DIELECTRIC CHARACTERIZATION OF THE BIOACTIVE COMPOSITES COLLAGEN – GLYCOSAMINOGLYCANS FOR TISSUE ENGINEERING

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Abstract: The aim of the study reported was to identify the particular differences between unmodified collagen and hyaluronic acid (HA)– and chondroitin sulphate (CS)-modified collagen detectable by the dielectric spectroscopy. Measurements were performed over the frequency range from 100Hz to 100kHz and at temperatures from 25 to 260°C. The influence of HA and CS on the dielectric behaviour of collagen is manifested by a shift of the dielectric loss maximum associated with low temperature relaxation towards lower temperatures. The high temperature relaxation around 220-230°C for three materials is accompanied by an increase in the complex permittivity for each frequency and small changes in the ac conductivity for the low-frequency region. Power-low response are observed for the frequency dependence of ac conductivity for unmodified and modified collagen. Analysis of polarisation and conduction mechanisms for the materials studied was explained on the basis of proton transport.

Introduction

The ultimate goal of tissue engineering is to create functional and viable tissue constructs for patients requiring organ or tissue replacement. The main approaches towards tissue engineering involves the in vitro culturing of cells on biodegradable polymeric scaffolds to form neo-organs that are then implanted into the body at the necessary anatomical site. Both collagen and glycosaminoglycans (GAG), such as hyaluronic acid (HA) and chondroitin sulphate (CS), play important roles in the creation of functional scaffolds. Attachment of HA and CS to reconstituted collagen may offer the opportunity to exploit the biocharacteristics of these polysaccharides and valorize collagen as a biomaterial. Although numerous papers are devoted to collagen-GAG in literature does not report results concerning the electric behaviour of such valuable composite materials. Since collagen is constituted by polar repeating units -CO-CR-NH- as well as GAG contain polar groups (COO^-, OSO_3^-), the dielectric techniques are particularly sensitive to analyze relaxation phenomena. In this study we are interested in the investigation of the dielectric properties of collagen-HA and collagen-CS scaffolds. This work extends our earlier dielectric studies obtained for collagen [1]. The data obtained in this paper indicate the effect of water and electric field frequencies on the dielectric properties of collagen.

Materials and methods

Collagen type I was derived from purified bovine Achilles tendons by pepsin digestion and acetic acid dissolution. Further details of this procedure and methods of collagen investigation are given elsewhere [2]. All experiments were carried out using collagen films. For the film preparation, the dispersion after deaeration was cast on dish and then dehydrated by slow drying under a laminar air-flow. To prepare collagen modified by CS or HA two complementary cross-linking methods, dehydrothermal (DHT) pre-treated and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) have been introduced [3,4]. Some films without any further treatment were taken as controls.
Measurements of the complex permittivity ε^* ($\varepsilon^* = \varepsilon' - j\varepsilon''$) and conductivity σ_{ac} ($\sigma = 2\pi f \varepsilon_o \varepsilon''$) were carried out using an impedance analyser HIOKI 3522-50 LCR over the frequency range of 100Hz – 100kHz and temperatures from 25 to 260°C. Prior to the dielectric measurement the collagen sample was covered with silver paste electrodes and subjected to elimination of loosely bound water by keeping it in the measuring cell at a constant temperature of 120°C for 2h. After this time the sample was cooled to room temperature and was subjected to dielectric measurements in the cycle of heating from room temperature to 260°C at a rate of about 1°C/min.

Results

Fig. 1a and b show the plots of the relative permittivity ε' and dielectric loss ε'' for unmodified collagen and CS- and HA- modified collagen versus the temperature at a chosen frequencies of 1 and 50kHz. These parameters

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are much higher for HA- modified than for the other materials. However, little difference is observed in ε' over the entire temperature range between CS-modified and unmodified collagen. In addition, the curves for unmodified and modified collagen reveal the maxima in ε" occurring much below 140°C for 50kHz and at lower T than those used in the experiments for 1kHz, and a weak maximum at around 220°C-230°C. The appearance of these peaks is accompanied by changes in ε' for all the materials.

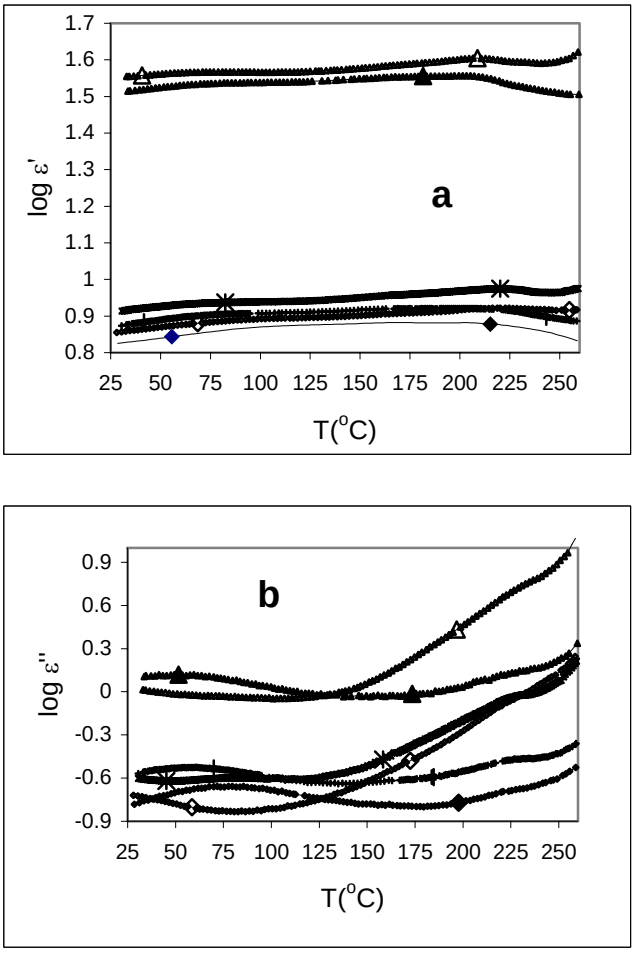

Figure 1: The variation of ε' (a) and ε" (b) vs. temperature for collagen: unmodified (1kHz ◊, 50kHz ♦), CS- modified (1kHz ∗, 50kHz +) and HA- modified (1kHz △, 50kHz ▲).

Fig.2 (a-c) show the frequency dependencies of the relative permittivity ε', dielectric loss ε" and conductivity σ_{ac} at several temperatures for unmodified collagen and CS- and HA-modified collagen. The plots of ε" for three materials reveal two dispersion processes. The first and second are manifested by the ε" maxima which shift to higher frequencies with increasing temperatures and significant increase in ε" above 140°C at low frequencies, respectively. In unmodified collagen, and CS- and HA-modified collagen the first dispersion appears in the range of 3-100kHz and 30-100kHz, and at temperatures from 33-88°C, 51-67°C and 42-61°C, respectively. The plots of ε' for each material show a weak dispersion and little variation in

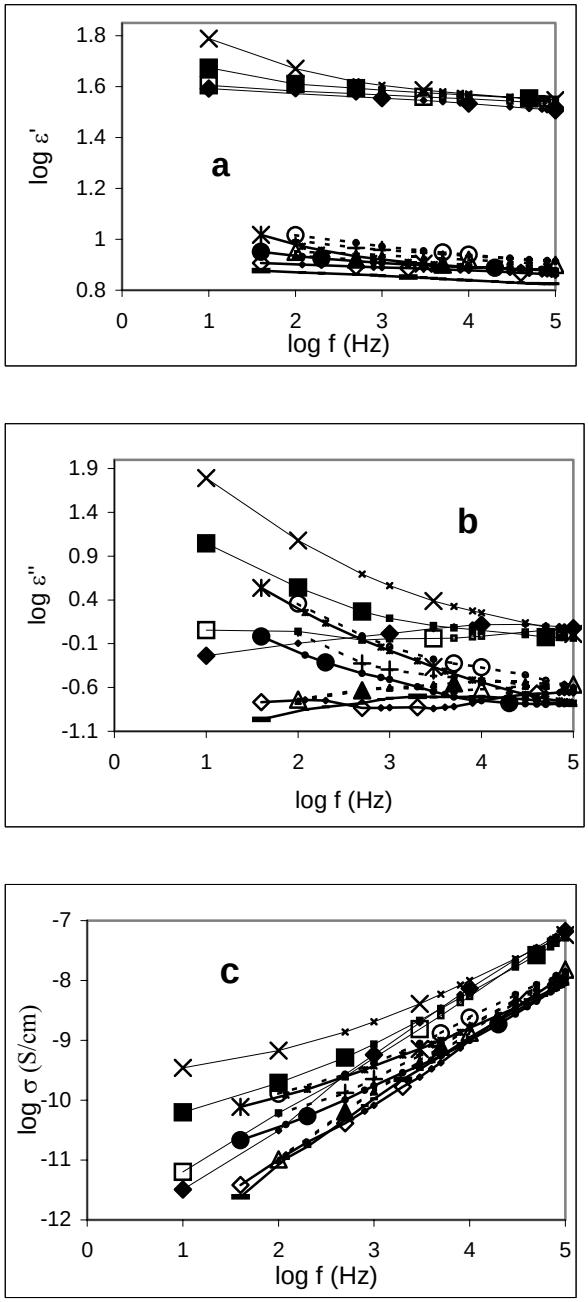

Figure 2: The variation of ε' (a), ε" (b) and σ_{ac} (c) vs. frequency for collagen: unmodified (30°C —, 90°C ◊, 170°C •, 210°C ∗), CS- modified (30°C▲, 90°C △, 170°C +, 210°C ○) and HA- modified (30°C♦, 90°C □, 170°C ■, 210°C x).

the temperature range 30-140°C, but above 140°C present a remarkable dispersion in the low frequency. In addition, the ε' and ε" values of HA- modified collagen are much higher than those of unmodified collagen and CS- modified collagen. The conductivity curves for the three materials studied exhibit a power-

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low behaviour of the form $\sigma_{ac} = Af^{n}$, where A is a constant and n is the power law exponent. The values of n were obtained from the linear fit of all the curves at the frequencies above 500Hz. For the coefficient of determination, r^2, greater than 0.995, the n values are similar for all materials and are about 0.94 -1.06 in the temperature range of 30-140°C. At higher temperatures, linear fits of spectra with r^2>0.993 yield the exponent n values decreasing from 0.90 at 170°C to 0.69 at 210°C. The values of σ_{ac} (Fig.2c) in the low-frequencies and in the whole temperature range are lower than $10^{-8}(\Omega cm)^{-1}$ for unmodified and modified collagen, so the electrode polarization effects are negligible.

Discussion

The dielectric results presented in the Figs.1and 2 for unmodified collagen, and CS- and HA-modified collagen, correspond to the processes of release of the water below 140°C and decomposition for higher temperatures, respectively. These two molecular processes for the collagen and other biological proteins have been confirmed by differential scanning calorimetry (DSC) and thermogravimetric (TG) analysis [4-6]. From the Arrhenius plots of the logarithm of the frequency maximum against (1/T), not shown here, we obtained the activation energy ΔH for water release in the region of the first dispersion for the three materials. Although the curves for CS- and HA- modified collagen appear at lower temperatures than for the unmodified collagen, the ΔH takes a similar value about 56kJ/mol for these materials. In an earlier paper [7], we obtained, a similar value of ΔH for bone and horn. This suggests the same polarisation and conduction mechanisms involving hopping protons interacting with the bound water molecules. The occurrence of these mechanisms is supported by the values of n~1 for σ_{ac}. In biological materials, the polarization mechanisms are a result also of the accumulation of protons on the border of the collagen-water system, i.e. Maxwell-Wagner polarization, which is manifested as an increase in ε' at low frequencies (Fig.2a). Most probably, a significantly higher values of the dielectric parameters in HA-modified collagen than in unmodified collagen are due to a large density of charge carriers and the formation of additional hydrogen bonds for charge transport in the former as a result of the cross-linking reactions [8]. In these reactions, EDC and NHS activates carboxylic groups of glutamic and aspartic acid residues in collagen or carboxylic group of glucuronic acid residues in CS or HA. The reaction between NHS-activated carboxylic group of collagen, CS and HA with ε-amino groups from lysine and hydroxylysine of collagen induces the formation of amide bonds -CO-NH-. However, the lower values of the dielectric parameters at the same temperature for the CS-modified collagen than for the HA-modified collagen would suggest that the cross-linking reactions are weaker in the former. Further in this paper, analysis of the thermal decomposition of unmodified and modified collagen is performed on the basis of ac conductivity σ_{ac}.

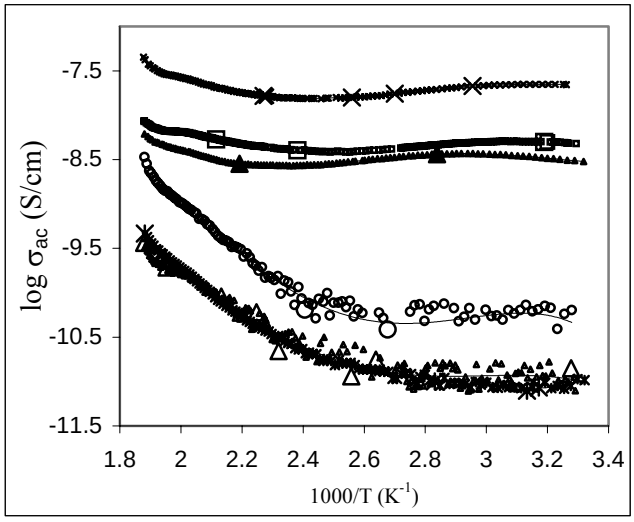

Figure 3: Plots of log σ_{ac} vs. 1/T for unmodified collagen (*100Hz, ▲30kHz), CS- collagen (△100Hz, □ 30kHz) and HA-collagen (o100Hz, x30kHz).

Fig. 3 presents the Arrhenius plots of σ_{ac} against T^{-1} for the studied materials at 100Hz and 30kHz, so for the frequencies from the low- and high-frequency range. In the temperature range $3.3K^{-1}$(30°C)-$1.87K^{-1}$(260°C), the values of σ_{ac} are significantly higher for HA- modified collagen than unmodified collagen at the same temperature. However, small differences in the conductivity appear for low-frequencies between CS-modified collagen and unmodified collagen. Only for higher frequencies, the values of σ_{ac} are a little bit higher in CS- modified collagen than in unmodified collagen. Above 180°C for these curves, we can obtain the activation energy ΔH of ac conductivity for the high temperature relaxation of unmodified and modified collagen. For unmodified and HA-modified collagen, and CS-modified collagen, the values of ΔH significantly increase from about 22kJ/mol (r^2>0.9287) and 13kJ/mol (r^2>0.9117) at 30kHz to 60kJ/mol (r^2>0.9763) and 37kJ/mol (r^2>0.9306) at 100Hz, respectively. This energy is necessary to break of intra- and intermolecular hydrogen bonds and a rearrangement of the triple helix into a random configuration.

Conclusions

Dielectric spectroscopy has been applied to compare the low and high temperature behaviour between unmodified collagen and hyaluronic acid (HA)– and chondroitin sulphate (CS)-modified collagen.
The results of this study indicate that CS and HA affect the dielectric properties of collagen. Most probably, a higher values of the dielectric parameters in modified than in unmodified collagen are due to a large density of charge carriers and the formation of additional hydrogen

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bonds for charge transport in the former as a result of the cross-linking reactions. Although the low T relaxation for modified collagen appear at lower temperatures than for the unmodified collagen, the activation energy ΔH responsible for the water release takes the value about 56kJ/mol for all materials. For decomposition process, the ΔH exhibits frequency dependence. In the range 100Hz-30kHz, the changes in ΔH are higher for unmodified and HA-modified collagen when compared to the CS- modified collagen.

Acknowledgements

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