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Study of the heat-induced denaturation and water state of hybrid hydrogels based on collagen and poly (N-isopropyl acrylamide) in hydrated conditions

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ABSTRACT

Hybrid hydrogels of collagen and poly (N-isopropyl acrylamide) (pNIPAM), various nanoclays and hydroxyapatite, were prepared by chemical cross-linking. These bioartificial polymeric materials were studied to establish the influence of collagen, pNIPAM, cross-linking agent and inorganic nanoparticles content on the thermal properties and heat induced denaturation in hydrated state by differential scanning calorimetry. It was established that the denaturation of collagen is an irreversible kinetic process that is governed by the temperature history, nanoparticle type in hybrid hydrogels, specimen hydration, and cross-linking agent amount, amongst other variables. The interaction of water with hybrid collagen and water state in semi-interpenetrated polymer network depends on hydrogel composition and thermal history. The hybrid hydrogels keep the temperature responsiveness. Contact angle and swelling measurements demonstrated the correlation between hydrophobicity/hydrophilicity balance and state of water in the polymer network and the denaturation temperature values.

1. Introduction

Collagen belongs to protein class of the human body and is used in the manufacture of biomaterials. The studies of physico-chemical and biological properties are essential because the collagen-based materials are synthesized and destined mainly for medical applications. Some of their properties are interesting also for other applications, such as tanning treatments for animal skins, textiles, tailoring leathers and parchments from heritage objects [1,2]. The sensitivity of this protein in the manufacturing step such as sensitivity to acidic or basic environments, changes in temperature or presence of radiation, enzymes, and so on, has led to interest in chemical modifications and thus to improve the physico-chemical and biological properties of collagen [3]. The most common and effective treatment is chemical cross-linking of the peptide chain using various cross-linking agents. The efficiency of cross-linking procedures on the thermal behavior of collagen-based materials have been studied on gels, sponges and collagen films using different types of cross-linkers, such as glutaraldehyde, hexamethylene diisocyanate, cyanamide, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide and acyl azide [4,5]. Polymeric hydrogels designed for medical applications are sometimes prepared with toxic cross-linking agents, which cannot be easily and completely removed, thus leading to the occurrence of side effects at contact with living tissue. As well, the inconvenience of using common cross-linking agents for bioproducts synthesis is related to their low compatibility with human tissue, which limits further applications of the cross-linked polymeric materials.

The compatibility assessment studies at cellular level, followed by *in vitro* and *in vivo* compatibility evaluation revealed that a number of chemical cross-linking agents, along with natural enzyme cross-linkers, are compatible with the human body, which increase the applications of such collagen-based products [6]. In addition to improved enzymatic resistance, swelling ability, rheological properties, cross-linking agents can enhance the thermal stability of modified collagen [7]. In our previous studies, from biocompatibility tests performed on rats, it has been shown that the diethylene glycol diacrylate used as cross-linking agent is nontoxic even if the LD_{50} of hybrid hydrogels exceeds 3200 mg/kg and the subcutaneous implanted biomaterials presents normal response of rats' immune parameters [6].

Under thermal stimulus, the transition of collagen from native to denatured state is a cooperative phenomenon with significant uptake of heat, observable on DSC curves as an endothermic peak.

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The thermal transition is responsible for the rupture of intermolecular hydrogen bonds and appearance of the unfolded triple helix. Transition to the amorphous state of peptide chains occurs with changes in protein conformation and certainly of other physical properties too. These processes are stamped on the calorimetric behavior by variation of the enthalpy [8–12].

Studies on the thermal behavior of collagen-based hydrogels showed their good thermal stability compared with the native protein [13–15] or they demonstrated that the functionalized collagen-based hydrogels preserved triple helix conformation because covalent net-points were established during hydrogel formation, so that collagen triple helices were successfully retained and stabilized. As mentioned above the collagen denaturation is related to the unfolding of collagen triple helices into randomly coiled chains with appreciable heat absorption; it is therefore expected to be highly affected by the formation of a covalent network [16].

Also, it has been reported that nanocomposites based on clay exhibited improved thermal stability [17,18] due to the interaction and surface activity of clay layers with polymeric chains. This strongly depends on the degree of inorganic particles exfoliation/intercalation through the polymeric mass, intrinsic thermal resistance of the polymer chains, inorganic filler content and in case, by chemical constitution of organic modifier inorganic particles and the chemical character of polar compatibilizers.

A better understanding of the thermal denaturation of collagen-based materials included in polymeric systems is of a significant interest from the standpoint of medical practical applications such as laser surgery, thermal therapy, tissue engineering and drug-loading manufacturing process.

For the practical application of such materials the interaction with water is of utmost importance. The correlation between equilibrium water content and state of water of hydrogels and their properties is well established, since the water has a key role on mechanical properties and other characteristics of hydrogels [19]. Water is responsible for some of peculiar characteristics of hydrogels, one of which is their injectability, which makes these hydrogels suitable as matrices for mini-invasive surgery and localized therapy. The water state in hydrogels is categorized into three different types [20], namely: (1) free water – that is water not intimately bound to the polymer chain and behaves like bulk water undergoing a thermal transition at temperature analogous to bulk water (at 0 °C), (2) freezable bound water–water that is weakly bound to the polymer chain or interacts weakly with nonfreezing water and undergoes a thermal phase transition at a temperature lower than 0 °C. These two first types of water are so-called freezing or free water and (3) bonded water (non-freezing water) which is tightly bound to the polymer and does not exhibit a first order transition over the temperature range from −70 °C to 0 °C.

Differential scanning calorimetry (DSC) was widely used to characterize the water state in hydrogel membranes being a convenient and informative method [21]. DSC was used to assess the water state in polysaccharide hydrogels such as hyaluronic acid or xanthan [22], chitosan [23,24], chitosan/polyvinyl alcohol [25], chitosan/collagen [26], poly (vinyl alcohol) hydrogels [27], acrylamide/acrylic acid copolymeric hydrogels [28], salvia hydrogels [29], hydrogels based on poly (hydroxyethyl acrylate) [30]. According to literature data [23,31] the effect of hydrogel composition and morphology is discussed mainly in respect with the bonded water content. The bonded water content in different polymer/water systems depends on both chemical and higher order structure of the polymer, the changes in hydrophilicity or hydrophobicity as well as crystallinity, degree of cross-linking, a.s.o. By means of differential scanning calorimetry and cell proliferation assay it was demonstrated that the state of water in the silk hydrogel influences the cytotoxicity. Namely, the bonded water supports cell-adhesion proteins in the cellular matrix to interact with the surface of the silk hydrogels [32].

Analysis of the thermodynamic properties of bulk and interfacial water of different types of hydrogels containing collagen was also useful for their applications as soft tissue scaffolds, artificial skin substitutes and other biomaterials [33].

The interest in hybrid systems originates from the widely debated thermo-responsive hydrogels, especially based on poly(N-isopropylacrylamide) (pNIPAM) for bioengineering applications, due to their capacity to respond to temperature changes as a trigger for conformational modifications. Additionally, inorganic nanoparticles were found to improve the physical and chemical properties of hybrid materials. Moreover by using two sensitive polymers, respectively collagen with sensibility to pH and temperature and pNIPAM sensitive to temperature, allows the preparation of a biomaterial with controlled sensitivity suitable for tissue engineering or as drug-loaded polymeric system with targeted release of therapeutic agents.

This paper deals with the study of the heat-induced denaturation and the water state of some nanohybrid responsive hydrogels by means of differential scanning calorimetry (DSC). The hybrid hydrogels consist in collagen and pNIPAM with various kinds of nanoclays or hydroxyapatite nanoparticles embedded. This study focuses on the influence of the hydrogel composition and of nanoparticles embedded in the matrix on denaturation temperature and bonded water content. The results have been correlated with the swelling behavior and the variation of the contact angle. Studies were performed in hydrated conditions taking into account the strong correlation between water content of hydrogels and their properties.

2. Experimental

2.1. Materials

Collagen matrix was purchased from Lohmann & Rauscher International GmbH & Co KG – Germany. N-isopropyl acrylamide (NIPAM) purified in toluene/n-hexane mixture and diethylene glycol diacrylate (DEGDA) were acquired from Sigma–Aldrich – Germany. Ammonium persulfate and N, N, N, N-tetramethylethylene diamine supplied by Merck were used as promoters of the crosslinking reaction. Dellite® 67G and Dellite® HPS nanoparticles provided from Laviosa Chimica Mineraria S.r.l. – Italy, Cloisite® 93A nanoparticles from Southern Clay Products – USA and hydroxyapatite nanoparticles purchased from Sigma–Aldrich – Germany, represent the inorganic materials of hybrid hydrogels.

The hybrid hydrogels have been prepared using a two steps procedure described in a previous paper [3]. In brief the procedure occurs by the dispersion of montmorillonite nanoparticles in water which preceded the hydrogels synthesis step. Insertion of nanoparticles was possible by immersing the collagen matrices in the nanoparticles dispersion. The hybrid hydrogels have been prepared by crosslinking the pNIPAM with diethylene glycol diacrylate in the collagen membrane loaded with inorganic nanoparticles.

Semi-interpenetrated polymeric support fabricated by insertion of poly (N-isopropyl acrylamide-co-diethylene glycol diacrylate) onto collagen (ND) was used as reference for all nanocomposites hydrogels.

The study underlines the effect of natural nanoclay – Dellite® HPS (purified montmorillonite) (HPS) compared with chemically modified montmorillonite nanoparticles, respectively chemically modified with dimethyl dihydrogenated tallow ammonium – Dellite® 67G (G) or methyl dehydrogenated tallow ammonium – Cloisite® 93A (C) and hydroxyapatite (HA) nanoparticles on the