Maleimide-grafted cellulose nanocrystals as cross-linkers for bionanocomposite hydrogels

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ABSTRACT

This article deals with the preparation of bionanocomposite hydrogels from natural polymers and nanoentities, an emerging class of materials for biotechnological and biomedical applications. Herein, the applicability of the Diels-Alder "click" reaction to the design of bionanocomposite hydrogels from furan modified gelatin using maleimide-functionalized cellulose nanocrystals as multifunctional cocross-linkers is demonstrated. The functionalization of cellulose nanocrystals with maleimide moieties was confirmed by XPS. The swelling and rheological properties of the resulting bionanocomposite confirmed the formation of hydrogel networks with covalently embedded nanoentities. The Diels-Alder reaction resulted in the formation of stiffer networks with lower swelling ratios due to the formation of additional cross-linking points. The designed "click" strategy proved to be a promising candidate for the formation of fully renewable bionanocomposite hydrogels.

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1. Introduction

Hydrogels represent an interesting class of materials with a broad range of applications in the pharmaceutical and biomedical field, as well as in agriculture and cosmetics. Among the different types of hydrogels, hydrogels from bio-derived polymers are particularly interesting since they are easily made biodegradable, promote tissue growth, mimic the extracellular matrix and possess a renewable character (Yang, Han, Duan, Xu, & Sun, 2013; Balakrishnan & Banerjee, 2011; Van Vlierberghe, Dubruel, & Schacht, 2011). During the last decade, the design of bionanocomposite hydrogels including nanoentities such as carbon nanotubes, metallic nanoparticles or clays have attracted great interest from the scientific community (García-Astrain, Ahmed et al., 2015; García-Astrain, Chen et al., 2015; Sabaa, Abdallah, Mohamed, & Mohamed, 2015; Derkus, Emregul, & Emregul, 2015; Haraguchi, 2007). The interactions between the nanoentities and the polymeric matrix at the nanoscale give rise to new materials with enhanced mechanical, thermal, biological, magnetic, optical or electronic properties (García-Astrain, Ahmed et al., 2015; García-Astrain, Chen et al., 2015; Gaharwar, Peppas, & Khademhosseini, 2014; Daniel-da-Silva, Salgueiro, & Trindade, 2013; Kamoun & Menzel, 2012; Shin et al., 2012; Barbucci, Giani, Fedi, Bottari, & Casolaro, 2012; Dvir et al., 2011; Narayana Reddy et al., 2011).

Moreover, in order to achieve superior environmental compatibility as compared to inorganic nanofillers, renewable bionanoentities are being incorporated into novel hydrogel formulations. In particular, cellulose nanocrystals have proven to be effective reinforcements for various hydrogels (Mohammed, Grishkewich, Berry, & Tam, 2015; Araki & Yamanaka, 2014; Dai & Kadla, 2009; McKee et al., 2014; Wallenius et al., 2015). Cellulose nanocrystals (CNCs) are nanoentities 100–500 nm in length and 5–30 nm in width which can be obtained from the partial hydrolysis of a variety of cellulosic materials (Lin & Dufresne, 2014; Yang et al., 2015). This natural nanoscaled material possesses numerous advantages including rod-like morphology, high aspect ratio, crystallinity, non-toxicity, biodegradability, high mechanical strength and surface reactivity (Lin & Dufresne, 2014). Generally, bare CNCs are physically incorporated as reinforcements into the hydrogel matrix (Dai & Kadla, 2009; Mohammed et al., 2015; Wallenius et al., 2015). However, recent work showed that surface-modified CNCs can act both as reinforcement and cross-linking agent within a polymeric matrix. In this way, the performance of the material is improved via the covalent linkage which enhances the interfacial adhesion and reinforces the effect of the filler (Chen, Lin, Huang, & Dufresne, 2015).

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Following this strategy, aldehyde-functionalized CNCs were used as cross-linkers for carboxymethylcellulose/dextran hydrogels and also for the preparation of injectable hyaluronic acid-based formulations (Domingues et al., 2015; Yang, Bakaic, Hoare, & Cranston, 2013). Xylan and gelatin-based hydrogels cross-linked with CNCs or oxidized CNCs, respectively, have also been reported (Köhnke, Elder, Theliander, & Ragauskas, 2014; Dash, Foston, & Ragauskas, 2013). However, to the best of our knowledge, little work has been done on the use of these renewable bionanofillers as hydrogel cross-linkers via means of "click" chemistry.

We have recently reported the applicability of the Diels-Alder (DA) "click" reaction for the preparation of biopolymer-based nanocomposite hydrogels (García-Astrain, Ahmed et al., 2015; García-Astrain, Chen et al., 2015). Herein, we demonstrate the role of maleimide-functionalized CNCs as cross-linkers for the formation of a completely renewable bionanocomposite hydrogel based on gelatin and chondroitin sulfate (CS). Gelatin, composed of a large variety of aminoacids, allows for a broad range of chemical modifications and, due to its sol-gel transition, is a suitable hydrogel precursor (García-Astrain et al., 2014; Van Vlierberghe et al., 2011). On the other hand, CS is a structural component of the extracellular matrix and its use is mainly focused on the synthesis of novel biomaterials.

The aim of this work was to explore the applicability of the Diels-Alder reaction for the preparation of fully biobased nanocomposites using modified CNCs as nanofillers. CNCs were surface-modified by reaction with a maleimide-functionalized aminoacid and the Diels-Alder cycloaddition was then employed as a mild covalent strategy for their binding with furan-modified gelatin. In order to stabilize the hydrogel, second cross-linking based on the amide coupling between CS and gelatin was performed. The effect of functionalized CNCs on the swelling and viscoelastic properties was analyzed, as well as the role of CNCs as stabilizer within this completely renewable bionanocomposite formulation.

2. Materials and methods

2.1. Materials

Gelatin (from porcine skin Type A, 300 Bloom), furfuryl glycidyl ether (FGE, 96.0%), chondroitin sulfate A sodium salt from bovine trachea (CS, 60.0%), N-hydroxysuccinimide (NHS, 98.0%), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC, 99.0%), β-alanine (99.0%), maleic anhydride (99.0%), 4-(dimethylamino)pyridine (DMAP, 99.0%) and microcrystalline cellulose were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS) solution was prepared from PBS tablets from Panreac (pH = 7.4). Acetic acid, sulphuric acid (96.0%) and ethyl acetate were also purchased from Panreac and toluene and dichloromethane from Lab Scan. Deionized water was employed as solvent. All reagents and solvents were employed as received. Gelatin was modified by reaction of its free ε-amino groups with furfuryl glycidyl ether (FGE) in aqueous solution as described in our previous work (G-FGE) (García-Astrain et al., 2014).

2.2. Synthesis of 3-(2,5-dioxo-2H-pyrrol-1(5H)-yl)propanoic acid (AMI)

AMI was prepared following a reported procedure with some modifications (Scheme 1) (Mantovani et al., 2005). An acetic acid solution of maleic anhydride (5.00 g in 50 mL) was added dropwise to an acetic acid solution of β-alanine (4.54 g in 50 mL). The mixture was stirred for 3 h at room temperature and a white suspension was obtained. After that period, 70 mL of AcOH were added, the temperature was raised until 115 °C and the mixture was stirred overnight. After one hour of reaction a limpid colourless solution

was observed. At the end of the whole process, an orange oil was obtained. The solvent excess was removed under reduced pressure and the product was washed with toluene (3 × 30 mL), which was again removed under reduced pressure. The product was then purified by flash chromatography using a silica column (DCM/ethyl acetate 9:1). A white solid was obtained. Yield: 29.15%.

2.3. CNCs functionalization (CNC-Mal)

CNCs were isolated from microcrystalline cellulose after acid hydrolysis with sulfuric acid, removing the disordered or paracrystalline regions of cellulose and leaving crystalline regions intact (González, Retegi, González, Eceiza, & Gabilondo, 2015). 5 g of CNC were hydrolyzed in a water bath at 45 °C using a 64% (w/w) sulfuric acid solution for 30 min. After that time, the solution is poured into a large excess of water and washed with successive centrifugations. The solution was dialyzed against water until neutral pH was reached and finally, nanocrystals were freeze-dried. For the CNC functionalization, 0.5 g of CNC were suspended in 30 mL of N,N-dimethylformamide (DMF) and 0.5 g of AMI (2.9 × 10^{-3} mol) were added (Scheme 2) (Cateto & Ragauskas, 2011). The mixture was cooled in ice and 0.399 g of DMAP (3.3 × 10^{-3} mol) were added at 0 °C. Then, a 10% (w/w) DMF solution containing 0.567 g of EDC (2.9 × 10^{-3} mol) was added dropwise. The mixture was stirred at room temperature for 24 h. After that time, the surface functionalized CNC (CNC-Mal) were precipitated in a dilute aqueous acid solution and then dialyzed against water until neutral pH was attained. The sample was washed several times with water, ethanol and hydrochloric acid 0.5 M. Finally, the product was recovered after freeze-drying.

2.4. Bionanocomposite preparation

For hydrogel formation, furan modified gelatin (GF, 70 mg) was dissolved in a previously ultrasonicated 0.5 wt.% suspension of maleimide-grafted CNCs. The final concentration of CNC-Mal in the bionanocomposite was 6.3% wt. Finally, CS was incorporated to the mixture (1:2 weight ratio with respect to gelatin) in the presence of EDC (2.3 × 10^{-3} mol) and NHS (2.0 × 10^{-3} mol). The mixture was allowed to gel for 24 h (Scheme 3). Control hydrogels without CNCs (G-CS hydrogel) or using bare CNCs (G-CS-CNC hydrogel) were also prepared for comparison following the same procedure described above.

2.5. Methods

Fourier Transform Infrared Spectroscopy (FTIR) was performed in a Nicolet Nexus spectrophotometer at room temperature. KBr pellets were used in the range from 4000 to 400 cm^{-1}, with a 4 cm^{-1} resolution. Proton and carbon Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) spectra were recorded with an Avance Bruker equipped with BBO z-gradient probe. Experimental conditions were as follows: (a) for ^{13}C NMR: 125.75 MHz, number of scans 14 000, spectral window 25 000 Hz, and recovery delay 2s; (b) for ^{1}H NMR: 500 MHz, number of scans 64, spectral window 5000 Hz, and recovery delay 1s. The solvent employed in all cases was D$_2$O. X-ray Photoelectron Spectroscopy (XPS) was carried out using a SPECS (Berlin, Germany) system equipped with a Phoibos analyzer 150 1D-DLD and a monochromatic source Al-Kα (1486.7 eV). An initial analysis of the present elements was performed (wide scan: step energy 1 eV, dwell time 0.1 s, pass energy 80 eV) and detailed analysis of the present elements was carried out (detail scan: step energy 0.1 eV, dwell time 0.1 s, pass energy 50 eV) with a take-off angle of 90° for the photoelectron analyser. Curve fitting was performed using a Gaussian–Lorentzian peak shape function with a straight base line throughout the analysis using a CasaXPS software 2.3.16.