A thermoanalytical study of cartilaginous tissues

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An endothermic process was detected in cartilaginous tissues near 70 °C using differential scanning calorimetry, and the heat effect 1.5±0.5 J g^{-1} decreased to 0.24±0.03 after laser irradiation (λ = 1.56 μm).

Laser heating of cartilage can induce a change of its shape without dramatic destruction of the tissue structure.[1] Simultaneous measurements of temperature and tension showed[2] that the internal stress relaxation of cartilage occurs at about 70 °C. Differential thermal analysis (DTA) demonstrated an endothermic peak at about the same temperature.[3] The peak area is much less than that of the second peak at 100 °C, which corresponds to the enthalpy of water evaporation. Several hypothetical mechanisms of the laser-induced shaping of cartilage based on 'bound to free' water transformation have been proposed.[2–5]

The main goal of this study was to estimate the endothermic effect in cartilage and cornea heated to 20–100 °C or exposed to laser radiation. This temperature range was not examined in biological tissue calorimetry.[6–7]

The nasal septum and cornea tissues were collected from 8-month-old domestic pigs. The samples of about 50 mg were studied by differential scanning calorimetry (DSC). Additionally, two cartilage samples (discs 5 mm in diameter and 1.6–1.8 mm in thickness) irradiated with an erbium-doped fiber laser (λ = = 1.56 μm, 3.5 W) for 10 s were used for DSC analysis.

The measurements were carried out in air using a simultaneous (TG-DSC) thermoanalyser (STA-409, Netzsch) at a scanning rate of 10 K min^{-1}. The samples were heated in a hermetically sealed brass pan (0.12 cm^{-3}) to avoid the contribution of water evaporation. Diphenylamine (mp 54 °C, ΔH = 108.08 J g^{-1}) was used as a calorimetric standard. Glycerine in a brass pan was used as a reference sample. The DSC examination of water in a hermetically sealed brass pan was carried out and no changes were observed in the range 20–95 °C.

The DSC thermograms exhibited a weak endothermic peak in the temperature range 65–78 °C and the absence of mass losses, for all of the samples (Figure 1). The DSC examination of irradiated porcine cartilage has shown an about six-fold decrease of the enthalpy effect with respect to the non-irradiated samples. At the second heating scan of the same sample, the enthalpy effect at 70 °C almost disappeared in DSC measurements. The shapes of DSC curves clearly indicate a complete character of the thermochemical process occurring in the tissue under heating. The average heat effects and their standard deviations are summarised in Table 1.

Endothermic processes could be responsible for the heat effect observed. It is known that cartilaginous tissue involves an extracellular matrix and a sparse population of chondrocytes. The matrix is mainly composed of water (60–85 wt.%), collagen (15–22 wt.%), and proteoglycan (4–7 wt.%). The matrix is generally believed to be a gel, while solid matrices of the gel consist of a network of collagen II fibrils in which large proteoglycan aggregates (PGA)[8–10] are trapped. Water in cartilage forms solvent sheaths around the polar and ionised groups of macromolecules. This is mainly related to proteoglycan aggregates since their composition involves sulfate and carboxyl groups, which effectively attract the dipole-polarised molecules of water.

Table 1 The heat effects and temperature ranges of thermochemical processes in cartilaginous tissues measured using DSC.

Type of tissue	Temperature/°C	Heat effect/J g^{-1}
Porcine nasal septa	65–75	1.5±0.5
Porcine cornea	68–78	0.9±0.5
Porcine nasal septa after laser treatment	65–75	0.24±0.03

It is expected that the heating of tissues can result in chemical processes such as collagen denaturation and decomposition of large proteoglycan aggregates. Indeed, under prolonged heating at 70 °C, the disruption of native PGA takes place. However, this process, as demonstrated by quasielastic laser-light scattering, is quite slow. The particle size diminishes by a factor of two in about 1 h.[11] If PGA in cartilage has a similar rate of decomposition, its contribution to the total enthalpy effect could be neglected owing to the short heating time of a DSC experiment. The destruction of the triple helical structure of collagen occurs in a wide temperature range (43–240 °C) depending on pH, water content and cross-linking degree.[12–16] The enthalpy of collagen denaturation is 30–70 J g^{-1} on a dry collagen basis[12–16] and is independent of water content if the water content exceeds 0.3 g per gram of dry collagen.[15,16] The estimation of the enthalpy effect referred to the collagen content of cartilage gives about 5–14 J g^{-1}. This value is much higher than the measured enthalpy (1.5 J g^{-1}). Assuming that the observed process is completed (in accordance with the shape of a DSC peak) and collagen II has the same enthalpy as collagen I, we can conclude that the change in cartilaginous tissue at 70 °C cannot be attributed to the total denaturation of collagen.

The weak enthalpy effect observed in our experiments may also be related to transitions and relaxations in cartilage macromolecules. Such a kind of relaxations occurring in the polymer–solvent system is attributed to conformational transitions of macromolecules or *ll* (liquid–liquid) transition (local destruction of hitching in a polymer network).[17] It is believed[18] that the association and interaction in the collagen–proteoglycan system help to maintain the shape and to drive an elastic deformation in connective tissues. The secondary and tertiary structures are determined by a multihydrogen-bond array (directly between proton-donor and proton-acceptor groups of macromolecules or as water bridges).[19]

Our DSC analysis has shown that for cartilaginous tissues

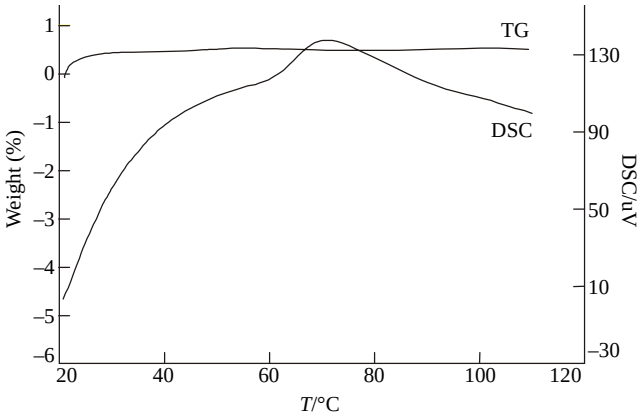

Figure 1 Typical thermograms of porcine cartilage.

exposed to laser or conventional heating the endothermic effect almost disappeared. Conventional heating and laser treatment did not lead to the total denaturation of the tissue, but they may result in the destruction of hydrogen bonds and solvate sheaths. This process can alter the mobility of macromolecule segments and improve the cartilage flexibility. Thus, the desolvation may be a starting point for further stress relaxation. The following cooling of the biopolymer enhances inter- and intramolecular interaction of unsatisfied proton-donor and proton-acceptor polar groups, and results in the stability of the three-dimensional network of a cartilage matrix. This consideration is also in agreement with an atomic force microscope investigation,[20] which did not show any denaturation of a cartilage matrix under laser irradiation resulting in stress relaxation in the tissue.

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