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# EFFECT OF THE LONG-TERM STORAGE METHODS ON THE STABILITY OF CARTILAGE BIOMECHANICAL PARAMETERS

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Abstract. Long-term stability of the tissue product in terms of mechanical parameters is a key factor for its expiration date. For the investigation of storage effects on the cartilage tissues the experimental mechanical loading test combined with XCT scanning for the irregular shape inspection was performed. The samples were preserved according to three different protocols using the deep-freezing and two types of saline solution preservation. The stability of the biomechanical parameters was tested within annual intervals. All samples were subjected to uni-axial compression loading using the in-house developed compact table top loading device in displacement-driven mode. Based on the measurements, the results are represented in the form of stress-strain curves and quantified as elastic modulus and ultimate compression stress. It can be concluded that no significant difference was found in neither the mechanical properties of the samples nor in the effects of each preservational method.

Keywords: Stability study, cartilage, biomechanical characterisation, compression loading, XCT imaging.

## 1. Introduction

Storage of tissues of human origin for long-term banking is an essential operation of Tissue Establishments. Long-term stability of the tissue product is the key parameter for determining its expiration date. The acceptable method of tissue preservation must be capable of retaining the natural properties of the graft (e.g. viability, structural integrity) for the time interval of the maximum possible storage time.

One of the most common preservation methods which has been used for decades is deep-freezing. The grafts without cryoprotectants are stored in the range of temperatures from −80 °C to −40 °C. For this storage procedure only little impact on the biomechanical parameters was reported [1, 2]. Alternatively, the chemical preservation could be used which involves the conservation of cartilage using saline solution with the concentration typically in range 0.9–3.0 % at room temperature [3]. This method is not widely used yet and in general there is a lack of publications investigating the stability of biomechanical parameters of products stored in this way. This method brings potential risk of a significant decrease of elastic modulus and yield stress in cartilages as uniquelly reported in [4].

During the pre-storage tissue treatment the allografts are commonly subjected to antibiotic-based disinfection [1] and for this study the samples stored using the saline solution were also sterilized using ionizing radiation. Gamma rays irradiation sterilization itself can influence the biomechanical properties of allografts and induce changes in the molecular structure [5, 6]. In addition, to avoid potential risk, both storage methods were examined and compared in terms of costal cartilage biomechanical stability. The aim of this study is to assess the effect of long-term storage of cartilages preserved by the two different methods, using three different protocols. These findings will be useful for a comprehensive assessment of the suitability of the alternative saline preservation method for costal cartilage storage. The problem of irregular shape of the tested cartilage sample [7, 8] was solved by subjecting the sample to XCT imaging to obtain full 3D geometrical model for estimation of the cross-section area together with employing of closed-loop controlled precise system for mechanical testing. For that reason the set of biomechanical experiments coupling XCT imaging and uni-axial testing [9] was performed to evaluate the time-dependent trends in changes of biomechanical parameters.

## 2. Materials and methods

### 2.1. Samples

For costal cartilages of human origin three types of tissue preservation for the long-term storage were used:

(i) deep-freezing and storage in range temperatures

from −80 °C to −40 °C and cooled solid $CO_2$ during the transportation,

(ii) preserved by 3.0 % NaCl solution and stored at room temperature,

(iii) preserved by 0.9 % saline and stored at room temperature.

Before the storage process, all the samples were treated by antibiotic-based disinfection bath. Moreover, the samples stored in the saline solution were sterilised by gamma rays. Two testing samples were prepared by manual sectioning from each cartilage with height of 5–7 mm and plan-parallel (± 0.1 mm) contact faces fitting in circumscribed circle of 12 mm. Because of the irregular shape of the samples, 3D models were used for identification of cross-section area $A_c$ used in stress calculation. Individual samples were subjected to X-ray microtomography (XCT) scanning just before the loading experiment. The scanning procedure consisted of 800 projections with acquisition time of 400 ms, in total 8 mins including rotation and read-out time. The sample was scanned in polymer container partly filled with water but the sample was surrounded only by air. This setup ensures that the imaging process does not affect the structure and biomechanical properties of the sample, utilizing an X-ray source with an acceleration voltage of 75 kV and tube current of 120 $\mu$A. The sample placed in XCT scanned is depicted in Figure 1.

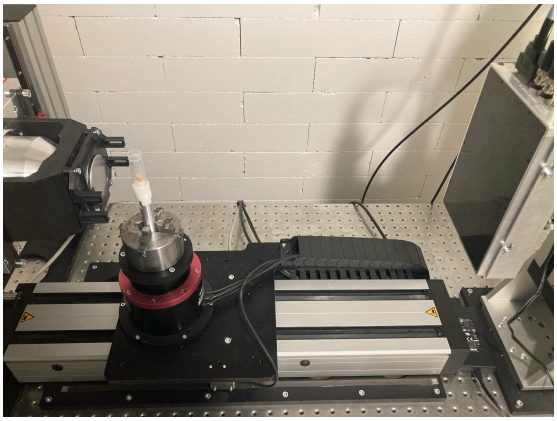

FIGURE 1. The environmental container with the sample placed in XCT scanner. X-ray source (left), sample with container and holder on rotary table (middle), flat panel detector (right).

To obtain proper $A_c$ size, volumetric data were reconstructed using cone-beam FDK algorithm [10] implemented in VGStudio Max (Volume Graphics, Germany) software and exported as image stack with pixel size of 9.5 $\mu$m. On the volume of interest the tissue was segmented by thresholding and application of gaussian smoothing and erosion filtering (see Figure 2). Minimal $A_c$ was taken for further stress calculations. In all cases minimal $A_c$ reached at least 95 % of median cross-sectional area. This fact minimizes the risk of corrupted data processing.

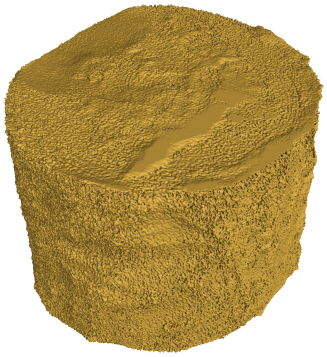

FIGURE 2. Visualization of the cartilage sample based on XCT imaging.

FIGURE 3. Compression test arrangement

## 2.2. Biomechanical analysis

Biomechanical parameters, namely compressive modulus $E$, ultimate stress $\sigma_u$ and corresponding strain level $\varepsilon_u$ were taken for evaluation of the long-term storage effect on the tissues. For that purpose uniaxial quasi-static compression tests were performed using in-house developed compact loading device (see Figure 3) in detail described in [9]. To ensure the optimal accuracy of the measurement load-cell LCM300 (Futek, USA) with the nominal capacity of 1.1 kN was employed. For the displacement driven experiment the loading rate was set to 10 $\mu$m s$^{-1}$ and maximal displacement 3500 $\mu$m corresponding to 50 % strain for the highest sample. Read-out frequency was set to 200 Hz for raw data logging employing in-house developed control system [11]. Automated Matlab (MathWorks, USA) script was used for the data processing to obtain the stress-strain curves representing the deformation behaviour and assess to the biomechanical parameters.

For deformation behavior the stress was considered as engineering stress obtained using

$$\sigma_{\text{eng}} = \frac{F}{A_c}, \tag{1}$$

where $F$ is load-cell output. Engineering strain $\varepsilon_{\text{eng}}$ was calculated by the following formula

$$\varepsilon_{\text{eng}} = \frac{u}{h_0}, \tag{2}$$

where $u$ represents displacement measured by encoder and $h_0$ is the initial height of the sample.

## 3. Results and discussion

To evaluate effect of the long-term storage of cartilages under deep frozen condition, biomechanical characteristics assessed from uni-axial compression test performed in February 2022 and February 2023 were used. The deformation behaviour of all tested samples is represented by stress-strain curves in Figure 4.

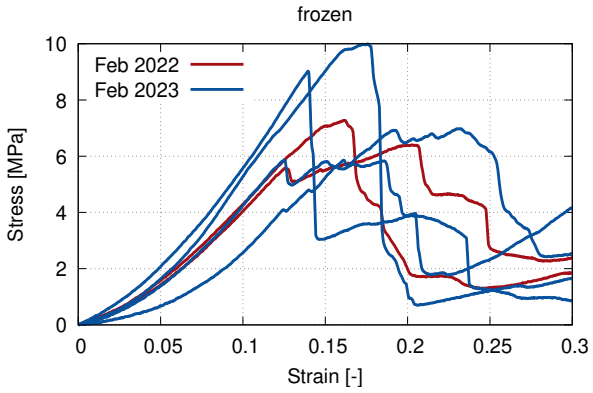


Figure 4. Deformation characteristic of all deep frozen samples

The mean values of material parameters with corresponding standard deviation are summarized in Table 1. Based on the obtained results, no significant

| Date | Feb 2022 | Feb 2023 |
|---|---|---|
| $E$ [MPa] | $57.24 \pm 0.87$ | $65.45 \pm 13.69$ |
| $\sigma_{\text{u}}$ [MPa] | $6.84 \pm 0.62$ | $7.97 \pm 1.88$ |
| $\varepsilon_{\text{u}}$ [-] | $0.1821 \pm 0.0286$ | $0.1582 \pm 0.0311$ |

Table 1. Time dependence of biomechanical parameters of deep frozen samples

long-term storage effect was proven. The difference in biomechanical parameters of each individual sample is higher than the influence of the storage effect.

To evaluate the effect of the long-term storage of cartilages preserved in 0.9 % NaCl, biomechanical characteristics were assessed using the same tasting procedure. Resulting stress-strain curves are presented in Figure 5.

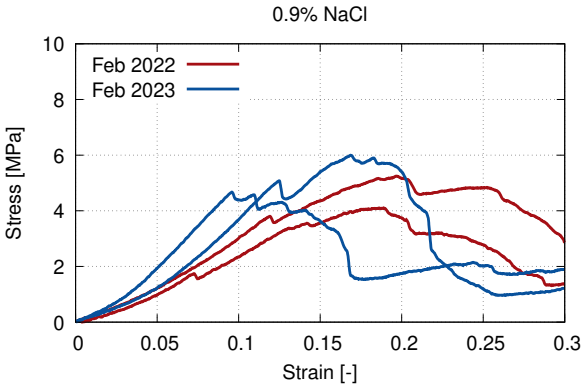


Figure 5. Deformation characteristic of 0.9 % NaCl preserved samples

The mean values of material parameters with corresponding standard deviation are summarized in Table 2.

| Date | Feb 2022 | Feb 2023 |
|---|---|---|
| $E$ [MPa] | $46.80 \pm 11.24$ | $53.38 \pm 4.38$ |
| $\sigma_{\text{u}}$ [MPa] | $5.00 \pm 0.81$ | $4,88 \pm 0.25$ |
| $\varepsilon_{\text{u}}$ [–] | $0.1625 \pm 0.0459$ | $0.1305 \pm 0.0537$ |

Table 2. Time dependence of biomechanical parameters of 0.9 % NaCl preserved samples

From the obtained results a slight increase of the magnitude of the elastic modulus over a longer time can be seen. This finding is in contrary to the hypothesis of time-dependent degradation of the biomechanical parameters. There is negligible difference in ultimate stress and non-significant drop of the strain at the ultimate strain level. Because of the nearly 40 % strain variation ($0.1322 \pm 0.0515$) result it can't be taken as a proof of the time effect.

Finally the effect of saline saturation was evaluated. Resulting stress-strain curves are presented in Figure 6.

The mean values of material parameters with corresponding standard deviation are summarized in Table 3. Based on the obtained results no significant

| Salinity | 0.9 % NaCl | 3.0 % NaCl |
|---|---|---|
| $E$ [MPa] | $37.55 \pm 5.66$ | $56.04 \pm 2.25$ |
| $\sigma_{\text{u}}$ [MPa] | $4.67 \pm 0.81$ | $5.33 \pm 0.93$ |
| $\varepsilon_{\text{u}}$ [-] | $0.1928 \pm 0.0056$ | $0.1322 \pm 0.0515$ |

Table 3. Biomechanical parameters depending on saline solution

long-term storage effect was proven. The difference in biomechanical parameters of each individual sample is higher than the influence of the storage effect. The ultimate stress difference is minimal (under 3.0 %). Slight

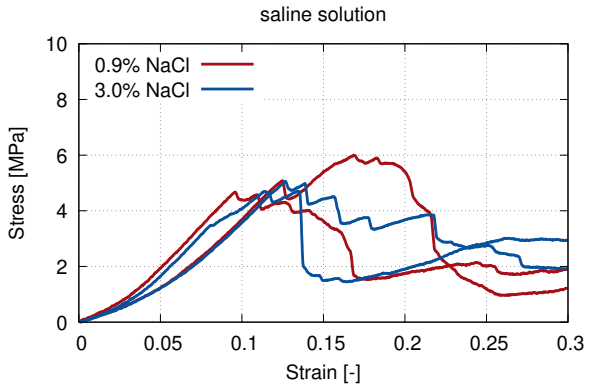


FIGURE 6. Deformation characteristic of 0.9 % NaCl and 3.0 % NaCl preserved samples

increase of elastic modulus together with decrease of the strain for ultimate stress could be recognized but on negligible level. But it has to be kept in mind that this study is not statistically relevant because of limited number of delivered samples.

## 4. CONCLUSIONS

It can be concluded that no significant difference in mechanical properties was observed within one year measurement interval despite the findings for saline preservation presented by Zhang et al. [4]. For that reason all proposed preservation methods are suitable for long-term storage of the allografts. The gamma dose caused by XCT scanning cannot influence long-term biomechanical parameters because the imaging was performed just before the mechanical testing. The same testing procedure will be applied on the cartilage samples in the next two years to complete the stability study.

### ACKNOWLEDGEMENTS

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