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The influence of low temperatures on dynamic mechanical properties of animal bone

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Different preservation methods are currently used in bone banks, even though their effects on allograft quality are not fully understood. Freezing is one of the most popular methods of preservation in tissue banking. Yet, there is not a lot of data on dynamic mechanical properties of frozen bone. Material used in this study was femoral bones from adult bovine that were machine cut and frozen to the temperature −140°C. Both elastic modulus and loss modulus were measured at 1, 3, 5, 10, and 20 Hz in the temperature range of 30-200°C. Differences between frozen and control samples were observed. The frequency increase always led to the increase in elastic modulus values and decrease in loss modulus values. Freezing reduced the elastic modulus values of about 25% and the loss modulus values of about 45% when measured at 20°C.

Key words: dynamic mechanical analysis, bones, freezing.

Introduction

Bone graft is used in a wide range of operative procedures [4]. Each year, an estimated 450,000 allografts are transplanted in the USA alone and they are coming into ever more frequent use [1, 14]. Allograft bone has been used as a stimulus for new bone formation and as a replacement or reinforcement for structural components of the skeletal system. Although autograft has long been considered the golden standard for achieving the required remodeling and stability [4, 17]. Bone allografts are available in great quantity for large reconstructions and diminish the discomfort patient in comparison with autografts [2]. Bone banks supply a wide range of allograft bones, including massive bone allografts, cortical bone allografts, and milled bone [15, 16]. Autologous bone grafts are usually transplanted fresh, while allografts are subjected to preservation and sterilization [12, 16]. One drawback to using allografts is the potential risk of disease transmission. Tissue banks evaluates the donor's social and medical history and screen for infectious markers (HIV-1, HIV-2, hepatitis B, hepatitis C, HTLV-I, HTLV-II, and syphilis) [3, 7]. At present, the preparation of these grafts includes removal of marrow and cells, treatment with solvent – detergent, prion inactivation, freezing or freeze-drying and gamma irradiation. Even though cumulative effect of these steps make the grafting material very safe for human, such processing methods are likely to affect both the mechanical and biological properties of bone [5, 12]. Bone banks typically store bones in sterile conditions at temperatures below −40°C for periods longer than 6 months or between −18°C and −28°C for periods shorter than 6 months [14].

Dynamic Mechanical Analysis (DMA) is the method accepted in studies on mechanical properties of bone but there is not many data on dynamic mechanical properties of frozen bone. The modulus measured in DMA is, however, not exactly the same as the Young's modulus of the static analysis. In DMA complex modulus (E^*), elastic modulus (E') and loss modulus (E'') are measured. These different moduli allow to examine the ability of the material to return or store energy (E') and its ability to lose energy (E''). The principles of DMA are based on the fact that the sinusoidal oscillations of stress generate the response of bone – the sinusoidal oscillation in strain. The strain oscillations have the same frequency but different amplitude. Usually stress and strain are phase shift [11, 13, 20]. The purpose of the study was to determine of the temperature dependence of the elastic modulus (E') and loss modulus (E'') of frozen bone. Furthermore the effect of applied frequency on temperature dependence of E' and E'' was determine as well.

Material and methods

Femoral bones from adult bovine (2-3 years old animals) were machine – cut from the central part of the diaphyses using a diamond saw. Specimens of 20 × 5 × 2 mm were dried and frozen to the temperature −140°C. Fresh bones samples were used as controls. DMA testing was performed by means on dynamic mechanical analyser DMA

242, Netzsch at the frequency of 1, 2, 5, 10 and 20 Hz, in the temperature range of 30-200°C. The dynamic load of 7 N was applied using a three point bending configuration.

Results

The results were digitized and analyzed by computer. The values of E' and E'' are presented respectively in Figure 1 and Figure 2. Figure 3 presents the changes in E' and E'' related to the control samples. E' and E'' were measured for 5 different frequencies. However only 1, 5 and 20 Hz are showed to the better transparency of figures. The differences between frozen and control samples were observed in E' values, which were temperature and frequency dependent. The frequency increase always led to the increase in E' values. At the ambient temperature E' for the frozen bone was reduced about 25% in the comparison with the control samples. Above the temperature of 50°C the values of E' increased linearly and reached the E' values of control samples between 160 and

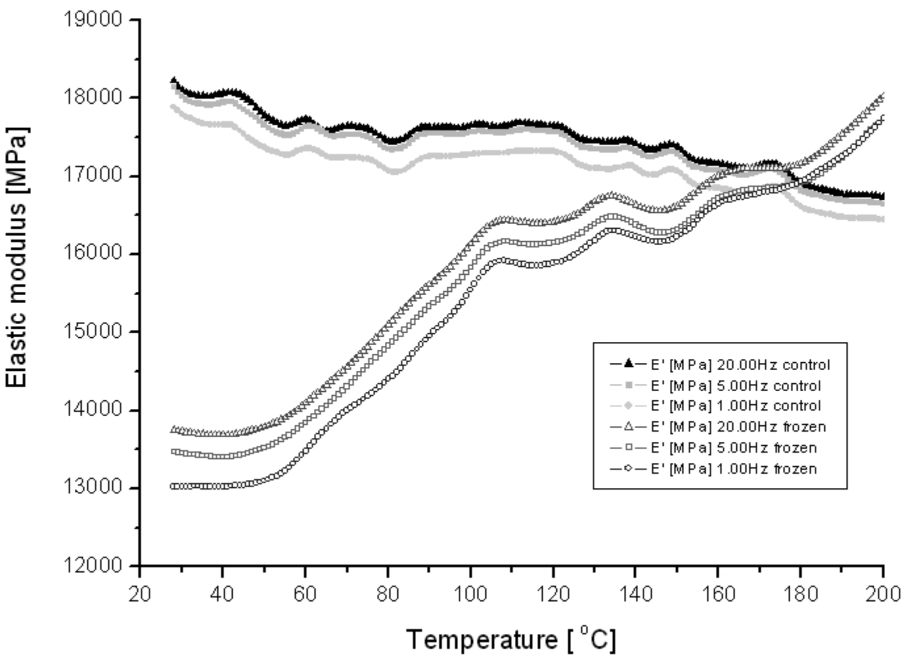

Figure 1. E' values for frozen and control samples measured for frequency of 1, 5 and 20 Hz.

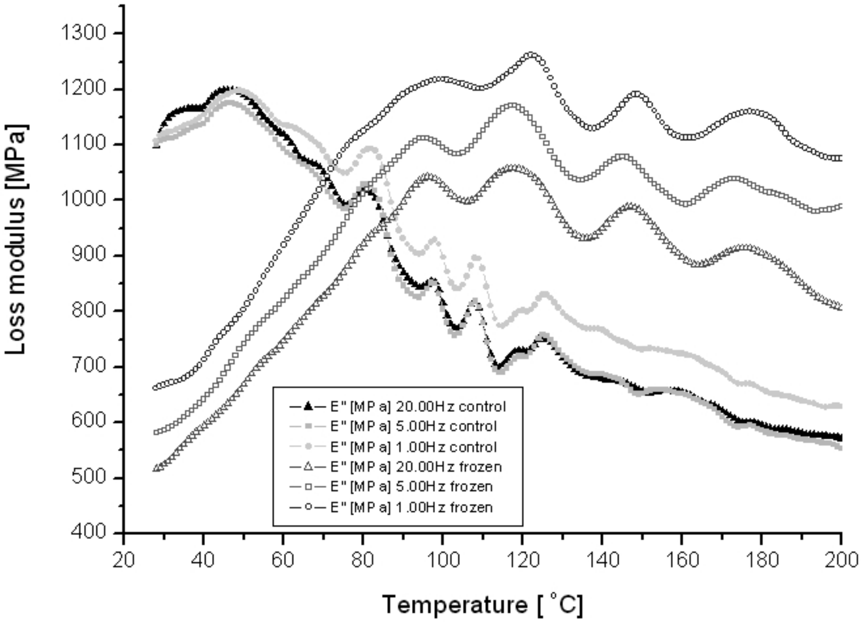

Figure 2. E'' values for frozen and control samples measured for frequency of 1, 5 and 20 Hz.

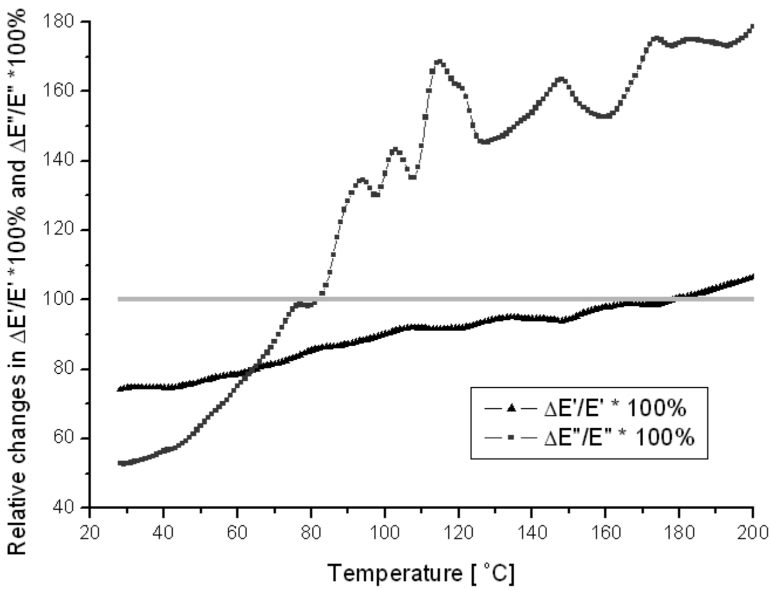

Figure 3. The effect of freezing on E' and E'' values expressed as the percent of control samples E' and E'' values, frequency 5 Hz.

180°C. E'' values were also temperature and frequency dependent, but the frequency increase led to the decrease in E'' values. Freezing affected also E'' values. Reduction by about 45% was observed in room temperature for the E'' values. The increase in temperature led to the increase in E'', which reached the values of control samples in the temperature range of 60-90°C.

Discussion

Freezing preserves the bone and allows for long-term storage [9]. At the same time freezing decreases also the antigen response to allograft and slow down the enzymatic degradation of collagen [9].The data from this study indicates that freezing at the temperature of liquid nitrogen deteriorates the biomechanical properties of bones, contrary to the results reported by Frankel [6] and Sedin [19] who found no differences in bending strength in samples frozen to the temperature of −25°C and in controls. Komender [10], on the other hand, reported the reduction of 10% in bending strength and compression but not in torsion for bones frozen at the temperature of −78°C. Also Kang et al. [9] proved a mean decrease of 7.2% in bending strength and decrease of 11% in compressive strength at the freezing temperature of −80°C. Only Pelker et al. [17, 18] showed increase in compression of 10-20% at freezing temperatures of −20°C, −70°C and at the temperature of liquid nitrogen. They reported no changes in torsion for all the measured temperatures. Presented experiments were performed using the static tests. The present study is the first work that discuss the influence of low temperatures on dynamic mechanical properties of bone. Diverse results reported in the mentioned studies can account for differences in bone treatment (e.g. different conditions of freezing), its physicochemical characteristics (e.g. content of minerals) and different measuring methods.

Conclusions

Taking into consideration the above mentioned facts further studies to explain the role of freezing on dynamic mechanical properties of bones are needed. Freezing deteriorates the dynamic mechanical properties of bone but at the same time it preserves the bone and allows long-term storage. Freezing at −140°C deteriorates elastic modulus by 25%

and loss modulus by 45% when measured at 25°C . Since bone is less elastic and less capable to lose energy it seems to be optimal to use another temperatures to freeze bones.

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