THE CHANGES IN MECHANICAL PROPERTIES OF LIVER TISSUE DURING THE COLD ISCHEMIC PRESERVATION

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Introduction

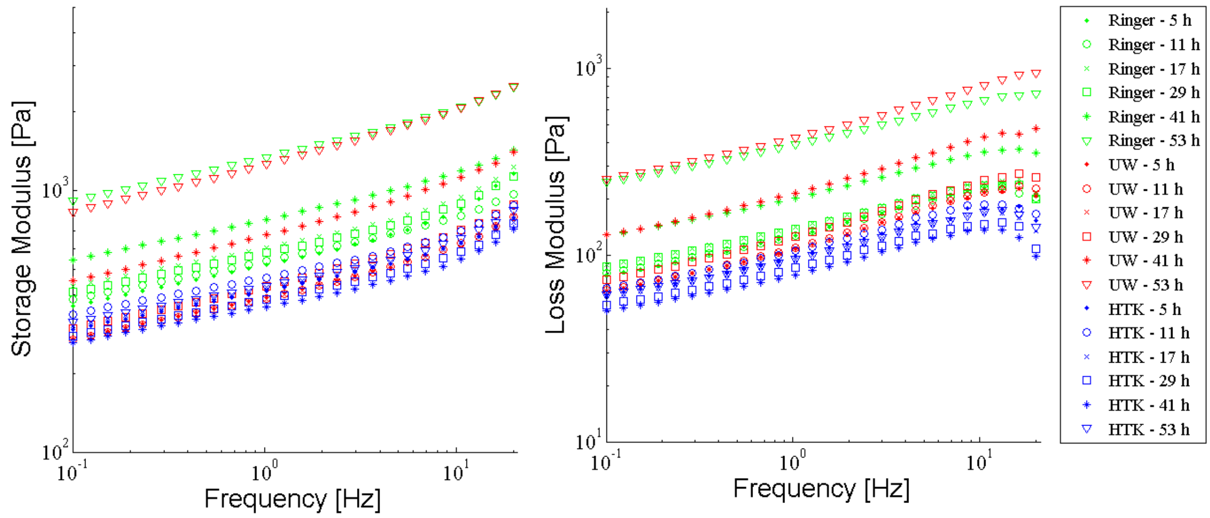
In liver transplantation, most of the time, the donor and recipient are in different locations and longer preservation periods are inevitable. Hence, the choice of the preservation solution and the duration of the safe preservation period are critical for the success of the transplant surgery. In this study, we examine the changes in mechanical properties of bovine liver preserved in different chemical solutions as a function of preservation period to investigate the efficiencies and safe preservation periods of these solutions. Although, the material properties of animal and human livers have been investigated in the past [1], to our knowledge, the number of studies investigating the effect of preservation solution and period on the material properties of liver is very limited [2].

Methods

3 fresh bovine livers are obtained from a slaughterhouse and transported to our laboratory in a Lactated Ringer's solution at +4^{o}C immediately after the harvesting. A total of 54 cylindrical tissue samples having a diameter of 25 mm and a thickness of 2 ± 0.5 mm are prepared from the left lobe of each liver (3 samples for repetition × 3 preservation solutions × 6 preservation periods). The samples are divided into 3 groups and preserved in 3 different solutions: Lactated Ringer (control group), Custodiol (HTK), and Wisconsin University (UW). Using a rheometer (Anton Paar, MCR 102), dynamic shear loading experiments (ω=0.1-20 Hz, γ=0.5%), and static shear loading experiments (γ =0-15%, $\dot{\gamma}$ =0.0005 s^{-1}) are performed on the cylindrical tissue samples preserved for 5, 11, 17, 29, 41, and 53 hours. Before the experiments, the samples are pre-conditioned to reduce the variability in measurements (ω=1 Hz, γ=0.5%, n=50 cycles). During the experiments, the temperature is set to T = 4^{o} C and controlled by the Peltier module (P-PTD200/56/AIR) of the rheometer.

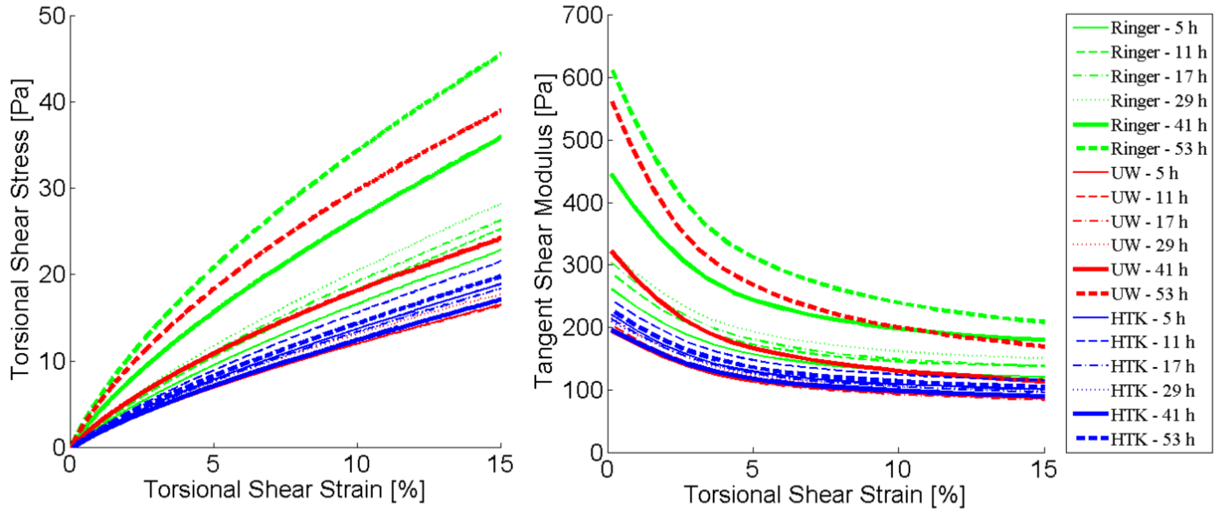
Results

The storage and loss moduli of the liver samples preserved in Ringer, HTK and UW solutions for 5 – 53 h are presented as a function of oscillation frequency in Figure 1. The storage and loss moduli of bovine liver increase as a function oscillation frequency independent of the preservation solution and period. The storage modulus of the samples stored in Ringer, HTK and UW solutions vary between 361-2084 Pa, 262-701 Pa and 266-2073 Pa for the frequency range of 0.1-10 Hz, respectively. The loss modulus of the same samples preserved in Ringer, HTK and UW solutions vary between 78-675 Pa, 51-187 Pa and 64-815 Pa, respectively. The torsional shear stress and tangent shear modulus of the bovine liver samples preserved in Ringer, HTK and UW solutions for 5 – 53 h are given as a function of torsional shear strain in Figure 2. While the torsional shear stress of the tissue samples increases as a function of torsional shear strain, the tangent shear modulus of the samples decreases and reaches to a steady state value independent of the preservation solution and period. At small torsional shear strain (0.1%), the tangent shear modulus of the samples preserved in Ringer, HTK and UW solutions varies between 265 – 631 Pa, 224-261 Pa and 190-443 Pa, respectively. At large torsional shear strain (15%), the tangent shear modulus of the samples stored in Ringer, HTK and UW solutions vary between 123 – 242 Pa, 103 - 130 Pa and 84-166 Pa, respectively. In order to investigate the effect of the preservation solution and period on the storage and loss moduli of the liver tissue, two-way ANOVA tests are performed. The results show that the preservation period, solution and their interaction have significant effects on the storage modulus, loss modulus, torsional tangent shear stress and torsional tangent shear modulus of the livers ($p<0.05$). Also, Bonferroni corrected t-tests are performed to assess the statistical difference between the measurements conducted at different preservation periods. The results show that the storage modulus of the liver samples preserved in Ringer, UW, and HTK solutions change significantly after 11th ($p<0.05$), 29th ($p<0.001$) and 11th ($p<0.001$) h , respectively. On the other hand, the loss modulus of the same samples preserved in Ringer, UW, and HTK solutions change significantly ($p<0.001$) after 17th, 29th and 29th h, respectively. In particular, the storage moduli of the samples preserved in Ringer and UW solutions increase to almost two-folds at the 41st h and to almost four-folds at the 53rd h when they are compared to the moduli values obtained at the 5th h, respectively. However, the storage and loss moduli values of the samples preserved in HTK solution show relatively small changes throughout the whole preservation period. Additionally, Bonferroni corrected paired t-tests showed that the torsional shear stress and tangent shear modulus of the liver samples preserved in Ringer, HTK and UW solutions change significantly at 11th h ($p<0.001$). At small torsional shear strain (0.1%), the tangent shear modulus of the samples preserved in Ringer and UW solutions for 53 h increases 2.38 fold ($p<0.001$) and 2.24 fold ($p<0.001$), respectively, compared to the values obtained for 5 h. However, the increase in tangent shear modulus is only 1.17 fold ($p<0.001$) for the samples stored in HTK solution.



a) b)

Figure 1 a) The storage and **b)** loss moduli of bovine liver as a function of perservation period, solution and oscillation frequency.



a) b)

Figure 2 a) The torsional shear stress and **b)** tangent shear modulus of the bovine liver samples as a function of preservation period, solution and torsional shear strain.

Discussion

We measured the dynamic and static mechanical properties of the bovine liver tissue preserved in Ringer, HTK and UW solutions as function of preservation period using a rheometer. We observed that the liver tissue becomes stiffer and more viscous as the liver samples spend more time in the preservation solution. While the storage, loss and tangent moduli of the liver samples preserved in Ringer solution have the largest change, those stored in HTK solution have the smallest change. The results of the dynamic mechanical experiments show a good agreement with the early findings reported in the literature [2-9]. Also, the results of the static mechanical experiments are in line with the results reported by [10-13]. However, in most of the earlier studies, the investigators have neglected the effect of post-mortem time on the measurements, which could be the reason for the variations in the values reported for storage, loss and tangent moduli of liver. Moreover, we performed statistical analyses on the mechanical properties to suggest safe preservation periods for the most commonly used preservation solutions. Based on our analyses of the mechanical properties, we conclude that the bovine liver tissue is preserved well in UW and HTK solutions for up to 29 h. However, the material properties of the liver tissues stored in UW solution change drastically when preservation period is longer than 29 h. Hence, if preservation longer than 29 h is required, HTK should be preferred over UW. In the future, we are planning to investigate the changes in histological properties of the same liver samples to correlate them with the changes in material properties.

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