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Bioactive glasses-incorporated, core–shell-structured polypeptide/polysaccharide nanofibrous hydrogels

Jian Chen[a], Xiaoyi Chen[b], Xianyan Yang[b], Chunmao Han[a], Changyou Gao[b,c], Zhongru Gou[b,*]

[a] Department of Surgery, The 2nd Affiliated Hospital, Zhejiang University of School of Medicine, Hangzhou 310009, China
[b] Zhejiang-California International NanoSystems Institute, Zhejiang University, Hangzhou 310029, China
[c] MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

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ABSTRACT

Although the synthetic hydrogel materials capable of accelerating wound healing are being developed at a rapid pace, achieving inorganic–organic hybrid at nanoscale dimension in nanofibrous hydrogels is still a great challenge because of its notorious brittleness and microstructural stability in wet state. Here, we developed a new nanofibrous gelatin/bioactive glass (NF-GEL/BG) composite hydrogel by phase separation method and followed by arming the nanofibers network with counterionic chitosan–hyaluronic acid pairs for improving microstructural and thermal integrity. We achieve this feature by carrying an optimal balance of charges that allows the inorganic ion release in aqueous solution without minimal structure collapse. Therefore, such NF-GEL-based, polysaccharide-crosslinked bioactive hydrogel could afford a close biomimicry to the fibrous nanostructure and constituents of the hierarchically organized natural soft tissues to facilitate chronic, nonhealing wound treatment.

1. Introduction

Synthetic hydrogels, cross-linked materials that typically consist of over 50% water, are notoriously brittle and have poor microstructural and mechanical stabilities (Tanaka, Gong, & Osada, 2005). Considerable efforts have been recently made to develop advanced polypeptide- and/or polysaccharide-based nanofibrous hydogel for enhancing wound repair (Guo et al., 2009; Jonker, Lowik, & van Hest, 2012). In wound operations, the purpose of dressing the wound is to promote an optimal healing environment by providing pain relief, protection from trauma and infection, a moist environment, and removal of debris. By simultaneously maximizing the patient's nutritional status and providing meticulous wound care, most wounds will heal appropriately (Lewis, Whitting, ter Riet, O'Meara, & Glanville, 2001). However, when the wound tissue is edematous and/or nonhealing because of severe tissue damage, poor blood flow, inflammation and infection, repair by conventional wound dressing and antibiotic administration may become technically unreliable and compromise wound healing (Boatenog, Matthews, Stevens, & Eccleston, 2008; Brem et al., 2003). Chronic, nonhealing wounds are involved progressively more tissue loss and bacterial colonization, particularly in venous stasis, diabetic ulcer, and bed sores, and thus give rise to the biggest challenge to wound-care hydrogel researchers (Ehrenreich & Ruszczak, 2006; Gurtner, Werner, Barrandon, & Longaker, 2008).

Multiple studies have shown that the local release of some inorganic ions from wound dressings can suppress microoganisms and accelerate wound repair process (Ali, Rajendran, & Joshi, 2011; Kawai et al., 2011; Neel, Ahmed, Pratten, Nazhat, & Knowles, 2005; Youk, Lee, & Park, 2004; Xu & Zhou, 2008). The ionic form of silver (Ag^+) is a well-known highly antibacterial material, and the silver-loaded dressing is an increasingly popular approach in the control of wound bioburden (Ali et al., 2011). However, a high concentration of silver impairs the functioning of the central and peripheral nervous systems (Chopra, 2009; Leaper, 2006). It is well recognized that calcium is an important factor in the wound healing of skin and suspect that it is required for the migration of epidermal cells (Lansdown, 2002). Clinically, the direct topical application of calcium to chronic wounds through calcium alginate dressings has been shown to be beneficial (Motta, 1989). Recently, it has been found that some bioactive glasses (BGs) readily form a soft tissue bond and their ion release products stimulate collagen production (Hench, 2006). Wilson, Pigott, Schoen, and Hench (1981) firstly showed that soft connective tissues could form a bond to 45S5 Bioglass® and established the safety of use of particulate forms in soft tissue if the interface was immobile. More recent studies demonstrated the beneficial effects of 45S5 and borate containing

* Corresponding author at: Zhejiang-California International NanoSystems Institute, Zhejiang University, Kaixuan Road 268, Hangzhou 310029, China.
Tel.: +86 571 8697 1782; fax: +86 571 8697 1539.
E-mail address: zhrgou@zju.edu.cn (Z. Gou).

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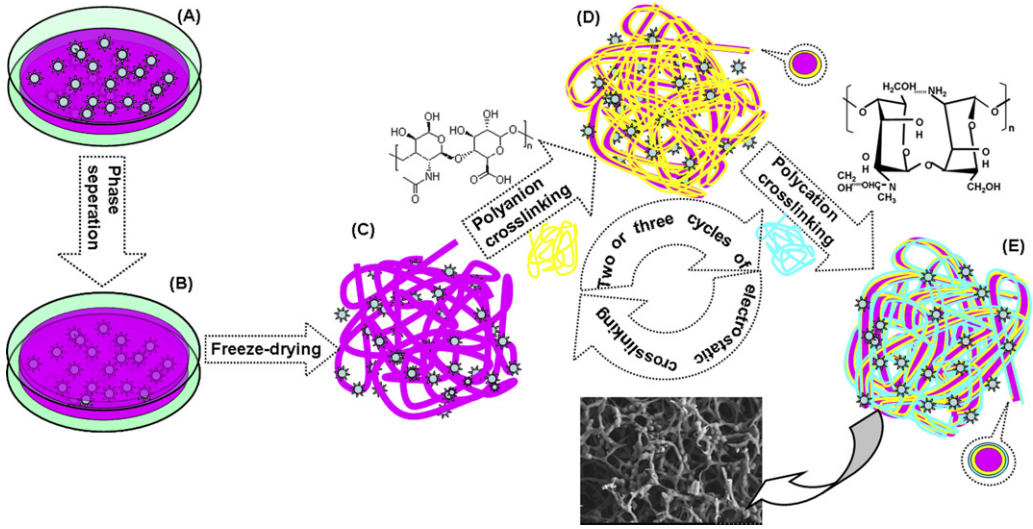

Scheme 1. Experimental procedure for preparing the nanofibrous organic–inorganic hybrid hydrogels via phase separation (A and B) and fabricating core–shell-structured nanofibrous polypeptide–polysaccharide composite hydrogels via electrostatic crosslinking (C–E).

13–93 BGs to promote angiogenesis, which is critical to the healing of soft tissue wounds (Gorustovich, Perio, Roether, & Boccaccini, 2010; Rahaman et al., 2011). Thus, the development of BG-loaded hydrogels with antimicrobial activity is highly desired.

The biomimetic biopolymeric nanofiber hydrogels closely mimic the microstructure and porosity of extracellular matrices (ECMs) in which growth factors, drugs and nutrients freely diffuse in scaffolds at very slow rates (Van Vlierberghe, Dubruel, & Schacht, 2011). Among the preparation methods, the self-assembling peptide nanofiber hydrogels are of particular interest due to the attractive extracellular matrice-mimicking porous architecture which may promote wound healing, skin regeneration and enhance angiogenics (Sun et al., 2011; Schneider, Garlick, & Egles, 2008). Unfortunately, the self-assembly methods usually require multiple labor-intensive steps associated with peptide preparation and purification, and slow assembly in specific electrolyte environment (Hartgerink, Elia, & Stupp, 2002; Zhang, Gelain, & Zhao, 2005). Furthermore, these procedures are not enough to meet the scalable yield for wound management, and maybe result in instable individual fibrous structure when incorporating with dissolvable particles. Electrospinning has been studied widely because of its efficiency and simplicity in fabricating of nanofibrous structures. Natural polymers such as gelatin (GEL) (Rujitanaroj, Pimpha, & Supaphol, 2008), chitosan (CS) (Tchemtchoua et al., 2011), and hyaluronic acid (HA) (Li et al., 2006; Uppal, Ramaswamy, Arnold, Goodband, & Wang, 2011) have been used to produce nanofibrous hydrogel mats as bioactive dressings. However, the usage of cytotoxic solvents, the size limitation of inorganic particles, three-dimensional (3D) structure maintenance, and limited spinnable conditions are all drawbacks in organic–inorganic hybrid electrospinning (Xie, Li, & Xia, 2008). On the other hand, some nanofibrous hydrogel's capacity to act as the porous matrices of BG microparticles for wound care is limited due to its poor mechanical and thermal stabilities at physiological temperature, and particularly the conventional chemical crosslinking make the hydrogels bind tissue rapidly and tightly, which is catastrophic to skin wound treatment (Liang, Chang, Liang, Lee, & Sung, 2004). Thus a further challenge in the design of bioactive dressings is how to produce scalable biomimetic nanofibrous hydrogels for accelerating the chronic wound healing, without involving environmentally/biologically harmful additives or inert materials.

Herein we developed a reliable, facile way to fabricate the novel GEL/BG@CS–HA hydrogels based on thermally induced phase separation and electrostatic crosslinking techniques, which would create stable nanofibrous 3D porous architecture and integrate the antibacterial and bioactive properties (Scheme 1). This method may be easily scaled-up to prepare large quantities of BG-incorporated core–shell nanofibers with GEL as core and CS–HA complex as shell. The superb structural and thermal integrity and chemically tunable bioactivity and antimicrobial activity provide tremendous opportunities for their use as an efficient wound dressing in non-healing wounds.

2. Materials and experiment

2.1. Chemicals and materials

High-purity grade inorganic salts, GEL (type B, from bovine skin), trishydroxymethyl aminomethane (Tris), and ethanol (≥99.8 wt.%) were purchased from Sinopharm Chemical Reagent Co. Ltd., CS ($M_W \sim 50$ kDa; degree of deacetylation: 85%; Shandong Haidebei Marine Bioengineering Co. Ltd.), Tris(hydroxyllmethyl) aminomethane (Tris; Bio-Rad), and HA (Freda Biochem Co. Ltd.) were used as received. Ultrapure MiniQ water (18.2 MΩ cm^{-1}) was used in experiments. The 45S5 BG particles (with similar composition to Biolgass® 45S5) were prepared by a sol–gel method with chemical composition (wt.%): 45.0 SiO_2, 24.5 CaO, 24.5 Na_2O, and 6 P_2O_5 as reported previously (Chen & Thouas, 2011).

2.2. Preparation of conventional GEL/BG composites

GEL (6.0 g) was dissolved in 120 mL deionized water at 50 °C to make a GEL solution of 5.0% (w/v). Then, the solution was divided into six equal parts (20 mL) and added 0, 4, 8, and 12 mg BG powders under magnetic stirring to for mixture solutions with BG/GEL mass ratio of 0, 0.4%, 0.8%, and 1.2%, respectively. The GEL solutions were added into the 6-well cell culture plates (CCP) and were kept at −80 °C for 24 h. After that, the frozen gels were lyophilized for 48 h. The dried GEL/BG porous composites were stored in a desiccator until characterization.

2.3. Preparation of nanofibrous GEL/BG porous composites

Gelatin was dissolved in 50/50 (v/v) ethanol/water mixture at 55 °C to make a GEL solution of 5.0% (w/v). Under continuous stirring, the 0, 4, 8, and 12 mg BG powders were added into 20 mL