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Hybrid collagen-based hydrogels with embedded montmorillonite nanoparticles

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ABSTRACT

Montmorillonite nanoparticles have been physically incorporated within a crosslinked collagen/poly(N-isopropyl acrylamide) network in order to adjust the properties of the stimuli-responsive hybrid systems. The research underlines both the influence of hydrogel composition and nanoparticle type on hybrid hydrogel properties. The dispersion of the montmorillonite nanoparticles in polymeric matrix have been visualized by SEM, TEM and AFM techniques and quantitatively and qualitatively estimated using near infrared chemical imaging. The electrical charge of the nanoparticles influenced the polymeric chain arrangement and the pore size. The morphologies of the nanoparticulated layers are partially exfoliated or intercalated and uniformly dispersed through the polymeric semi-interpenetrated network based on collagen and poly(N-isopropyl acrylamide). The hybrid hydrogels exhibit pseudoplastic behavior and the addition of nanoparticles has resulted in the increase of the complex viscosity. The adhesion capacity was affected mainly by the presence of organically modified montmorillonites.

1. Introduction

The field of hybrid hydrogels is fast growing both in terms of fundamental research and medical applications [1]. The possibility to incorporate nanoparticles (Np) in polymeric matrices was explored in order to improve and control the hydrogel properties [2,3]. Natural or synthetic, organic or inorganic nanoparticles were attached to hydrogel by physical or covalent bonds by many researchers [4,5]. Special attention was given to the sensitive hydrogels due to their reversible volume transition which allows to identify, transmit or process the modification in their physical or chemical behavior because of action of the external stimuli and the response to this action. The main external stimuli are pH, temperature, magnetic or electric field, light, biological agents such as glucose and enzymes. Most polymeric hybrid hydrogels based on collagen are of great interest because they can maintain their configuration above 80 °C, have increased thermal stability and the crosslinking reaction has no negative influence on the biocompatibility so they would become promising scaffolds for tissue engineering [6]. Hybrid hydrogel collagen/chitosan demonstrated significantly enhanced mechanical strength and elasticity, excellent optical properties, optimum mechanical properties and good permeability to glucose and albumin. The collagen/chitosan hydrogel had excellent biocompatibility with successful regeneration of host corneal epithelium, stroma, and nerves [7].

Hybrid hydrogels consist from a polymeric network loaded with inorganic nanoparticles, which are dispersed between the polymer chains [8,9] or obtained from the dispersion of at least two types of polymers. What is essential is that the inorganic components to be homogeneously interposed in the polymer mass start with a good dispersion of nanoparticles. Usually the montmorillonite nanoparticles used in synthesis of hybrid materials leads to materials with higher mechanical and thermal stability, chemical resistance, barrier properties, controlled inflammability, [10–12] etc.

The hydrogels based on collagen offer high biocompatibility, excellent intrinsic cellular interaction, biodegradability, controlled degradability, and low toxicity of bioproducts but they present low mechanical properties, failure strength and they can cause inflammation at contact with a living tissue. Thereby, hybrid hydrogels based on synthetic and natural polymers are of interest due to the advantages of combining polymers with controllable properties and specific interactions induced between cells and material [8,11,12].

Nanocomposites are characterized by the coexistence of two distinct phases, a continuous organic phase of macromolecules and a nanometer-sized inorganic phase dispersed through the polymer phase. The content of inorganic phase is optimal at lower concentrations, up to 5% in respect to the amount of polymer. Usually, the inorganic parts of hybrid hydrogels are hydroxyapatite, montmorillonite, magnetic nanoparticles, etc. [13]. Furthermore, the montmorillonite nanoparticles were involved along with macromolecules to generate performed polymeric structures [14]. The montmorillonite nanoparticles, with general formula $(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot nH_2O$, are inorganic materials, environmentally friendly, easily available and at low cost. Primarily the benefit of montmorillonite nanoparticles inserted into polymeric systems is the reinforcement effect induced by the presence of nanoparticles. The

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Table 1
The composition of the hydrogels.

Sample code	Nanoparticles type and content	NIPAM, %	Collagen		DEGDA		APS, %	TEMED, %
			% from entire reaction amount	% in respect with NIPAM	% from entire reaction amount	% in respect with NIPAM		
ND	–	61.4	30.72	50	6.14	10	1.5	0.1
1 HPS	1% Dellite® HPS	54.3	38.74	71.2	5.43	10	1.5	0.1
2 HPS	1% Dellite® HPS	61.4	30.72	50	6.14	10	1.5	0.1
3 HPS	1% Dellite® HPS	60.2	30.10	50	8.02	13.5	1.5	0.1
1 G	1% Dellite® G67	54.3	38.74	71.2	5.43	10	1.5	0.1
2 G	1% Dellite® G67	61.4	30.72	50	6.14	10	1.5	0.1
3 G	1% Dellite® G67	60.2	30.10	50	8.02	13.5	1.5	0.1
C	1% Cloisite® 93A	61.4	30.72	50	6.14	10	1.5	0.1

studies concerning montmorillonite-based materials demonstrated their improved properties; respectively these materials show improvement of the swelling behavior, large surface area contact, adsorptive, ion exchange properties, and ability to transport drug molecules [15]. The collagen/chitosan/montmorillonite membranes have been designed to develop new bio-nanocomposites [16].

In our previous studies, semi-interpenetrate polymeric networks based on collagen and poly(N-isopropylamide) with decreased rate of biodegradability comparative with pure collagen and improved properties have been designed, regarding swelling capacity, thermal stability [17,18]. The effect of inorganic nanoparticles on hybrid hydrogel properties, as well as their biocompatibility with living cells and the immune response parameters followed by evaluation of chronic inflammation in rats, is presented in literature [19]. The present study is focused on the development and characterization of novel hybrid collagen/N-isopropylamide hydrogels with embedded inorganic nanoparticles targeted for tissue engineering applications, as scaffolds to cover the burned tissues with a design to support the healing and regeneration process of the damaged skin layers. With collagen as a major structural protein in the human body, the poly(N-isopropylacrylamide) attend to form a biomaterial with sensitivity to temperature [17,18], nearest the human body temperature, which enables the preparation of polymeric scaffolds as carriers for therapeutic agents essential in tissue engineering, as well as to release bioactive compounds in a controlled manner by its sensitivity to environmental stimuli. The structural, morphological and rheological characterizations are disputed along with the adhesion capacity of hybrid hydrogels, emphasizing the composition and nanoclay type influence.

2. Experimental

2.1. Materials

The collagen type I matrices were provided by Lohmann & Rauscher International GmbH & Co KG. The monomer, N-isopropylacrylamide (NIPAM), and diethylene glycol diacrylate (DEGDA) were purchased from Sigma-Aldrich. The reaction promoters, respectively the ammonium persulfate (APS) and N,N,N,N-tetramethylethylene diamine (TEMED) were supplied by Merck, Germany. Before the reaction, the N-isopropylacrylamide and ammonium persulfate were purified in toluene/n-hexane mixture and respectively in 75% alcoholic solution. Three kinds of nanoclays have been used namely: Dellite® HPS (HPS), Dellite® 67G (G), and Cloisite® 93A (C). Dellite® HPS nanoparticles are natural nanoclays, respectively a purified montmorillonite unmodified while Dellite® 67G is a nanoclay deriving from a naturally occurring montmorillonite especially purified and modified with a high content of quaternary ammonium salt (dimethyl dihydrogenated tallow ammonium). Cloisite® 93A nanoparticles are products of Southern Clay Products which are natural montmorillonites modified with a ternary ammonium salt, respectively methyl dehydrogenated tallow ammonium, for an organic modifier concentration of 95 meq/100 g clay.

2.2. Synthesis of hybrid hydrogels

The synthesis of hybrid hydrogels has been performed in two distinct steps — Fig. 2a. Firstly, the nanoparticles were dispersed for 24 h in water by mechanical stirring and alternatively using an ultrasonic bath. Constant montmorillonite concentrations of 1 wt.%, in respect with polymer mass, for all the hybrid hydrogels have been used. The insertion of nanoparticles was possible by immersing the collagen matrices in the dispersion of nanoparticles and keeping them in an ultrasonic bath for 2 h in order to ensure a good dispersion of nanoparticles. Collagen matrices embedded with nanoclay were lyophilized overnight. After this step, the hydrogels, with compositions given in Table 1, were prepared.

The hydrogel synthesis is described in a previous study [17]. Briefly, the hydrogels based on three-dimensional collagen matrix, N-isopropylacrylamide and diethylene glycol diacrylate in various weight ratios — Table 1 — were synthesized through a radicalic process. The polymerization process was initiated by the redox pair ammonium persulfate and tetramethyl ethylene diamine, which allows the development of the reaction even at reduced temperature of 24 °C [17,20]. According to the proposed recipe, the hybrid hydrogels have different embedded nanoparticles (Dellite® HPS, Dellite® 67G, Cloisite® 93A) and crosslinking agent content of 10% (2 HPS and 2 G), 13.5% (3 HPS and 3 G) or collagen content 71.2% (1 HPS and 1 G), 50% (2 HPS and 2 G). The reaction medium was double distilled water and the final products were gels with good physical consistency. Successively, the obtained samples were washed for 48 h in double distilled water, until the washing water shows no absorption band on UV–vis spectra corresponding to unreacted NIPAM monomer, crosslinker and traces of initiators and the hydrogels were freeze-dried at −50 °C at a pressure of 0.040 mbar with an Alpha 2–4 LD plus system to create porous polymeric scaffolds. Before characterization, samples were maintained in dried atmosphere over $CaCl_2$ for at least 48 h.

2.3. Characterization methods

2.3.1. Clay nanoparticle characterization

The size distribution, electrical charge and conductivity of montmorillonite nanoparticles were determined, in triplicate, using a Malvern Zetasizer Nano ZS instrument. Based on electrophoretic mobility, the zeta potential (ζ) of the clay particles (1 wt.%), dispersed in water, was calculated with Smoluchowski equation and the hydrodynamic

Table 2
The physico-chemical characterization of nanoparticles in water.

Np type	Average size, nm	Zeta potential, mV	Electrical conductivity, mS/cm
Dellite® HPS	398 ± 20	−23.7 ± 1.4	0.0893 ± 0.004
Dellite® G67	1450 ± 27	+46 ± 5	0.0296 ± 0.005
Cloisite® 93A	163 ± 12	−0.47 ± 2.2	0.0443 ± 0.007