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Biocomposites of copper-containing mesoporous bioactive glass and nanofibrillated cellulose: Biocompatibility and angiogenic promotion in chronic wound healing application

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ABSTRACT

Biocomposites of copper-containing mesoporous bioactive glass (Cu-MBG) and nanofibrillated cellulose (NFC) were designated as potential dressing material for chronic wound healing. The phase composition and mesoporous micro-structure of the synthesized Cu-MBGs were elaborately characterized by combining several techniques, including TEM, SEM, XRD, SXAS and N_2 physisorption. High bioactivity of the Cu-MBG was confirmed in stimulated body fluids *in vitro*. A controlled dissolution of Cu from the glass suggests Cu-MBG a suitable source for Cu release in wound healing dressings. Depending on the content of Cu-MBG in the composite formulation, the composites were fabricated as membranes and aerogels. In biocompatibility assessment of the composites, a dose-dependent cytotoxicity of Cu^{2+} on 3T3 fibroblasts was found. Importantly, a critical biological level of Cu^{2+} below 10 mg/L was suggested for the survival and growth of 3T3 fibroblasts. The Cu^{2+} released from the composite aerogel of NFC and Cu-MBG showed a profound angiogenic effect in the 3D spheroid culture system of human umbilical vein endothelial cells. Moreover, the angiogenic gene expression of 3T3 fibroblast was upregulated in the real-time quantitative PCR analysis, which also confirms that the incorporation of Cu-MBG into NFC matrix enhances the proangiogenic potential of the biocomposites. In addition, composites of NFC and Cu-MBG also showed an inhibiting effect on the growth of *E. coli*.

Statement of Significance

To address an urgent need in clinics on developing a new generation of therapeutic dressings with advanced functionalities, this study has exploited the utilization of Cu-containing mesoporous bioactive glass in the nanocellulose matrix to release Cu^{2+} as therapeutic ions for its angiogenic effect on promoting wound healing. This manuscript reports research work on biomaterial design, fabrication development, material characterizations and bioassessments in 2D cellular studies. To utilize nanocellulose derived from the wood resource in biomedical applications is of great significance, due to its vast availability and bioeconomy competence. The use of Cu-containing bioactive glass in tissue engineering scaffolds, including wound healing, is an intriguing research topic, which has been recently discussed in the field of biomaterials. I think that our manuscript title with 'Biocomposites of copper-containing mesoporous bioactive glass and nanofibrillated cellulose: biocompatibility and angiogenic promotion in chronic wound healing application' will make its own contribution on understanding the complex effects of Cu^{2+} on wound-healing-relevant events with acceptable novelty for *Acta Biomaterialia*.

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1. Introduction

Chronic wounds are a major health problem around the world. They produce a great burden to patients and health care systems [1]. Acute wounds normally heal in a very orderly and efficient manner characterized by four distinct but overlapping phases: hemostasis, inflammation, proliferation and remodeling [2]. When wound repair process is abnormally interrupted by some local factors including ischemia infection, tissue maceration or presence of foreign body, chronic wounds may occur. In order to promote the healing of wounds, there is an urgent need to develop dressing materials with therapeutic functionalities in the clinics of chronic wound management [3]. Moisture control over the wounds and angiogenic conditions are two important factors that may significantly affect the wound healing. A moisture environment is crucial for healing of chronic wounds, as it can speed up epithelialization and collagen synthesis [4]. Neovascularization is critical since a reduced ability to re-establish the blood supply to the injury site ultimately leads to wound chronicity [5]. Despite the development of numerous therapies, impaired angiogenesis still remains a significant challenge for chronic wound healing. Another aspect addressed in modern chronic wound management is microbial biofilm formation over the chronic wounds [6]. Clinically, sixty percent of chronic wound specimens are characterized as containing biofilm, which is usually associated with prolongation of the inflammatory phase of repair [7]. Thus, an antibacterial capacity is desirable for the wound dressing in order to disrupt the biofilm formation in the wounds [8].

Mesoporous bioactive glasses (MBGs) refer to the sol-gel-derived bioactive glasses (SiO_2-CaO-P_2O_5 system) featured with highly ordered mesoporosity [9,10]. The sol-gel route to synthesize MBGs involves an evaporation induced self-assembly (EISA) process using surfactants or non-ionic block copolymers as structure directing agents. It leaves replicate mesopores of molecular aggregates after the removal of all organics in calcination. Compared with melt-derived bioactive glasses, MBGs can have a broader range of compositions and they possess superior bioactivity due to the high surface area and large pore volume [11]. The unique mesoporous structures also embow MBGs with improved function as carriers for targeted biomolecule delivery in the physiological processes, including antibiotics and growth factors [12]. Similarly to the melt-derived bioactive glasses, extensive research effort has focused on the application of MBGs in bone tissue engineering scaffolds in the recent decade [13–15].

As recent studies have also revealed the capacity of bioactive glasses to stimulate vascularization and to heal soft tissue wounds [16], MBGs are also highly interested materials to be applied in chronic wound healing. Chronic wounds are potentially caused by deficiencies or imbalance of ionic concentration profile in the wounded tissue. Ca^{2+}, one of the ionic dissociate products of MBGs, is recognized as an initial trigger in our immune response to healing process [17]. Moreover, the composition of MBGs of SiO_2-CaO-P_2O_5 system can be doped with a small amount of oxides of trace elements. Zinc, copper, cerium, gallium, zirconium and iron have been incorporated into MBG network [18–21]. The release of these cations, the so-called therapeutic ions, into physiological fluids has been found to promote certain relevant biological functions, such as osteogenesis, angiogenesis or antibacterial capacity. Notably, Cu^{2+} is known as an essential participant of angiogenesis [22]. The ability of Cu^{2+} to promote wound healing has been associated with its interactions with many factors involved in the wound healing signaling cascade, including upregulation of express of vascular endothelial growth factor (*Vegf*) and integrin, stabilization of fibrinogen and upregulation of copper-dependent enzymes, which are important for matrix remodeling [22,23]. In addition, Cu^{2+} also endows the bioactive glasses with antibacterial capacity [24,25]. Administration of Cu from impregnated wound dressing is an emerging concept in wound care products and the Cu-containing wound dressing holds significant promises to promote wound healing for future clinical use [16,26].

Cellulose, a polysaccharide consisting of linear chains of β-(1→4) linked D-glucose units, is the most abundant natural polymer on Earth. In recent years, nanocellulose, such as nanofibers and nanocrystals of cellulose, has been increasingly investigated for various biomedical applications due to its unique material properties, e.g., high mechanical strength, broad chemical modifying capacity, biocompatibility and biodegradability [27,28]. Nanocellulose can be extracted from various cellulosic resources, including high plants, crop residues and bacterial sources. As topical wound healing dressing materials, different types of nanocellulose have also been extensively exploited, particularly those originating from bacterial sources [29,30]. Nanocellulose offers great features for enhancing wound healing, e.g., ability to absorb and retain moisture over the wound and beneficial effects on the biological processes involved in the wound healing cascade, such as lowering inflammatory response and promoting the fibroblast proliferation [30,31]. More lately, the extraction of nanocellulose from high-plants has become feasible with chemical and mechanical technology advances [32,33] and thus it has boosted the study of utilizing nanocellulose from this vast source in constructing functional materials in biomedical applications [34–36].

In this work, we present the development of biocomposites of nanofibrillated cellulose (NFC) and copper-containing MBG (Cu-MBG) and their potential to be used as wound dressing materials. The dissolution behavior and mineralization of Cu-MBG as particles and in the composite were investigated *in vitro* by several characterizing techniques. The cytotoxicity of the composites and their angiogenic performance were assessed in cellular studies. Finally, the antibacterial properties of the composites were preliminarily evaluated with the clinically important Gram-negative bacteria, *Escherichia coli* (*E. coli*).

2. Materials and methods

2.1. Synthesis and material characterizations of Cu-MBGs

MBGs (SiO_2-CaO-P_2O_5-CuO) were synthesized by the EISA method as previously reported by Wu et al. with some modifications [37]. The precursors to form a sol were tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), calcium nitrate tetrahydrate and copper(II) nitrate hemi(pentahydrate) dissolved in ethanol/water. Hydrochloric acid was used as catalyst and Pluronic®F108 was included in the sol as a structure directing agent. In a typical synthesis, 6.92 g of F108 (Mw = 14600, Sigma) was dissolved to 36.2 ml ethanol (99.9%) in a water bath at 40 °C to get solution (I). 2.48 g $Ca(NO_3)_2{\cdot}4H_2O$, 0.637 g of TEP(99.8%), 11.66 g of TEOS, 1.9 ml 1 M HCl, 15.24 ml ethanol and 5.72 ml H_2O were mixed thoroughly under stirring to get solution (II). Solution (I) and solution (II) were mixed in one bottle and stirred overnight in a water bath at 40 °C with a closed lid. Then the sol was cast in a petri dish and subjected to EISA process under room ambient in water bath at 40 °C. The obtained gel was left at 40 °C for 6 days to age. The collected xerogel was then calcined at 600 °C for 5 h to remove the structure-directing agents and nitrates. The obtained sample was denoted as MBGSi80 (molar ratio Si/Ca/P = 80/15/5). In the synthesis of MBGSi78Cu2 (molar ratio Si/Cu/Ca/P = 78/2/15/5) and MBGSi75Cu5 (molar ratio