Carbohydrate Polymers 195 (2018) 63–70

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Antibacterial and hemostatic hydrogel via nanocomposite from cellulose nanofibers

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ARTICLE INFO

Keywords:
Nanoellulose
Cellulose nanofibers
Hydrogel
Nanoparticle
Nanocomposite

ABSTRACT

Bacterial infection and uncontrolled bleeding are the major challenges facing the wound treatment. In order to solve these problems, we have devised a green nanocomposite hydrogel by introducing the aminated silver nanoparticles (Ag-NH_2 NPs) and gelatin (G) to carboxylated cellulose nanofibers (CNF). Interpenetrating polymeric network (IPN) was formed by interaction of multicomponent, leading to the non-covalent (dynamic ionic bridges) crosslinked hydrogel CNF/G/Ag. The produced hydrogel dressing with 0.5 mg/mL Ag-NH_2 NPs (CNF/G/$Ag_{0.5}$) demonstrated stronger mechanical, self-recovery, antibacterial properties, satisfactory hemostatic performance, and appropriate balance of fluids on the wound bed (2093.9 g/m^2 per day). More importantly, the wound healing model evaluation *in vitro* and *in vivo* of CNF/G/$Ag_{0.5}$ showed an outstanding biocompatibility (~100% infected cell viability) and wound healing efficacy (~90% healed and 83.3% survival after 14 days). Our study paved a highly promising approach to improve the performance of cellulose-based hydrogel dressing and would also be useful for developing ideal skin wound dressings by other green materials.

1. Introduction

The skin is the essential interface between the body and its environment which can heal itself when the wound is narrow and small. However, the large wound of human skin is prone to infection and difficult to heal. Therefore, to help wound healing, appropriate treatments to the wound are necessary (Li et al., 2015). The conventional wound dressing in clinical application is natural or synthetic bandages, cotton wool, and gauzes, which may need long-term treatment, be ineffective, or even adhere to desiccated wound surfaces (Radhakumary, Antonty, & Sreenivasan, 2011). In order to overcome many of these drawbacks, numerous wound dressing materials were being investigated (Çalamak, Erdoğdu, Özalp, & Ulubayram, 2014; Fan et al., 2014; Ignjatović et al., 2016; Kang et al., 2017; Mei et al., 2017). Among them, hydrogel-based wound dressing have been paid special attentions due to that it can possess many essential properties, such as providing a cooling sensation, a moist environment, allowing gaseous exchange and wound exudate absorption (Cheng et al., 2017; Li et al., 2017; Zhao et al., 2017). Unfortunately, up to now, there is almost no clinically used hydrogel satisfying with all the requirements of the ideal skin wound dressing.

The leading challenge facing the wound dressing is the uncontrolled hemorrhage which is often the direct cause of the trauma deaths (Gaston, Fraser, Xu, & Ta, 2018; Konieczynska et al., 2017). Many clinical hemostatic formulations work with the use of hemostatically active proteins such as fibrin, collagen, and thrombin, but may be difficult to store and use out of the hospital. Gelatin (G) is an interesting candidate as a hemostatic based on its low prices, widely distribute, hydrolysis into simple byproducts, and reliable hemostatic effect (Chen, Guo et al., 2016; Lan et al., 2015; Mele, 2016). For the aspect of wound contamination, the major reason is bacterial infection causing by *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which can easily enter the body through the wounds, reach into deeper portions of the tissue, furthermore, even lead to septicemia and death. In order to reduce the abuse of broad-spectrum antibiotic, many antibiotics alternatives, such as silver nanoparticles (Ag NPs) with low bacterial resistance and side effects, have been widely used as topical antibacterial agents (Chen, Zhang et al., 2016; Dhand et al., 2016; Nie et al., 2016). But the development and application of nanomaterials has generated public debate on the safety of nanotechnology and has been intervened by some supervisory departments. This issue could potentially be overcame by incorporation of nanoparticles into hydrogels resulting in decreased risks to human health and the environment (Foss Hansen et al., 2016; Thoniyot, Tan, Karim, Young, & Loh, 2015).

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https://doi.org/10.1016/j.carbpol.2018.04.085
Received 26 February 2018; Received in revised form 19 April 2018; Accepted 21 April 2018
Available online 22 April 2018
0144-8617/

Interpenetrating polymeric network (IPN) is a 3D network composed of two or more different types of materials, which are partially or fully interlaced on a molecular scale but not covalently bonded to each other and cannot be separated unless chemical bonds are broken (Dragan, 2014). Multicomponent hydrogels based on IPNs have shown structural diversity, versatility, significant enhancement in the mechanical performance for tissue healing. Cellulose nanofibers (CNF), a kind of green functional material, have been widely studied in the field of food and biomedical field due to their nontoxic, biocompatible, biodegradable, and environmental and economic sustainability (Fernandes, Pires, Mano, & Reis, 2013; Liu et al., 2018; Saito, Kimura, Nishiyama, & Isogai, 2007). TEMPO-oxidized cellulose nanofibers (carboxylated CNF) have good hydrophilicity and water retention, which can helpful to achieve good performance on blood absorption and water vapor transmission. To the best of our knowledge, cellulose-based multifunctional hydrogel dressing was rarely reported. It is well accepted that cellulose and other biopolymers are promising building blocks of sustainable materials with tailored characteristics (Fan et al., 2017).

In this article, we presented a novel design for a CNF/G/Ag nanoparticles hydrogel as a wound dressing which did meet the controlling of evaporative water loss, the stopping hemorrhage, and the good antibacterial and biocompatible properties to promote wound healing. The physical and chemical properties, bactericidal activities, safety as well as the wound healing efficiency were comparatively investigated.

2. Experimental details

2.1. Reagents and materials

TEMPO-oxidized cellulose nanofibers (carboxylated CNF, surface carboxylate density 1.1 mmol/g) were provided free by Dr. Yangbing Wen (Tianjin University of Science and Technology, China). Polyvinyl pyrrolidone (PVP, K15, M_W = 10000), $AgNO_3$, ethylene glycol (EG), 3-aminopropyltriethoxysilane (APTES), gelatin (G) were bought form bought from Sinopharm Chemical Reagent Co. Simulated body fluid (SBF, pH 7.4, 142 mM Na^+, 5 mM K^+, 2.5 mM Ca^{2+}, 148 mM Cl^-, 4.2 mM HCO-3, 1 mM HPO2-4, and 5 mM SO2-4) was obtained from Qingdao Jisskang Biotechnology Co., Ltd. Dulbecco's Modified Eagle's Medium (DMEM, with high glucose, L-glutamine, sodium pyruvate), fetal bovine serum (FBS), amphotericin B, phosphate-buffered saline (PBS), streptomycin, and penicillin were all purchased from Invitrogen. Cell-Counting Kit-8 (CCK-8) was received from the Dojindo Laboratories (Beijing, China).

2.2. Animals and ethics

Adult Kunming mice (male, 18–22 g) used in the study were obtained from Beijing HFK Bioscience Co., Ltd. (Beijing, China). The mice are housed in the animal laboratory of Institute of Process Engineering (Chinese Academy of Sciences, Beijing, China) in a controlled environment (22 ± 2 °C, 55 ± 5% relative humidity level, a 12 h light/dark cycle). The animal experiments were in accordance with the guidelines set by the National Institutes of Health (NIH Publication No. 85-23) and were approved by the Beijing Experimental Animal Ethics Committee.

2.3. Preparation of aminated Ag NPs (Ag-NH_2 NPs)

Ag-NH_2 NPs were accomplished by the procedure reported in literature (Wang, Gao, Sun, Su, & Gao, 2016; Zhang et al., 2010). 1 g $AgNO_3$ completely dissolved in 25 g PVP/200 mL EG solution at room temperature. Then, the mixture solution was heated to 120 °C at the rate of 1 °C/min, and kept the reaction at 120 °C for 1 h. After natural cooling, the solution was added was precipitated with acetone. The Ag NPs were obtained by ultracentrifugation and continuous reacted in 100 mL NaOH aqueous solution (0.5 mol/L) at 70 °C for 12 h. The resulting product was ultracentrifugated, washed, redispersed in methanol/water (9/1 v/v) solution, and followed by amination with APTES at 70 °C for 12 h. Finally, the solids were collected, washed with water (2 × 500 mL), and dried under vacuum at 30 °C to give Ag–NH_2 NPs.

2.4. Preparation of CNF/G/Ag hydrogel dressings

Carboxylated CNF (200 mg) was completely dissolved in 14 mL deionized water under room temperature and disposed by ultrasonic to obtain the uniformly CNF hydrogel. Then 0.5 mL gelatin solution (80 mg/mL) and different amounts of Ag-NH_2 NPs solution were added into the CNF hydrogel at the same time. The entire mixture kept under vigorous stirring for 2 h at room temperature to form non-covalent crosslinked hydrogels. Finally, the hydrogels were poured into a petri-dish and freeze-dried overnight to obtain wound dressing and named as CNF/G/Ag. The samples containing 0.2 mg/mL and 0.5 mg/mL Ag-NH_2 NPs were abbreviated as CNF/G/$Ag_{0.2}$ and CNF/G/$Ag_{0.5}$, respectively.

2.5. Characterization

After coating with 5 nm of Pt/Pd, the morphology of samples was observed with a Hitachi S-4800 field-emission scanning electron microscope (FE-SEM). The structure and phase composition of samples were characterized by Shimadzu XRD-6100 wide-angle X-ray diffraction (WAXD) using Ni-filtered CuKα radiation (40 kV, 30 mA) with 4°/min scanning rate at room temperature. Diffraction intensity was measured in a range of 2θ = 5–70°. The changes in the functional groups were studied by Varian 670-IR fourier transform infrared spectroscopy (FTIR) at room temperature in the spectral range of 4000–400 cm^{-1}. Rheological characterization of the hydrogels was measured and analyzed using a TA Instruments AR2000 rheometer with a 25 mm diameter parallel plate. Samples were placed between the 37 °C pre-heated plates of the rheometer. The self-recovery properties of CNF/G/$Ag_{0.5}$ were detected by dynamic strain amplitude cyclic test (γ = 1% for 120 s and γ = 80% for 60 s) at 37 °C.

2.6. Blood clotting assay

The fresh whole blood samples (1 mL) were collected from mice, and heparin was added immediately. The whole blood clotting assay was according to Ong, Wu, Moochhala, Tan, and Lu (2008). The hydrogel dressings (0.5 cm square) were pre-warmed to 37 °C and fully immerse into the blood solution, and then 10 mL of 0.2 M $CaCl_2$ solution added. All the samples were placed at 37 °C with 30 rpm shaken for 15 min. Free erythrocytes were hemolyzed with 20 mL of deionized water, and the absorbance of the resulting hemoglobin solution was measured at were detected at 541 nm using an Infinite M200 microplate spectrophotometer from three independent tests.

2.7. Platelet adhesion

The fresh whole blood samples (1 mL) were centrifuged at 200 rpm at 4 °C for 15 min. The pellets were further centrifuged at 1500*g* at 4 °C for 10 min, and then removed and resuspended in a 2.5 mM $CaCl_2$/1.0 mM $MgCl_2$.soultion. The platelet adhesion tests were adapted from Lih, Jung, Joung, Ahn, and Han (2016). The hydrogel dressing samples (0.5 cm square) were immerse into 2 mL platelet suspension, incubated at 37 °C for 1 h, washed twice to remove the nonadherent and loosely attached platelets with PBS buffer. Adhered platelets on the samples were lysed using 1 mL of 2% (v/v) Triton X-100 at 37 °C for 15 min. Then, 100 μL adhered platelet solution was seeded in 96-well plate with adding 100 μL lactate dehydrogenase activity assay kit and detected at 450 nm.