Effect of freezing storage time on the elastic and viscous properties of the disc of porcine temporomandibular joint

Jose L Calvo-Gallego[a,*], Maria S Commisso[b], Jaime Domínguez[a], Eiji Tanaka[c], Javier Martínez-Reina[a]

[a]*Department of Mechanical Engineering, University of Seville, Camino de los Descubrimientos s/n, Seville 41092, Spain*
[b]*University of Lyon, ECAM Lyon, INSA-Lyon, LabECAM, MATEIS, F-69005 Lyon, France*
[c]*Department of Orthodontics and Dentofacial Orthopedics, Institute of Biomedical Sciences, Tokushima University Graduate School, Japan*

Abstract

This is a preprint of the paper, written before peer review. The reader is referred to the published version of this paper: https://doi.org/10.1016/j.jmbbm.2017.03.035. Please, cite this work as: J.L. Calvo-Gallego, M. Commisso, J.Dominguez, E. Tanaka and J. Martínez-Reina. Effect of freezing storage time on the elastic and viscous properties of the porcine TMJ disc. Journal of the Mechanical Behavior of Biomedical Materials. 2017; 71:314-319.

The correct characterisation of the articular disc of the temporomandibular joint (TMJ) is key to study the masticatory biomechanics. For the interval from extraction until testing, freezing is the most used preservation technique for biological tissues, but its influence on their behaviour is still unclear. An important error can be committed in the characterisation of such tissues if freezing has any effect on their mechanical properties. Thus, the aim of this study was to determine whether the freezing storage time causes any change in the mechanical properties of the TMJ discs. To check that, the specimens were stored in a $-20°C$ freezer during different time intervals: 1 day, 1 week, 1 month and 3 months. Fresh specimens, tested right after extraction, were used as the control group. Compressive stress relaxation tests were carried out on the specimens and a quasi-linear viscoelastic (QLV) model was used to fit the experimental curves. A statistical analysis detected significant differences among the groups. Post-hoc tests determined that freezing the specimens more than 30 days may lead to changes in the viscoelastic properties of the tissue.

Keywords:

Articular disc, temporomandibular joint, freezing storage time, stress relaxation test, quasi-linear viscoelasticity

Reference	Type of tissue	Type of test	Objective	Freezing effect
Linde and Sørensen [20]	Human trabecular bone	Unconfined compression cycles	Comparing different storage methods	No change in stiffness. Significant change in viscoelastic properties
Clavert et al. [5]	Human biceps brachii tendons	Stress relaxation under uniaxial tensile test	Comparing immediate testing and testing after freezing at $-30°C$ during 2 weeks	Altered ultimate tensile strength and Young's modulus
Kennedy et al. [15]	Bovine articular cartilage	Indentation cycles	Comparing a fresh group with specimens frozen at $-20°C$ and $-80°C$	Decrease in stiffness
Chow and Zhang [4]	Bovine aortic tissue	Biaxial tensile cycles	Comparing different storages temperatures and times	Changes in the mechanical properties
Ternifi et al. [25]	Porcine kidney	Shear wave elastography	Comparing different storage temperatures	Decrease of the shear modulus in the surface but not in the interior
Kiefer et al. [16]	Bovine articular cartilage	Indentation cycles	Comparing different cryopreservation protocols	No effect noticed on the mechanical properties
Allen and Athanasiou [1]	Porcine TMJ discs	Unconfined compression stress relaxation tests	Analyzing the effect of multiple freezing - thawing cycles	Material properties retained after 5 freeze-thaw cycles
Hongo et al. [13]	Porcine intervertebral discs	Lateral flexion, flexion - extension and rotation cycles	Analyzing the effect of multiple freezing - thawing cycles	No effect noticed on the mechanical properties
van Haaren et al. [28]	Goat cortical bone	Destructive torsion and 4-point bending and hardness tests	Comparing 5 different freezing storage periods	No significant differences
Nazarian et al. [21]	Murine bone	Cyclic compression and 4-point bending tests. Last cycle until failure	Comparing a fresh group with specimens frozen at $-20°C$ during 2 weeks	No change in elastic and viscoelastic properties
Szarko et al. [24]	Bovine articular cartilage	Indentation cycles	Comparing different freezing storage temperatures	No alteration in the mechanical properties for $-20°C$ and $-80°C$
Wex et al. [29]	Porcine liver tissue	Shear relaxation and rheometry tests	Comparing different temperatures	No influence on the mechanical properties
Torimitsu et al. [26]	Human skulls	Destructive 3-point bending tests	Comparing different freezing storage periods	Little effect on the mechanical properties
Wieding et al. [30]	Ovine cortical bone	Destructive 4-point bending tests	Comparing 3 different preservation techniques	No difference in the elastic properties and energy absorption

Table 1: Summary of the literature about the effect of freezing storage time.

1. Introduction

A great debate still exists about the influence of freezing on the mechanical properties of biological tissues. It is a common practice to store the specimens in a freezer from the moment of extraction until testing. The main reason is that it can be difficult to test all the specimens of an excised tissue sample few hours after the excision. Therefore

---
*Corresponding author. Tel.: +34-954487311; fax: +34-954460475.
Email address: joselucalvo@us.es (Jose L Calvo-Gallego)

the specimens pass hours or days waiting to be tested, with the likely degradation of the tissue at room temperature. Many authors claim that the cold storage causes changes in the mechanical properties of the tissues [20, 5, 15, 4, 25], whereas other state that freezing does not have any influence [16, 1, 13, 28, 21, 24, 29, 26, 30]. In table 1, a summary of the literature about the effect of freezing in different tissues is presented.

As can be seen in table 1, there is no clear consensus on how freezing affects the biological tissues, though the majority of studies state that it has no effect. Moreover, few studies analyse the effects of freezing on the elastic and viscous properties separately. It can also be observed that not many studies have been conducted on fibrocartilage, and that they are not conclusive either. For example, Kennedy et al. [15] and Szarko et al. [24] carried out similar studies on the same tissue (bovine articular cartilage). The first one found differences in the mechanical properties between fresh specimens and frozen ones, whereas the second one did not. In particular, few authors studied the influence of freezing on the mechanical properties of the TMJ articular disc (Allen and Athanasiou [1]), and they focused on the effect of freeze-thaw cycles, but not on the freezing storage time.

The mechanical behaviour of the TMJ disc was characterised in a previous article [6]. A quasi-linear viscoelastic (QLV) model with a hyperelastic response for the elastic function was used to fit compressive stress relaxation tests performed in the articular disc of the porcine TMJ, at different strain rates and strain levels. The validity of the QLV model was checked showing the independence of the fitted model with both parameters. The model was able to capture the behaviour of this tissue with enough accuracy. However, since the samples were frozen before testing, the validity of the proposed model is subjected to the independence of the fitted properties with the freezing conditions.

The particular aim of this work is to determine if freezing storage time has any influence on the elastic and viscous properties of the articular disc of the porcine TMJ.

2. Materials and methods

2.1. Extraction of samples

A total of 86 articular discs were taken from *large white* pigs (aged from 8 months to 1 year) immediately after slaughter. A single cylindrical sample was extracted from the central region of each disc, with an approximately circular cross sectional punch of diameter $\phi = 4mm$. After extraction, the area and average thickness (L) of the sample were measured as explained in a previous study [6]. The variation in thickness of a sample, ΔL, defined as the difference between its maximum and minimum thickness, must be small enough to ensure a uniaxial stress state, as established by the criterion provided by Commisso et al. [9]. This criterion establishes the rejection of specimens based on their dimensions (L, ϕ and ΔL). It is given in a graphical way and not shown here for brevity. Following this

Area (mm^2)	L (mm)	ΔL (mm)
12.59 ± 1.40	1.82 ± 0.21	0.28 ± 0.08

Table 2: Dimensions (mean ± SD) of the cross sectional area, average thickness and variation in thickness measured in samples of porcine articular discs.

criterion, 79 out of the 86 extracted samples were finally accepted. In table 2, the means and standard deviations of the area, thickness and variation in thickness of all samples are shown.

2.2. Storage conditions

After extraction, the samples were individually: 1) wrapped in saline-soaked gauze (0.9% w/v of NaCl), 2) enveloped in plastic film and introduced in hermetic vials to prevent dehydration and 3) frozen at −20°C until testing during different times: 1, 7, 30 and 90 days. Finally, the last group consisted of specimens that were tested fresh, e.g. immediately after extraction, without freezing. At least, 15 specimens were tested for each group. Before testing, they were submerged in saline solution at room temperature and allowed to thaw.

2.3. Test protocol

A servo-hydraulic testing machine (858 Mini Bionix II, MTS) was used to carry out relaxation tests, by uniaxially compressing the specimens between two metal platens. They were fixed to the inferior platen of the testing machine with a circular piece of double-faced adhesive of 1 mm in diameter in the center of the sample to prevent it from sliding off the platens. Vaseline was spread on the surface of both platens to reduce the friction in the non-fixed areas, as recommended by Commisso et al. [8]. During the test, the samples remained submerged in saline solution at 37 ± 1 ° C. The superior platen was positioned at the average thickness of the sample.

A preconditioning of 20 cycles from 0% to 10% strain at 1 Hz was applied to each sample [2], followed by a ramp from 0% to 50% strain. This strain was maintained for 15 *min* allowing for stress relaxation (see figure 1).

2.4. Material model

In a previous work [6], the validity of the QLV model [12] was checked for the articular discs of the temporomandibular joint. Moreover, an exponential strain energy function (leading to the elastic response function T^e of equation 3) was found the best to fit the behaviour of the discs, together with a fifth-term Prony series (see equation 2). See [6] for details.

The stress response to a general stretch history $\lambda(\tau)$ is:

$$\sigma(t) = \int_0^t \overline{G}(t - \tau) \, \frac{dT^e[\lambda(\tau)]}{d\lambda} \, \frac{d\lambda(\tau)}{d\tau} \, d\tau \qquad (1)$$
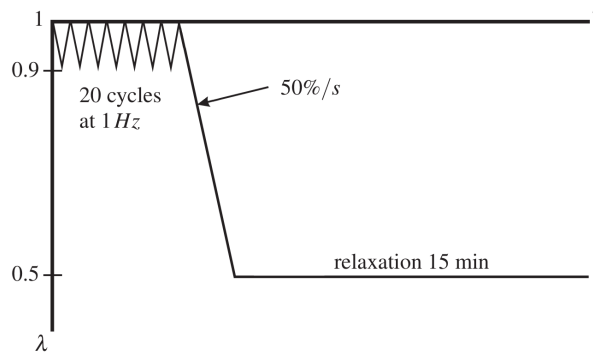
Figure 1: Stretch, λ, applied in the relaxation tests.

with:

$$\overline{G}(t) = g_\infty + \sum_{i=1}^{5} g_i \, e^{-t/\tau_i} \tag{2}$$

$$T^{(e)} = 2 \, A \, B \, e^{B(\lambda^2 + \frac{2}{\lambda} - 3)}(\lambda^2 - \frac{1}{\lambda}) \tag{3}$$

The relaxation times were taken in decades: $\tau_1 = 0.01s$, $\tau_2 = 0.1s$, $\tau_3 = 1s$, $\tau_4 = 10s$ and $\tau_5 = 100s$ [17], to ensure the uniqueness of the fitted $\overline{G}(t)$ [27].

In this work, the stretch history (figure 1) was a ramp of finite strain rate followed by the stress relaxation (the preconditioning cycles were not considered in the fitting). The procedure presented by Commisso et al. [6] was followed to fit the model constants.

From the force measured in the experimental tests, F, the experimental Cauchy stress, $\overline{\sigma}(t)$ was obtained assuming uniaxial compression [9]:

$$\overline{\sigma}(t) = \frac{F(t) \, \lambda(t)}{A_0}, \qquad \lambda(t) = 1 + \frac{u(t)}{L} \tag{4}$$

where A_0 is the initial cross-sectional area of the sample, $u(t)$ is the displacement of the upper platen, and L is the average thickness.

The raw stress record $\overline{\sigma}$, was filtered as explained by Commisso et al. [6]. The resulting stress record, $\tilde{\sigma}$, was fitted to the analytical stress record, σ, given by equation (1) using a least squares approach, that minimizes the following quadratic error:
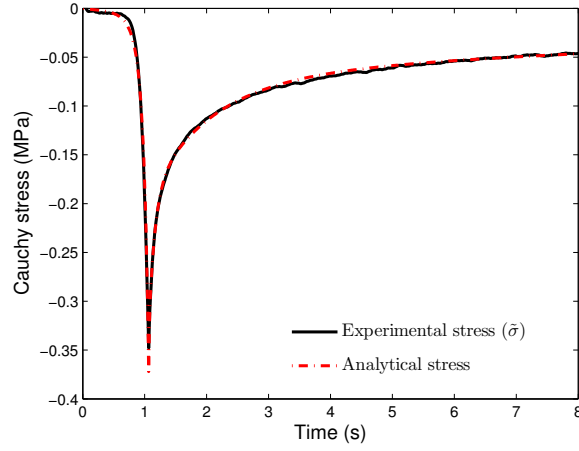
5

Figure 2: Example of an experimental stress record with its fitted curve.

$$e = \sum_{i=1}^{N} \left(\tilde{\sigma}(t_i) - \sigma(t_i) \right)^2 \tag{5}$$

The goodness of fit of the least squares procedure was evaluated by means of the coefficient of variation, CV:

$$CV(\%) = \frac{\sqrt{\dfrac{\sum_{i=1}^{N} \left(\tilde{\sigma}(t_i) - \sigma(t_i) \right)^2}{N}}}{\mu_{\tilde{\sigma}}} \times 100 \tag{6}$$

where $\mu_{\tilde{\sigma}}$ is the average of the temporal record $\tilde{\sigma}(t)$.

3. Results

An example of a typical experimental curve along with its fitted curve is shown in figure 2. The average CV is 17.69% if evaluated for the entire stress record and 24.28% if evaluated only in the loading ramp.

In table 3, the mean and standard deviation for each constant and each group are presented.

To investigate the influence of freezing storage time on the viscoelastic properties of articular disc, a multivariate analysis of variance (MANOVA) was planned, where the categorical independent variable was the storage time with five levels: fresh, 1, 7, 30 and 90 days; and the continuous dependent variables (DVs) were the seven QLV constants: A, B, g_1, g_2, g_3, g_4 and g_5. The material parameter g_∞ was not included in the statistical analysis because it is a linear combination of the other g_i arising from the normalization condition of $\overline{G}(t)$:

6

Storage time	A (MPa)	B	g_1	g_2	g_3	g_4	g_5	g_∞
Fresh	2.129	1.393	0.440	0.386	0.133	0.031	0.007	0.002
	± 3.638	± 0.580	± 0.173	± 0.120	± 0.047	± 0.014	± 0.005	± 0.002
1 day	1.061	1.544	0.540	0.304	0.119	0.029	0.005	0.003
	± 1.210	± 0.447	± 0.080	± 0.063	± 0.020	± 0.006	± 0.002	± 0.002
7 days	0.680	1.910	0.488	0.333	0.130	0.034	0.006	0.008
	± 0.841	± 0.642	± 0.092	± 0.063	± 0.028	± 0.014	± 0.006	± 0.012
30 days	2.277	1.089	0.561	0.307	0.102	0.022	0.005	0.003
	± 2.992	± 0.237	± 0.192	± 0.153	± 0.034	± 0.006	± 0.002	± 0.004
90 days	2.456	1.178	0.540	0.338	0.100	0.018	0.003	0.001
	± 2.855	± 0.319	± 0.098	± 0.076	± 0.023	± 0.005	± 0.001	± 0.001

Table 3: Mean ± standard deviation of the QLV constants for the different groups.

$$\overline{G}(0) = 1 = g_\infty + \sum_{i=1}^{5} g_i \tag{7}$$

Some assumptions need to be checked before proceeding with MANOVA. First, multinormality was checked using the test developed by Cardoso de Oliveira and Ferreira [3]. This test was significant ($p < .001$) and, thus, multinormality was violated, making necessary to perform a non-parametric MANOVA (NMANOVA).

NMANOVA (or MANOVA) is indicated only if the DVs are correlated, but not so strongly correlated that multi-collinearity may exists. This is not the case for all the variables in this study. In this last case, DVs must be grouped and regarded as the same variable, like occurred with variables A and B (Spearman R=-0.758). The same occurred with g1, g2 and g3. In conclusion, four DVs were compared in the NMANOVA: A, g_1, g_4 and g_5. The performed NMANOVA test was a multivariate extension of the Kruskal-Wallis test, developed by Katz and McSweeney [14].

Significant statistical differences were found among the five groups ($p < .001$). Thus, post-hoc tests were carried out to detect the origin of such differences. The post-hoc test also proposed by Katz and McSweeney [14] was followed. Significant differences were found for g_4 between 1 day and 90 days ($p = .0159$) and between 7 days and 90 days ($p = .0063$); and for g_5 between fresh specimens and 90 days ($p = .0236$). No statistical differences were found in the remaining constants.

4. Discussion

As can be seen from figure 2 and was stated by Commisso et al. [6], the algorithm there proposed is able to fit quite accurately the experimental curves. The stress relaxation is very quick as reflected by the high values of constants g_1, g_2 and g_3 (see table 3), which control the short-term relaxation.

The effect of freezing on biological tissues has been unclear, as presented in table 1. Even for the same tissue, different conclusions exist. Regarding the fibrocartilage, Kennedy et al. [15], Kiefer et al. [16] and Szarko et al. [24] studied the bovine articular cartilage. The first study found differences whereas the other two did not. However, none of them studied separately the elastic and viscoelastic properties.

Significant statistical differences were found in the QLV constants of different groups. So the freezing storage time was affecting the viscoelastic behaviour of the TMJ discs. To analyse the origin of the difference, post-hoc tests were conducted. The results showed that there are differences between the specimens tested after 90 days and the groups "fresh", "1 day" and "7 days". Moreover, these differences were found only in g_4 and g_5. Therefore, the elastic constants were not affected by the freezing storage time, but the viscous properties were, in particular, the long-term behaviour, represented by g_4 and g_5. Examining the values of these constants in table 3 (including g_∞), it can be seen that they are lower than those of the rest of groups. As a consequence, the importance of the long-term relaxation in the total relaxation in the "90 days" group is lower, or equivalently, the stress relaxation is faster in the "90 days" group.

The application of a compressive strain pressurizes the fluid within the tissue sample. Initially, it resists the load and then it flows out of the primary collagenous extracellular matrix (ECM). Mechanically, this is translated into an initial peak of stresses followed by a relaxation, as was shown.

If the viscous behaviour of the articular disc is modified by freezing, it is likely because some components which play a role in it may have changed. One possibility is a change of the proteoglycans that are present in cartilage. Proteoglycans consist of a core protein with one or more glycosaminoglycan (GAG) chains covalently attached to it. GAGs are strongly hydrophilic so producing an impedance to the flow of the interstitial fluid throughout the ECM. Chondroitin sulfate proteoglycans have several GAG side chains, whereas dermatan sulfate proteoglycans has only one (decorin) or two (biglycan). Thus, the impedance of chondroitin sulfate proteoglycans is higher and this may the reason why they are predominant in cartilage, where this impedance helps the tissue to bear the compressive loads. The impedance to fluid flow is an important factor affecting the viscoelastic behaviour of the tissue. Laouar et al. [19] found a proteoglycan loss due to freezing in porcine cartilage, suggesting that ice formation during cooling and warming caused alterations in the proteoglycan content of the ECM. Other authors reached the same conclusion [23, 31]. This loss could explain how the viscoelastic properties of the tissue are influenced by freezing. It must be admitted that the content in GAGs of the TMJ disc is lower than in hyaline cartilage (around 5.3% of the dry weight of porcine TMJ discs [10] versus the 25% of hyaline cartilage), what could minimize the effect of freezing on the viscoelasticity of the disc. However, GAGs are mainly concentrated in the intermediate zone of the TMJ disc [10], where the specimens of this study were extracted from.

Another possibility is that freezing causes a loss of interstitial fluid, therefore modifying the viscous behaviour of the tissue, as there is less fluid to circulate through the ECM. In this work, the specimens were frozen wrapped in saline-soaked gauze, enveloped in a plastic film and introduced in hermetic vials to prevent dehydration. So the amount of fluid should be the same when the specimens were removed from the freezer, but the structure which retains this fluid may have been modified, therefore leaving the ECM when the specimens were thawed just before testing. However, Qu et al. [22] found that the water content remained unaltered after freeze-thaw cycles in bovine cartilage. Therefore, it seems that the most probable cause of the change in the viscoelastic behaviour is the alterations in the proteoglycans structure.

In addition the statistical analysis suggests that the properties of the TMJ articular discs remain unaltered if they are frozen up to 30 days. If they are stored frozen during more than 30 days, the viscous properties of the tissue could have changed and its characterisation would be incorrect.

The main limitation of this study is the use of an isotropic model for the articular disc. The disc is anisotropic due to the presence of collagen fibres, which are mainly oriented in anteroposterior direction [11]. The anisotropic behaviour is revealed when the disc is loaded in different directions. However, in the tests performed in this study, the load was applied always in the same direction: a vertical compression, which is also the predominant type of load during mastication [7, 18]. Therefore, as reported in a previous work [6], the test used in this study is not appropriate to assess the anisotropic behaviour of the disc, but it is representative of the loads during normal activity.

Another limitation is not considering the compressibility of the disc. This tissue is usually considered an incompressible material, but the freezing storage could alter its bulk modulus, for the effect it has on proteoglycans. A rupture in the proteoglycans structure could allow an easier flow of water within the ECM. Different tests would be needed to confirm this alteration.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

References

[1] K. D. Allen and A. Athanasiou. A surface-regional freeze-thaw characterization of the porcine temporomandibular joint disc. *Ann. Biomed. Eng.*, 33(7):951–962, 2005.

[2] K.D. Allen and K.A. Athanasiou. Viscoelastic characterization of the porcine temporomandibular joint disc under unconfined compression. *J. Biomech.*, 39(5):312–322, 2006.

[3] I.R. Cardoso de Oliveira and D.F. Ferreira. Multivariate extension of chi-squared univariate normality test. *J. Stat. Comput. Sim.*, 80(5):513–526, 2010.

[4] M.J. Chow and Y. Zhang. Changes in the mechanical and biomechanical properties of aortic tissue due to cold storage. *J. Surg. Res.*, 171:434–442, 2011.

[5] P. Clavert, J.F. Kempf, F. Bonnomet, P. Boutemy, L. Marcelin, and J.L. Kahn. Effects of freezing/thawing on the biomechanical properties of human tendons. *Surg. Radiol. Anat.*, 23:259–262, 2001.

[6] M.S. Commisso, J.L. Calvo-Gallego, J. Mayo, E. Tanaka, and J. Martínez-Reina. Quasi-linear viscoelastic model of the articular disc of the temporomandibular joint. *Exp. Mech.*, pages 1–9, 2016.

[7] M.S. Commisso, J. Martínez-Reina, and J. Mayo. A study of the temporomandibular joint during bruxism. *International Journal of Oral Science*, 2014.

[8] M.S. Commisso, J. Martínez-Reina, J. Mayo, and J. Dominguez. Numerical simulation of a relaxation test designed to fit a quasi-linear viscoelastic model for temporomandibular joint discs. *Proc I Mech E Part H: J Eng Med*, 227(2):190–199, 2013.

[9] M.S. Commisso, J. Martínez-Reina, J. Mayo, J. Domínguez, and E. Tanaka. Effect of non-uniform thickness of samples in stress relaxation tests under unconfined compression of samples of articular discs. *J. Biomech.*, 47 (6):1526–1530, 2014.

[10] M. S. Detamore, J. G. Orfanos, A. J. Almazara, M. M. French, M. E. Wong, and K. A. Athanasiou. Quantitative analysis and comparative regional investigation of the extracellular matrix of the porcine temporomandibular joint disc. *Matrix Biol.*, 24:45–57, 2005.

[11] M.S. Detamore and K.A. Athanasiou. Structure and function of the temporomandibular joint disc:implications for tissue engineering. *J. Oral Maxil. Surg.*, 61:494–506, 2003.

[12] Y.C. Fung. *Biomechanics: Mechanical properties of living tissues*. Spinger-Verlag, New York, 1993.

[13] M. Hongo, R.E. Gray, J.T. Hsu, K.D. Zhao, B. Ilharreborde, L.J. Berglund, and K.N. An. Effect of multiple freeze-thaw cycles on intervertebral dynamics motion characteristics in the porcine lumbar spine. *J. Biomech.*, 41(4):916–920, 2008.

[14] B.M. Katz and M. McSweeney. A multivariate kruskal-wallis test with post hoc procedures. *Multivar. Behav. Res.*, 15:281–297, 1980.

[15] E.A. Kennedy, D.S. Tordonado, and S.M. Duma. Effects of freezing on the mechanical properties of articular cartilage. *Biomed. Sci. Instrum.*, 43:342–347, 2007.

[16] G.N. Kiefer, K. Sundby, D. McAllister, N.G. Shrive, C.B. Frank, T. Lam, and N.S. Schachar. The effect of cryopreservation on the biomechanical behaviour of bovine articular cartilage. *J. Orthop. Res.*, 7(4):494–501, 1989.

[17] J.H. Koolstra, E. Tanaka, and T.M.G.J. van Eijden. Viscoelastic material model for the temporomandibular joint disc derived from dynamic shear test or strain relaxation tests. *J. Biomech.*, 40:2330–2334, 2007.

[18] J.H. Koolstra and T.M.G.J. van Eijden. Combined finite-element and rigid-body analysis of human jaw joint dynamics. *J. Biomech.*, 38:2431–2439, 2005.

[19] L. Laouar, K. Fishbein, L.E. McGann, W.E. Horton, R.G. Spencer, and N.M. Jomha. Cryopreservation of porcine articular cartilage: MRI and biochemical results after different freezing protocols. *Cryobiology*, 54:36–43, 2007.

[20] F. Linde and H.C. Sørensen. The effect of different storage methods on the mechanical properties of trabecular bone. *J. Biomech.*, 26(10):1249–1252, 1993.

[21] A. Nazarian, B.J. Hermannsson, J. Muller, D. Zurakowski, and B.D. Snyder. Effects of tissue preservation on murine bone mechanical properties. *J. Biomech.*, 42:82–86, 2009.

[22] C. Qu, M. Hirviniemi, V. Tiitu, J.S. Jurvelin, J. Toyras, and M.J. Lammi. Effects of freeze-thaw cycle with and without proteolysis inhibitors and cryopreservant on the biochemical and biomechanical properties of articular cartilage. *Cartilage*, 5(2):97–106, 2014.

[23] S. Stevenson, G.A. Dannucci, N. A. Sharkey, and R.R. Pool. The fate of articular cartilage after transplantation of fresh and cryopreserved tissue-antigen-matched and mismatched osteochondral allografts in dogs. *J. Bone Joint Surg.*, 71(9):1297–1307, 1989.

[24] M. Szarko, K. Muldrew, and J.E.A. Bertram. Freeze-thaw treatment effects on the dynamic mechanical properties of articular cartilage. *BMC Musculoskel. Dis.*, 11:231, 2010.

[25] R. Ternifi, J.L. Gennisson, M. Tanter, and P. Beillas. Effects of storage temperature on the mechanical properties of porcine kidney estimated using shear wave elastography. *J. Mech. Behav. Biomed.*, 28:86–93, 2013.

[26] S. Torimitsu, Y. Nishida, T. Takano, Y. Koizumi, M. Hayakawa, D. Yajima, G. Inokuchi, Y. Makino, A. Motomura, F. Chiba, and H. Iwase. Effects of the freezing and thawing process on biomechanical properties of the human skull. *J. Legal Med.*, 16:102–105, 2014.

[27] K.L. Troyer, D.J. Estep, and C.M. Puttlitz. Viscoelastic effects during loading play an integral role in soft tissue mechanics. *Acta Biomater.*, 8:234–243, 2012.

[28] E.H. van Haaren, B.C. van der Zwaard, A.J. van der Veen, I.C. Heyligers, P.I. Wuisman, and T.H. Smit. Effect of long-term preservation on the mechanical properties of cortical bone. *Acta Orthop.*, 79(5):708–716, 2008.

[29] C. Wex, A. Stoll, M. Fröhlich, S. Arndt, and H. Lippert. Mechanics of fresh, frozen-thawed and heated porcine liver tissue. *Int. J. Hyperther.*, 30(4):271–283, 2014.

[30] J. Wieding, E. Mick, A. Wree, and R. Bader. Influence of three different preservatives techniques on the mechanical properties of the ovine cortical bone. *Acta Bioeng. Biomech.*, 17(1):137–146, 2015.

[31] S. Zheng, Y. Xia ad A. Bidthanapally, F. Badar, N. Duvoisin, and I. Ilsar. Damages to the extracellular matrix in articular cartilage due to cryopreservation by microscopic magnetic resonance imaging and biochemistry. *Magn. Reson. Imaging*, 27:648–655, 2009.