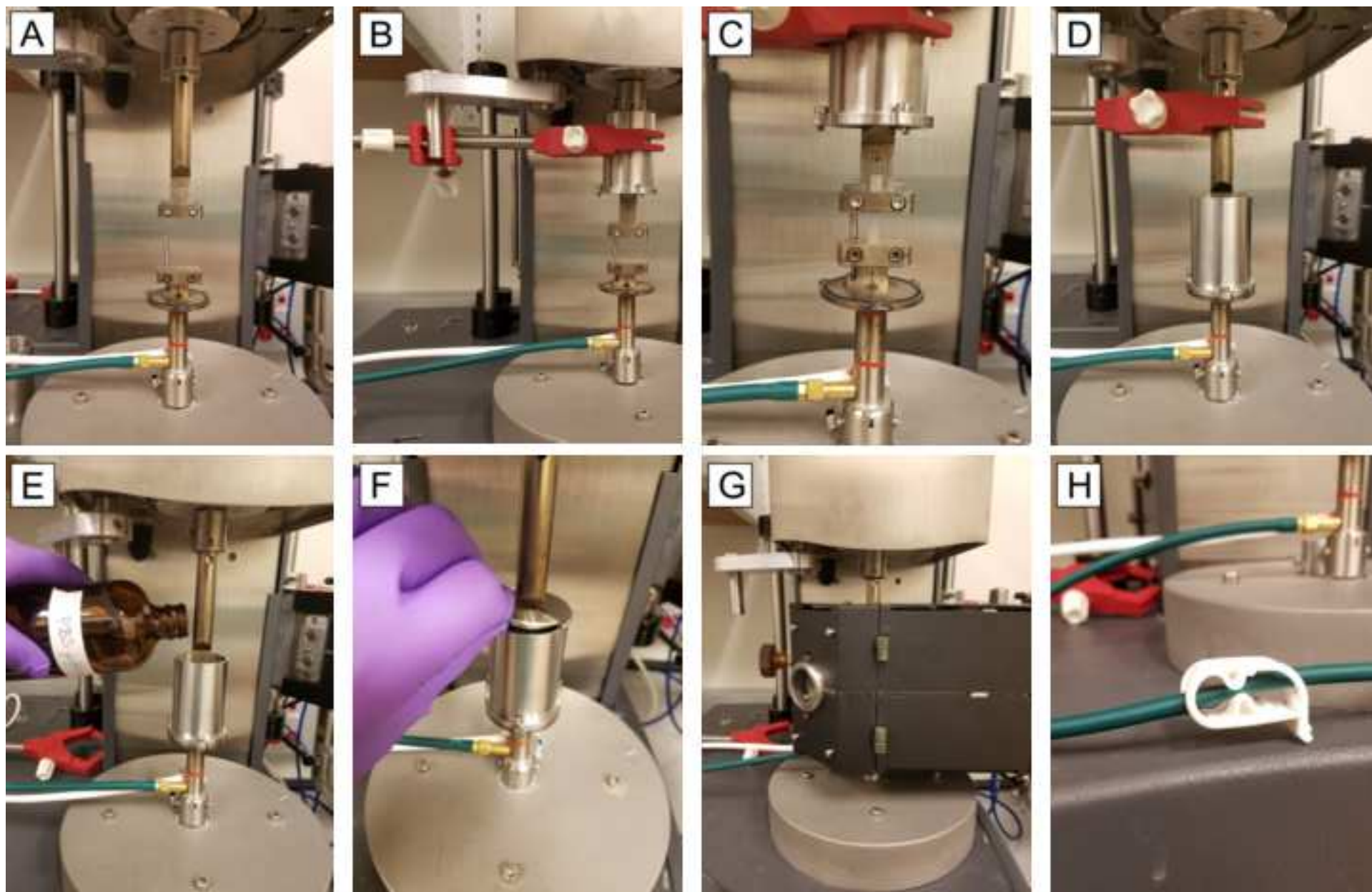
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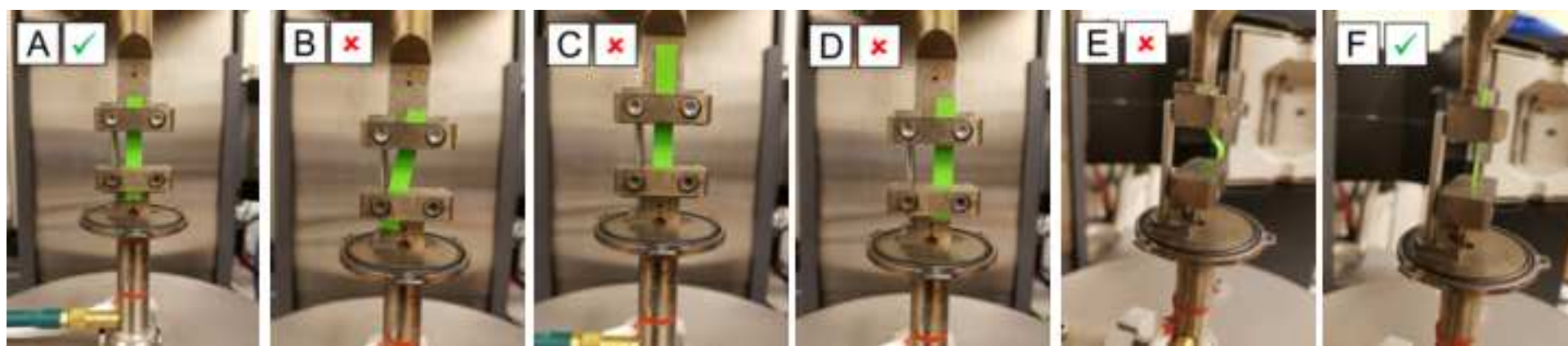


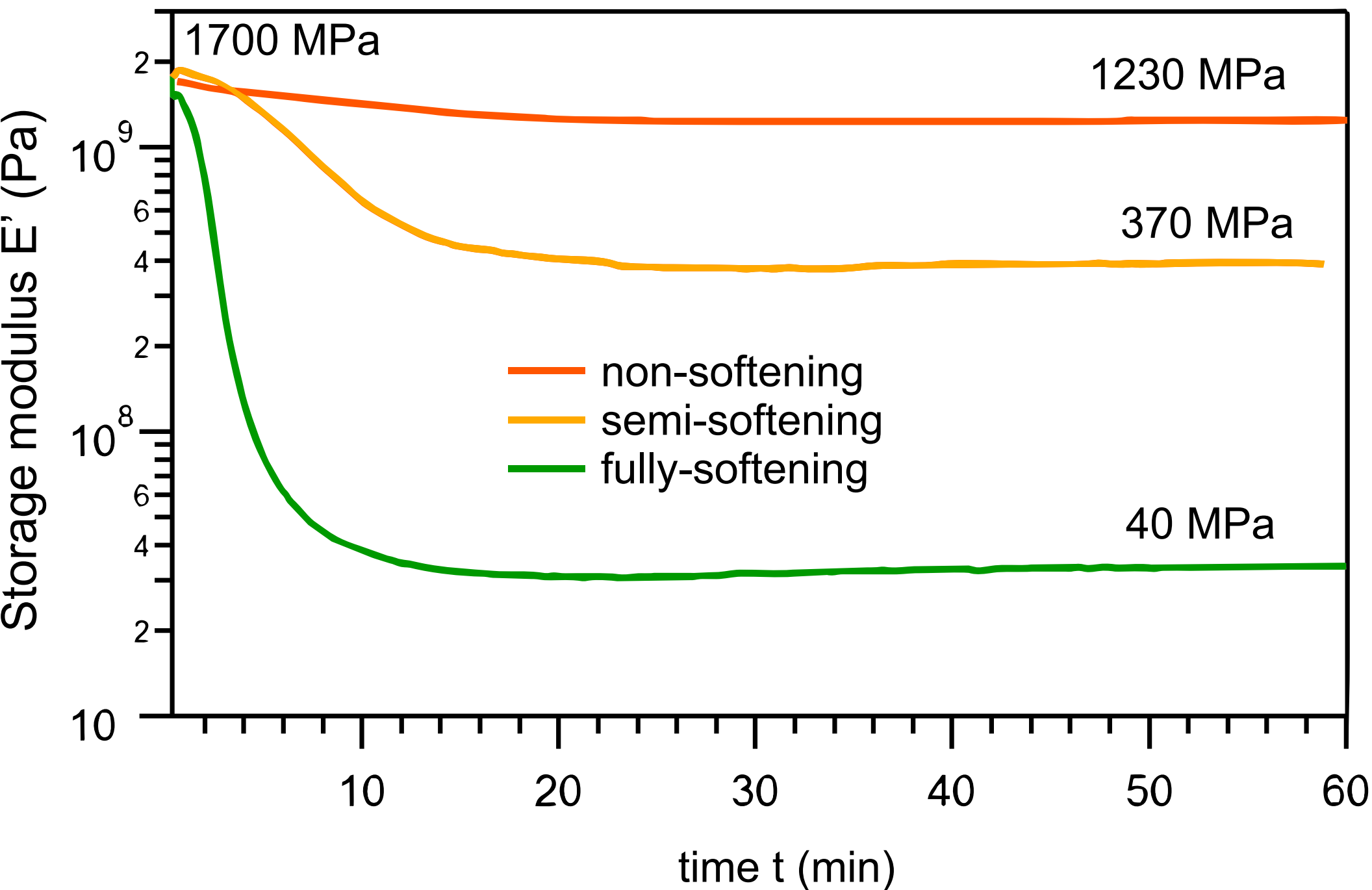


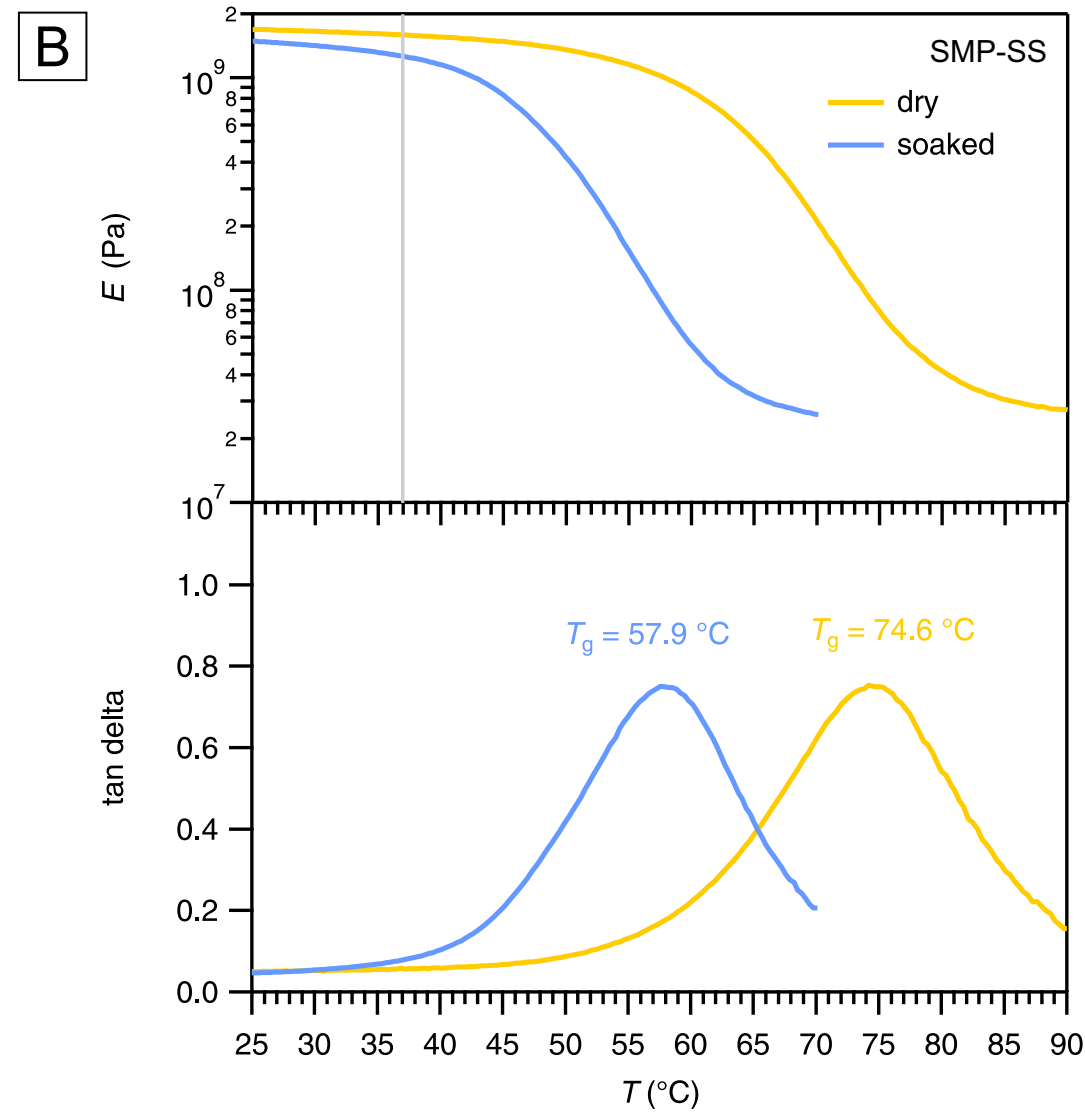
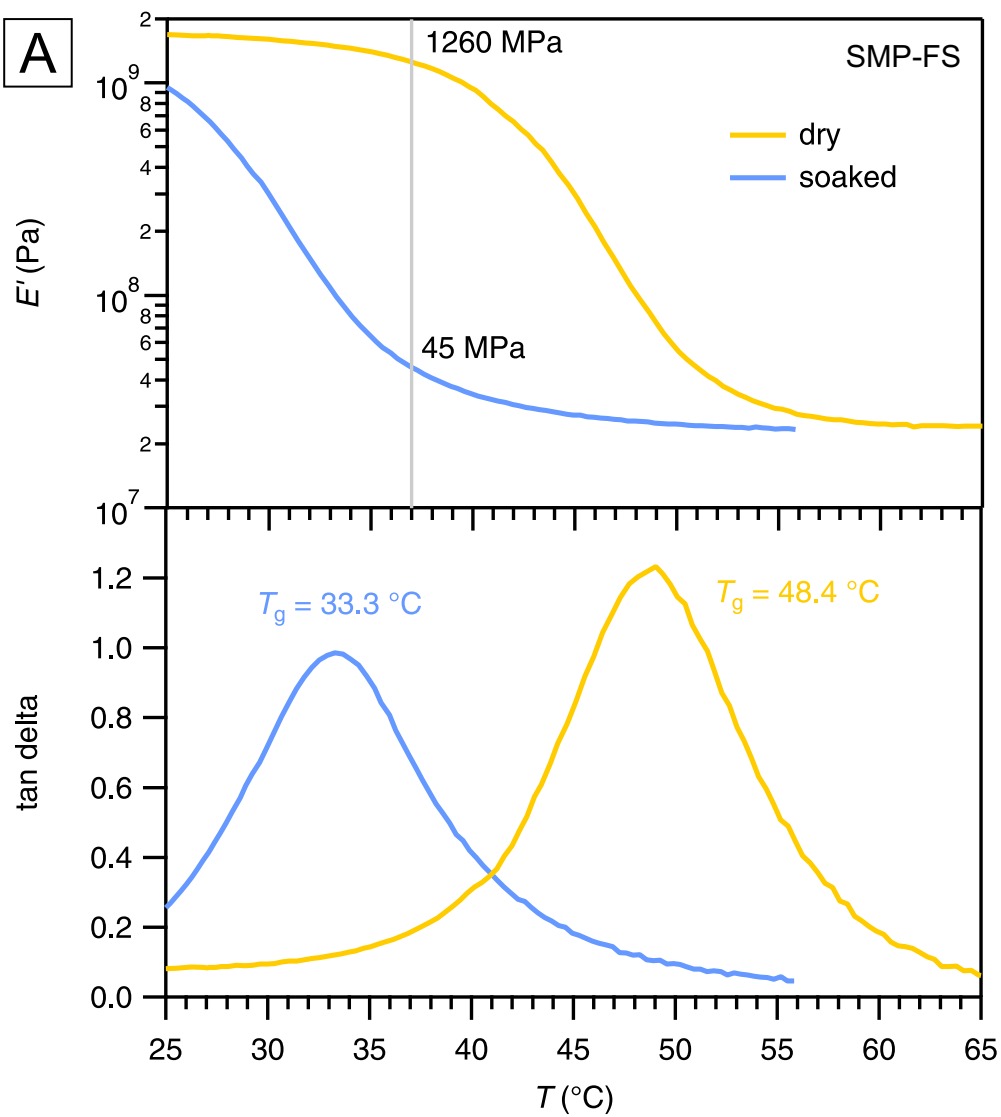
**A****B**











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1,3,5-Triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (TATATO)	Sigma-Aldrich	114235-100G
2,2-Dimethoxy-2-phenylacetophenone (DMPA)	Sigma-Aldrich	196118-50G
CO2 laser Gravograph LS100	Gravotech, Inc.	
Corning Large Glass Microscope Slides, 75 x 50mm	Ted Pella	26005
Environmental DMA: RSA-G2 Solids Analyzer	TA Instruments	
ESD Safe Plastic Tweezer, Tips; Flat, Duckbill, 11.5 cm	Cole Palmer	EW-07387-17
Laurell WS-650-8B spin coater	Laurell Technologies Corporation	
liquid nitrogen	Air gas	
PBS, 1X Solution, Fisher BioReagents	Fisher Scientific	BP243820
SHEL LAB vacuum oven	VWR International	89409-484
Silicon wafer	University Wafer	
The RSA-G2 Immersion System	TA Instruments	
Trimethylolpropane tris(3-mercaptopropionate) (TMTMP)	Sigma-Aldrich	381489-100ML
UVP CL-1000 crosslinking chamber with 365 nm bulbs	VWR International	21474-598

**Comments/Description**

Mechanical grade



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Author(s):

Seyed Mahmoud Hosseini, Walter E. Voit, and Melanie Ecker

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### CORRESPONDING AUTHOR:

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Department:	Center for Engineering Innovation	
Institution:	The University of Texas at Dallas	
Article Title:	The use of environmental dynamic mechanical analysis to predict the softening behavior of neural implants	
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## Answers to the Editors and to the Reviewers:

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Changes to be made by the author(s) regarding the manuscript:

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10. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.). **Changed accordingly**
11. Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion. **Changed accordingly**
12. 1.2: Please specify the spin speeds and times used in this step. **We have added Figure 2 showing spin curves and have added the spin speed for one thickness as an example.**
13. 4.1: Please point out the specific steps that are being repeated here. **The protocol was revised**
14. Please include single-line spaces between all paragraphs, headings, steps, etc. **Spaces were added**
15. After you have made all the recommended changes to your protocol (listed above), please

highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. **Parts have been highlighted in yellow.**

16. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

17. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted. **Steps 2.2.x and 2.3.x were not highlighted, because they include only detailed measuring parameters. Including them would exceed the 2.75 page limit.**

18. Discussion: As we are a methods journal, please also discuss critical steps within the protocol and any limitations of the technique. **Additional limitations were added.**

19. Reference 1: Please do not abbreviate journal titles. **Changed accordingly**

20. Table of Equipment and Materials: Please remove trademark (™) and registered (®) symbols. Please sort the items in alphabetical order according to the Name of Material/Equipment. **Trademarks were removed and items were ordered in alphabetical order.**

## **Reviewers' comments:**

### **Reviewer #1:**

Manuscript Summary:

The article presents a new in-situ method for characterisation of implant materials. Although compatible tests machines are commercial available for such tests, the simple modification a "standard DMA" machine is convincing. The visual presentation is plausible.

Minor Concerns:

Please introduce the abbreviation DMA in abstract and introduction. **We have added this accordingly.**

1. 209 - 212: Please comment the limitation of the machine for testing only of specific samples geometries. Mark this part less general.

**We have added the following paragraph to the Discussion section: "The herein described results were measured on polymer films in tension mode. However, the environmental DMA is also capable of measurements in compression and in shear when using the respective fixture, and therefore allows for the measurement of other sample geometries as well. It should be noted that the available space inside the immersion beaker is limited and therefore the size of samples used for measurements inside this beaker are restricted by that size.**

**Another limitation of this method is that the load cell which is used to detect the forces generated by the samples during the measurement (in dry and in wet conditions). The load cell can only measure forces up to 35 N and therefore limits the sample size/geometry as well."**

### **Reviewer #2:**

Manuscript Summary:

The manuscript discusses an experimental protocol for in vitro dynamic mechanical analysis of polymers for neural implants. The authors' work is based on their previous work on shape memory polymer-based implants and here they show how the softening of these polymers can be characterized experimentally. The in vitro approach could help predict softening of the

polymers in vivo. This is a well written manuscript, but this reviewer did have some concerns which the authors may want to address.

#### Major Concerns:

1. Protocol Step 1.2: Authors could give an idea (perhaps in the form of a plot) about the relationship between spin speeds/time and viscosity/thickness. We have added Figure 2 showing spin curves and have added the spin speed for one thickness as an example.
2. Protocol Step 3.4: Authors could elaborate on this step. Video would probably be helpful, but Figure 3C is difficult to understand and so could be replaced with a magnified image. In addition, it could be supplemented with an image of a centered and properly aligned specimen. We have added Figure 5 showing the correct alignment of the sample.
3. Authors could comment on how the corresponding variation in softening would be for an in vivo medium considering the difference in medium environment. We have added the following paragraph to the Discussion section: "However, measuring in PBS is just one approach to mimic the biological environments. In vivo conditions may vary in many aspects, such as ion concentrations, availability of antibodies, proteins, and other species inside the biological medium/tissue. Depending on the targeted area, one could also consider using different media for the environmental measurements, such as tris-buffered saline (TBS), TBS-T (TBS with Polysorbate 20), Bovine serum albumin (BSA), Cerebrospinal fluid (CSF), or other body fluids."

#### Minor Concerns:

4. Two sessions of measurements take place, one in PBS (Protocol Step 4.1) and two in air (Protocol Step 4.2). Measurement as stated in Protocol Step 4.1 supposedly takes place after Protocol Step 3.5. The step saying "following the above mentioned procedures" sounds a little confusing. The protocol was revised. Dry and wet measurements are now two separate sections.
5. Please update reference 9 since it has already been published. Reference was updated.

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Author of new article	Melanie Ecker
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