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Changes in denaturation and rheological properties of collagen–hyaluronic acid scaffolds as a result of temperature dependencies

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Abstract

This report describes the effect of temperature on the mechanical viscoelastic properties such as: storage modulus (E'), loss modulus (E''), and loss tangent ($\tan\delta$) of the collagen sponges modified with hyaluronic acid (HA). In order to detect collagen–HA copolymer denaturation and to assess its thermal stability, the differential scanning calorimetry (DSC) supplemented by thermogravimetric (TG) measurements was used. The denaturation temperature (T_d) of unmodified collagen samples increased from 69 to 86 °C for cross-linked samples, respectively. These temperature dependencies show remarkable changes in E' and E'' at selected temperature up to 226 °C for all samples due to the release of loosely and strongly bound water. The influence of HA on the viscoelastic behavior of collagen is manifested by a shift of the $\tan\delta$ peak associated with the process of decomposition towards higher temperatures resulting in a higher thermo-stability of the modified scaffolds.

Keywords: Collagen; Hyaluronic acid; Thermal stability

1. Introduction

Recently, the interest of the scientific community has been focused on the reconstruction and repair of bone and cartilage, given the frequency and importance of pathological situations [1]. It seems that both, collagen and hyaluronic acid (HA), which are essential components of natural bone and cartilage, play a pivotal role in the formation of biological scaffold for tissue engineering.

Collagen is a major component of the extra cellular matrix (ECM) in many tissues (skin, bone, cartilage, tendon, blood vessels, teeth), where it provides the principal structural and mechanical support [2]. Meanwhile, hyaluronic acid is natural polysaccharide that is most abundant in cartilage, vitreous humour and synovial [3]. Based on their unique physicochemical and biological properties, collagen and HA are currently used clinically. For example, the unique ability of HA to form a highly hydrated polymer matrix, with peculiar viscoelastic properties, rendered its use as a therapeutical agent feasible in viscosurgery and the treatment of arthritis through intra-articular injections [4]. Chemically modified HA has also been used as bio-interactive wound dressings [5].

The combination of collagen and HA has shown advantages over the use of either the material alone. When implanted in cranial defects in rats, the matrix which was synthesized by cross-linking collagen fibers with modified HA demonstrated good biocompatibility and exhibited greater osteoconductive potential than the matrices composed of either cross-linked collagen or cross-linked HA alone [6]. Tri-component, hydroxyapatite–collagen–HA composite, has offered new possibilities for application as bone implant material [7].

It seems that the composition of collagen with HA may also be used as a scaffold for tissue engineering. Tissue

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engineering offers the potential possibility to create in vitro functional and viable tissue constructs for patients requiring organ or tissue replacement [8]. A very important issue of the in vitro development of each tissue is two important components: a group of appropriate cells, and a biodegradable polymer scaffold on which they can grow. A critical element of such strategy is a scaffold. This matrix is a support for cells and provides appropriate signals to direct cellular processes that lead to tissue synthesis. Although HA in the native form is substantially non-adhesive to cells, the introduction of collagen shows dramatic changes in terms of cell adhesion and proliferation [9].

The formation of a new tissue in vitro is generally influenced by the chemical composition, porosity and three-dimensional (3D) structure of the scaffold [10]. Further attributes of scaffold include minimal toxicity, low immunogenicity and structural stability to withstand stress incurred during culturing in vitro and implanting in vivo. A potential problem in the application of such scaffolds for tissue engineering is cell-mediated contraction of the matrices that are employed [11]. As the scaffold contracts, there is a reduction in the pore volume that could restrict cell migration and proliferation. However, it has been demonstrated by in vitro experiments that the incorporation of HA into collagen gels is able to enhance the strength of the collagen gels, thus inhibiting fibroblast contraction on the collagen matrices [12]. This seems obvious to indicate that the rheological behavior and thermal stability of scaffold will determine its resistance to contraction. Although a number of investigations on collagen–HA scaffold materials have been done to characterize their biochemical and biological properties [13–16], the effect of temperature on the conformational and mechanical properties are not clearly elucidated.

In this study, particular attention has been paid to the preparation and characterization of cross-linked collagen–HA matrices with potential for use in tissue engineering. Differential scanning calorimetry (DSC) and thermogravimetric (TG) measurements were used in order to detect the denaturation of collagen–HA copolymer and to measure its thermal stability. In addition, to characterize rheological properties of this matrix, a dynamic mechanical analysis versus temperature (DMTA) was carried out.

2. Materials and methods

2.1. Materials

Collagen type I was derived from purified porcine tendons by pepsin digestion and acetic acid dissolution to prepare 0.45% (w/v) dispersion. Its antigenicity is low owing to the removal of telopeptide. The analysis of amino-acid composition (analyzer Jeol ILC—3 BC2) and infrared (IR) spectroscopy (BIO-RAD FTS 175C) confirmed a very high purity of the prepared system. The collagen solution was characterized by molecular weight, $M_v = 3.9 \times 10^5$, denaturation temperature, $T_d = 38.8\,^{\circ}C$ and pH = 3.5. Further details of this procedure and methods of collagen investigation are given elsewhere [17].

Hyaluronic acid, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysulfosuccinimide (NHS) and 2-morpholinoethane (MES) were purchased from Fluka BioChemika. All other reagents and solvents of analytical grade were supplied by POCh Poland and used as received.

2.2. Samples preparation

A sponge-shape matrix was prepared by freezing the collagen dispersion at $-70\,^{\circ}C$ and subsequently lyophilised. Some collagen sponges without any further treatment were taken as a reference material. Other sponges were cross-linked by dehydrothermal (DHT) pre-treatment in a vacuum oven at a temperature of $80\,^{\circ}C$ under a vacuum of 50 mTorr for 24 h. Then, the sponges were subsequently cross-linked by using water-soluble EDC/NHS system similarly to the method described previously for collagen and chondroitin sulfate (CS) [18] with a minor modification. Collagen matrices of 50 mg dry weight in presence of 0.5% (w/v) HA were cross-linked by immersion in 20 ml 50 mM MES at pH 5.5 containing 33 mM EDC and 6 mM NHS. After the reaction, the matrices were washed in 0.1 M Na_2HPO_4 and then with distilled water. Finally the cross-linked matrices were lyophilized. These samples in the air-dried state contained about 15–18% water in respect to dry weight.

2.3. Investigation on the thermal stability

2.3.1. Differential scanning calorimetry and thermogravimetric analysis

The thermal stability of collagen–HA matrix was assessed with a NETZSCH DSC 204 and TG 204 analyzer scanning from 20 to $450\,^{\circ}C$. The samples were weighed and introduced into aluminium pans. The pans were heated at a constant rate of $10\,^{\circ}C$/min in nitrogen atmosphere with an empty aluminum pan as the reference probe. The sample mass was in the range of 3–5 mg. All samples were run in duplicate. The denaturation temperatures were measured at the mid-point of the transition peak and the temperatures at the start of the process were measured at onset peak.

2.3.2. Dynamic mechanical thermal analysis

The dynamic mechanical analysis as a function of temperature was performed with a DMTA V apparatus, Rheometric Scientific, aided with computer system RSI Orchestrator. Seventeen-millimeter diameter samples were subjected to mechanical testing. Before testing, the thickness of each sample was measured using a micrometer. The measurements were carried out with a compression mode at a frequency of 1 Hz, a heating rate of $2\,^{\circ}C\,min^{-1}$ and a temperature range from 25 to $450\,^{\circ}C$. The set strain was 1.5% and the applied auto tension adjustment force was 50 g. The results were