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Short communication

Injectable composites *via* functionalization of 1D nanoclays and biodegradable coupling with a polysaccharide hydrogel

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ABSTRACT

The use of injectable materials in minimally invasive surgical procedures could help in facing the bone diseases connected to the ageing of world population. To this aim, materials integrating the rheological properties of biocompatible polymers with the mechanical properties of 1D inorganic nanostructures represent promising scaffolds. Here we describe the preparation of hydrogel composites made of carboxymethyl cellulose (CMC) and halloysite nanotubes (HNT) as injectable materials for the local treatment of bone defects. The rheology and injectability of the materials reflects their structural properties, showing the possibility of successfully injecting the prepared composites over a large range of operative conditions.

1. Introduction

The scientific research in bone medication and in the development of new and efficient methodologies for the treatment of bone diseases is becoming more and more relevant, mainly because of the progressive ageing of the world population [1]. For instance, in Italy the population ageing over 50 years is estimated to grow from 24 millions in 2010 to more than 29 millions in 2025. The number of fractures (both traumatic and pathologic) in Europe is expected to increase by more than 28%, with a total projected health care cost exceeding 46 billions € [2]. Any effective strategy to reduce those costs requires both a preventive action (typically achieved through a healthy life style) and the minimization of the post-surgery hospital stay. To this aim, minimal invasive surgery definitely represents a suitable strategy for the effective treatment of bone fractures and diseases [3]. In this perspective, the *in-situ* injection of properly designed biomaterials able to combine adequate mechanical properties with osteo-conductivity and osteo-inductivity can be of particular relevance.

The injectability of a material through a needle is typically assessed by evaluating the minimum pressure required for its injection, the evenness of its flow and the absence of clogging [4]. In the case of injectable composite materials, which typically are complex and non-Newtonian fluids (*i.e.*, hydrogels, gels, pastes, concentrated suspensions), the complete understanding of their rheological properties is crucial. Among them, shear thinning materials are especially appealing to minimal invasive surgery. Their viscosity decreases during the injection, while they eventually recover their initial rheological state at the injection site when the stress is relieved. As a result, the material is able to conform and eventually adhere to the site thanks to chemical affinity and microscopic interlocking [5].

Injectable biodegradable hydrogels provide an effective and convenient way for the controlled administration of a wide variety of actives (such as drugs, proteins, genes, and living cells) and they can be cleared from the body after accomplishing their mission [6]. A number of studies have already suggested the use of injectable composites in supporting the growth of damaged tissues, such as in bone tissue engineering [7], treatment of hemorrhage [8], and wound healing application [9]. Such a broad range of applications highlights the need for new materials (with tunable rheological properties) that could be injected with needles of different gauges.

In this work, we report on the preparation and the characterization of an injectable composite material made of halloysite nanotubes (HNTs) and carboxymethyl cellulose (CMC) conceived for the local treatment of bone defects. The choice of a polysac-

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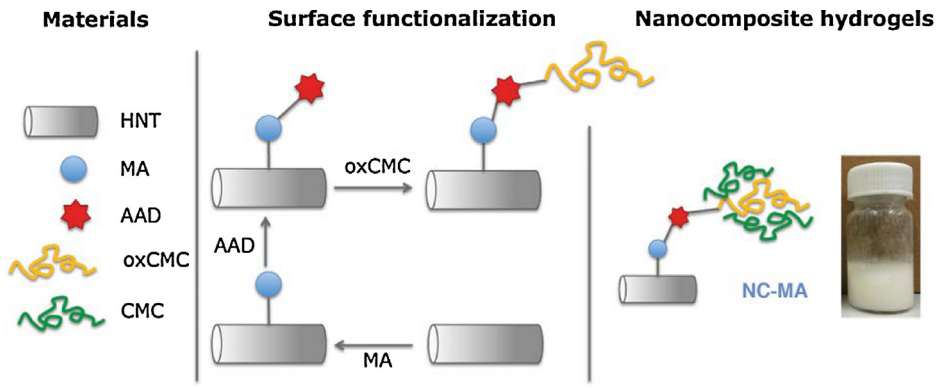

Fig. 1. Schematic representation of the HNT surface functionalization and of the formation of the nanocomposite hydrogels. The reaction scheme, including chemical structures, is given in the Supplementary material, Fig. S2.

charide matrix responds to the need of a biocompatible polymer with well-known rheological properties. CMC spontaneously forms a hydrogel and it is commonly adopted as a rheological modifier or additive in several water-based formulations in biomaterials and bio-based applications [10]. On the other hand, the use of nanotubular fillers introduces multiple advantages. HNTs are natural tubular aluminosilicates [11] which have been already proposed as reinforcing agents for composite materials in the field of bone medication thanks to their cytocompatibility [12], their mechanical properties [13], and their potentials as rheo-modifiers [14]. Compared to spheroidal and platelet particles, their 1D shape provides a stronger thickening power [15], while their hollow structure avoids a large increase in the density of the composite. Furthermore, their high surface area and the composition of the external surface allow for the adsorption of cations and positively charged species [16], while the presence of a hollow lumen of nanometric size can be exploited to accommodate and slowly release actives and drugs [17].

A crucial prerequisite to the reliable injection of our composite hydrogel through the needle gauges commonly employed in minimally invasive surgery is the homogeneous dispersion of HNTs in the CMC matrix. To this aim, we followed the hydrazide-aldehyde protocol successfully introduced by Hoare for the coupling of polymers and inorganic nanostructures [18,19]. HNTs were first functionalized with maleic anhydride and then reacted with adipic acid dihydrazide to obtain a hydrazide group. On the other hand, CMC was partially oxidized so to produce aldehyde groups. The two materials were finally mixed to spontaneously form a hydrazone bond.

2. Materials and methods

2.1. Materials

Halloysite nanotubes (HNT) were kindly provided by Imerys Minerals Ltd (Auckland, New Zealand). Sodium carboxymethyl cellulose (pre-hydrated TICALOSE® CMC 6000) was obtained from TIC Gums (Belcamp, MD, USA) and used as received. Adipic acid dihydrazide (AAD, purity ≥ 98%), (N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, commercial grade), ethanol (puriss grade), hydrochloric acid, sodium hydroxide, chloroform (purity ≥ 99.5%) were purchased from Sigma Aldrich (Sigma-Aldrich, Milan, Italy). Maleic anhydride (MA, purity 99%) and ethylene glycol (purity ≥ 99.5%) were purchased from Fluka (Buchs, Switzerland). Sodium periodate (purity 99.8%) was purchased from Merck (Darmstadt, Germany). MilliQ water (resistivity = 18.2 MΩ·cm) was used for the preparation of all the aqueous solutions and dispersions.

2.2. Purification of halloysites

The procedure for purifying the HNT batch used in this study was derived from the work by Shchukin et al. [20]. In details, 5 g of HNT were suspended in 500 ml of water and mixed with a blender for 20 min. The suspension was then transferred to a beaker and left to sediment. After a fixed amount of time, the supernatant solution was discarded and the sediment was re-suspended in 500 ml of water. The suspension was mixed with the blender for another 20 min and left to sediment again. The procedure was repeated three times. At the end, the HNT suspension was centrifuged for 10 min at 7500 rpm, and finally the precipitate was collected and dried in oven at 60 °C.

2.3. Methods

Surface area and porosity of HNTs were determined from N_2 adsorption isotherm with a SA 3100 analyzer (Beckmann-Coulter, Milan, Italy) utilizing BET and BJH calculations. ATR-FTIR measurements were performed on a Nexus 870 spectrometer (Nicolet, Madison, WI, USA), equipped with a liquid N_2-cooled mercury cadmium telluride (MCT) detector (650–4000 cm^{-1} range, resolution 2 cm^{-1}). Thermogravimetric analyses (TGA) was conducted on a SDT Q600 (TA Instruments, Newcastle, DE, USA) from room temperature to 1000 °C at 10 °C/min. Zeta potential (ζ, calculated according to the Smoluchowski model) was obtained with a 90Plus Particle Size Analyzer by Brookhaven Instruments Corporation. Morphological characterization was conducted by means of field emission scanning electron microscopy (ΣIGMA FE-SEM, Carl Zeiss Microscopy GmbH, Germany) on freeze-dried samples. Rotational shear measurements were carried out at 25 °C (Peltier temperature control system) on a Paar Physica UDS200 rheometer using a 2 cone and plate geometry (25 mm diameter) and working at controlled shear stress. After their loading, samples were equilibrated for 1 h at 25 °C prior to carrying out the experiments. Injectability tests were performed by means of a custom-made apparatus where controlled loads are applied to commercial syringes and the flow is evaluated from the amount of the composite which is injected over a known period of time. Apparent viscosity values (η) were calculated from the Poiseuille law (Eq. (1)) [21]:

$$F = \frac{32 D^2 l Q \eta}{d^4} \quad (1)$$

where F is the force applied to the syringe plunger, D is the syringe plunger diameter, d and l are the inner diameter and the length of the needle, respectively, and Q is the flow rate.