Nanoindentation of Hydrated Viscoelastic Materials

Introduction

The study of biological tissues poses many new challenges in the field of indentation\cite{1,2}. While most nanoindentation theory and apparatus have been developed for solid, homogeneous, elastic, or elastic-plastic materials, biological tissues represent a class of heterogeneous hydrated viscoelastic materials \cite{1,2}. As a further complication, biological tissues exhibit significant sample-to-sample variation, in addition to being limited in quantity. It follows that two key problems in the study of these complex materials are sample mounting to maintain hydration during testing and the identification of an appropriate control material to test different indentation techniques. To address these issues, a study was performed comparing quasi-static indentation of 20\% gelatin gels (gelatin), 5\% agarose gels (agarose), and strips of fresh porcine aorta (artery) using a hydration technique developed in our lab.

Materials and Methods

Sample Preparation

Porcine aorta was excised from a sacrificed pig one week prior to testing, and was stored in saline at 5 °C or on ice while not in use. For mechanical testing, 1.5 cm by 1 cm sections were cut from a ring of aorta that had been sliced open to lay flat. Sample thickness was approximately 1.5 mm. 5\% agarose gels were prepared by combining .5 grams of agarose (Low Melting, DNA Grade, Fisher Scientific, Pittsburgh, PA) and 10 ml of distilled water in a 150 ml glass beaker, microwaving the mixture until mixed, pipetting 5 ml of the mixture into a 60 mm diameter petri dish, and setting the gel at 5 °C overnight. 20\% gelatin gels were prepared using 2 grams of gelatin (Type B, bovine skin, 225 Bloom, Sigma, St. Louis, MO) in 10 ml of distilled water. Again, 5 ml of the mixture was pipetted into a 60 mm diameter petri dish and the gel was set at 5 °C overnight. Gels were stored inverted at 5 °C when not in use. 5 mm diameter disks of each gel were punched from the petri dishes as needed for testing. Sample thickness was approximately 1.8 mm. Tweezers were used to place samples of aorta or gel on polycarbonate blocks glued to metal platens prior to testing. Hydration of the samples during testing is outlined below.

Sample Hydration

Completely submerging samples in saline complicates testing as it obscures viewing with the optical microscope and requires specialized tips. For this reason, a method was developed to maintain hydration without submerging the sample. To achieve this, the commercial product OASIS floral foam (Smithers-Oasis USA, Kent, Ohio), a water-absorbent polymeric foam formed from phenolic resins, was utilized. In short, a slab of foam with a 5 mm diameter hole punched out of the center was used to maintain hydration of the gelatin and agarose samples. Gel samples were placed directly into these holes to allow hydration from the edges. For the artery samples, flat sheets of foam 1.5 mm thick and 1.5 cm long were brought into contact with the longer edges of the tissue sample to allow tissue hydration from the sides. Hydration of the foam sheets contacting the samples was maintained by resting them on a larger slab of hydrated foam which functioned as a fluid reservoir. All foam sheets...
were hydrated using saline. These techniques proved successful in maintaining hydration of sample materials for over 12 hours.

Sample Testing

Hydrated samples were tested on the Hysitron TriboIndenter® (Hysitron Inc., Minneapolis, MN) using a 100 μm radius of curvature conospherical diamond probe tip. All samples were tested at room temperature. A standard trapezoidal loading profile with a loading rate of 400 μN/s, a peak load of 400 μN, and a hold period of five seconds was applied to ten sites in each sample. Load-displacement curves were corrected for large displacements and the 3.5 μN force offset prior to analysis. Reduced modulus (E_r) and hardness (H) were calculated from the unloading curves following the method of Oliver and Pharr.

The reduced modulus is related to Young’s modulus, E, by 1/E_r = (1-ν_1^2)/E_1 + (1-ν_2^2)/E_2, where subscript 1 refers to the indenter material, subscript 2 refers to the indented material, and ν is Poisson’s ratio. The ideal spherical tip function was used to calculate projected contact area at the maximum load.

Results and Discussion

The results shown here indicate that 5% agarose gels exhibit load-displacement behavior that is both qualitatively and quantitatively similar to the mechanical behavior of porcine aorta under nanoindentation. Figure 1 depicts typical load-displacement curves for single indents in 5% agarose gel and porcine aorta samples using the 100 μm conospherical tip. As seen here, a 5% agarose gel exhibited the same qualitative behavior as the healthy porcine aorta. Further, as shown in Table 1, the calculated values of E_r and H for the artery were more comparable to values computed for agarose than for gelatin. In addition, similar adhesive behavior was observed as the tip approached agarose and artery samples, even though more adhesion was seen in the artery sample than the agarose sample during tip withdrawal.

One limitation to this study is that porcine aorta most likely has different quantitative mechanical properties from arteries in other mammals, as well as from other vessels in the same animal. However, the qualitative mechanical behavior of arteries from different organisms or locations in the body is expected to remain constant. Hence, agarose is likely to remain a valuable control material, although the exact percentage of agarose used may need to be varied based on the vessel being studied.

In conclusion, a conospherical tip was successfully utilized to indent hydrated viscoelastic materials. An OASIS-based hydration system was developed to maintain sample hydration, and results were compared for three different materials: gelatin, agarose, and artery. A 5% agarose gel proved to be the most similar in qualitative and quantitative behavior to the porcine artery tissue, and will be used as a control material for developing testing techniques in the future.

<table>
<thead>
<tr>
<th>Sample</th>
<th>E_r (MPa)</th>
<th>H (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery</td>
<td>0.77</td>
<td>0.12</td>
</tr>
<tr>
<td>Agarose</td>
<td>0.84</td>
<td>0.04</td>
</tr>
<tr>
<td>Gelatin</td>
<td>0.29</td>
<td>0.07</td>
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</tbody>
</table>

Figure 2: Reduced modulus (E_r) and hardness (H) values for porcine aorta (artery), 5% agarose gel (agarose), and 20% gelatin gel (gelatin) samples indented using a 100 μm radius of curvature conospherical tip.

References:

Acknowledgements:
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