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Wound dressings composed of copper-doped borate bioactive glass microfibers stimulate angiogenesis and heal full-thickness skin defects in a rodent model

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ABSTRACT

There is a need for better wound dressings that possess the requisite angiogenic capacity for rapid in situ healing of full-thickness skin wounds. Borate bioactive glass microfibers are showing a remarkable ability to heal soft tissue wounds but little is known about the process and mechanisms of healing. In the present study, wound dressings composed of borate bioactive glass microfibers (diameter = 0.4–1.2 μm; composition $6Na_2O$, $8K_2O$, 8MgO, 22CaO, $54B_2O_3$, $2P_2O_5$; mol%) doped with 0–3.0 wt.% CuO were created and evaluated in vitro and in vivo. When immersed in simulated body fluid, the fibers degraded and converted to hydroxyapatite within ~7 days, releasing ions such as Ca, B and Cu into the medium. In vitro cell culture showed that the ionic dissolution product of the fibers was not toxic to human umbilical vein endothelial cells (HUVECs) and fibroblasts, promoted HUVEC migration, tubule formation and secretion of vascular endothelial growth factor (VEGF), and stimulated the expression of angiogenic-related genes of the fibroblasts. When used to treat full-thickness skin defects in rodents, the Cu-doped fibers (3.0 wt.% CuO) showed a significantly better capacity to stimulate angiogenesis than the undoped fibers and the untreated defects (control) at 7 and 14 days post-surgery. The defects treated with the Cu-doped and undoped fibers showed improved collagen deposition, maturity and orientation when compared to the untreated defects, the improvement shown by the Cu-doped fibers was not markedly better than the undoped fibers at 14 days post-surgery. These results indicate that the Cu-doped borate glass microfibers have a promising capacity to stimulate angiogenesis and heal full-thickness skin defects. They also provide valuable data for understanding the role of the microfibers in healing soft tissue wounds.

1. Introduction

The skin (the largest organ of the human body) plays a critical role as a protective barrier against the environment, in preventing infection and the loss of water and electrolytes, and in regulating the body temperature [1]. The healing of large areas of full-thickness skin defects resulting is a significant clinical problem [2–4]. Current treatments based on the use of autografts, allografts, xenografts and bioengineered skin substitutes suffer from limitations such as antigenicity, potential risk of disease transmission and limited availability [5,6]. Recent advances in tissue engineering and regenerative medicine have resulted in an improved understanding of wound healing and the development of a variety of methods for skin repair and regeneration. Biomaterials-based wound dressings have been receiving considerable research interest because they can provide one of the most straightforward treatment options [7].

Wound dressings composed of electrospun polymer microfibers or nanofibers (natural or synthetic) are receiving significant interest because they have a structural similarity to the extracellular matrix (ECM), high surface area to volume ratio and tunable

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mechanical properties [8–11]. However, their use in clinical applications can suffer from limitations such as the release of acidic degradation products that could lead to a large decrease in the local pH, inflammation, blisters, scar hyperplasia and severe wound contraction [12]. The low bioactivity and typical hydrophobic nature of polymeric biomaterials could limit their integration with host tissue [13]. Furthermore, without the use of therapeutic agents, they lack the ability to stimulate vascularization, a key process in wound healing.

While bioactive glasses have been investigated and applied for decades in bone regeneration, recent studies have shown the capacity of bioactive glasses to stimulate vascularization and to heal soft tissue wounds [14–17]. Microfibrous borate bioactive glass has shown the capacity to heal soft tissue wounds in humans [18,19], while a commercial product is also available to treat soft tissue wounds in animals. Because of their high surface area, rapid degradation and conversion to hydroxyapatite (HA), ease of handling and shape flexibility, bioactive glass microfibers and nanofibers have been attracting growing interest in healing soft tissue wounds [20–23]. Liu et al. found that melt-derived borate bioactive glass microfibers (diameter = 0.2–5 μm) with the composition designated 13-93B3 ($6Na_2O$, $8K_2O$, 8MgO, 22CaO, $54B_2O_3$, $2P_2O_5$; mol%) converted to HA within 7–14 days in simulated body fluid (SBF) and released a high concentration of Ca and B ions into the medium [24]. A subsequent study using Cu-doped 13-93B3 microfibers (0.4 wt.% CuO) showed that the Cu was almost completely released from the microfibers into SBF glass within 7–14 days [25]. Liu et al. suggested that the high concentrations of Ca, B and Cu released from the microfibers could play a key role in their ability to heal soft tissue wounds.

Effective wound dressings should have the capacity to stimulate rapid angiogenesis because blood vessels are required for delivering oxygen and nutrients to the cells in the wound site. A variety of approaches have been developed to enhance angiogenesis by bioengineered constructs, including the incorporation of angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) [26–28]. However, the use of growth factors typically suffers from disadvantages such as high cost, potential adverse biological effects when used in supra-physiological doses [29–31] and loss of bioactivity [32,33].

Inorganic angiogenic factors such as Cu^{2+} ions are of interest because of their low cost, high stability and potentially better clinical safety when compared growth factors [34–36]. Copper is an essential component of the angiogenic response [35]. Copper ions have been reported to enhance angiogenesis by stabilizing the expression of hypoxia-inducible factor (HIF-1α), thus mimicking hypoxia, which plays a critical role in the recruitment and differentiation of cells and in blood vessel formation [36,37]. The release of Cu^{2+} ions has been shown to stimulate the expression of proangiogenic factors such as transforming growth factor-β (TGF-β) and VEGF in wounds created in diabetic mice [38,39], decrease the risk of ischemia in skin flaps and to induce a vascularized capsule around a cross-linked hyaluronic acid hydrogel [40]. In combination with VEGF and bFGF, Cu^{2+} ions were shown to enhance the vascularization of an implant as an alternative or a complementary approach to the use of growth factors in a three-dimensional (3D) angiogenesis culture system [36]. Lin et al. showed recently that subcutaneous implantation of Cu-containing borate bioactive glass (13–93B3) microfibers in rats significantly enhanced the growth of capillaries and small blood vessels when compared to silicate 45 S5 bioactive glass microfibers and sham implant controls [41].

In the present study, wound dressings composed of borate bioactive glass (13-93 B3) microfibers doped with varying amounts of Cu (0–3.0 wt.% CuO) were created and evaluated in vitro and in vivo. The release of Cu^{2+} ions from the Cu-doped microfibers and the conversion of the microfibers to HA in simulated body fluid (SBF) were studied. When compared to previous studies described above, the experiments of the present study were designed to improve our understanding of the role of borate bioactive glass microfibers in wound healing in two key areas. First, the effects of the soluble ionic product of the microfibers on the response of cells (HUVECs and fibroblasts) were studied in vitro. Second, we systematically investigated the capacity of the microfibers to stimulate angiogenesis and to heal full-thickness skin defects in a rodent model.

2. Materials and methods

2.1. Preparation and characterization of borate bioactive glass microfibers

The parent borate bioactive glass used in the present study, with the composition $6Na_2O$, $8K_2O$, 8MgO, 22CaO, $54B_2O_3$, $2P_2O_5$ (mol.%) and designated 13-93B3, has been used previously in the form of particles, 3D scaffolds and microfibers [25,42]. The glass has the same composition as silicate 13-93 bioactive glass but with all the SiO_2 replaced with B_2O_3. Glasses containing 0, 0.5, 1.0 and 3.0 wt.% CuO, designated BG, 0.5Cu-BG, 1Cu-BG and 3Cu-BG, respectively, were prepared by conventional melting and casting under conditions similar to those described previously for 13-93B3 glass [42]. Then the glass was re-melted and formed into microfibers by blowing a high pressure jet of gas onto the molten glass and quenching the fibrous material. The compositions of the BG and 0.5Cu-BG microfibers were almost identical to the microfibers used in a clinical trial to heal soft tissue wounds in humans and those available commercially for animal use [18,19].

The microfibers were examined in a field-emission scanning electron microscope (SEM) (Hitachi S-4700; Tokyo, Japan). The average diameter of each group of microfibers was determined by measuring the diameters of over 200 individual fibers in the FESEM images using software. The microfibers were coated with Au/Pd and examined in the SEM at an accelerating voltage of 20 kV and a working distance of 9.5 mm. The as-prepared microfibers were ground into a powder and analyzed using X-ray diffraction (XRD) (D/max- 2500V; Rigaku, Tokyo, Japan) to check for the presence of any crystalline phases. XRD was performed using CuKα radiation (λ = 0.15406 nm) at a scanning rate of 10° per min in the 2θ range 10–80°.

2.2. Degradation and conversion of the bioactive glass microfibers in vitro

The degradation of the microfibers and their conversion to HA were studied as a function of immersion time in SBF (pH = 7.4) at 37 °C. A ratio of 1.0 g of the microfibers to 100 ml of SBF was used in all of the conversion experiments. The concentration of Cu^{2+} ions released from the microfibers into the SBF as a result of the conversion process was determined using inductively-coupled plasma atomic emission spectroscopy (ICP-AES; Optima 2100 DV; USA). At selected immersion times, the weight loss of the microfibers was measured as described previously [43]. The formation of HA on the surface of the reacted microfibers was studied using FESEM fitted with an energy-dispersive X-ray (EDS) spectrometer and XRD.

2.3. In vitro response of cells to soluble ionic products of bioactive glass microfibers

2.3.1. Cells and cell culture

The response of cells to the bioactive glass microfibers was studied in vitro. However, the cells were not seeded directly on the microfibrous glass because of difficulties encountered in performing some assays. Instead, the cells were incubated in media containing the ions released from the microfibers (referred to as the ionic dissolution product of the microfibers). This method has been used in previous studies to evaluate the proangiogenic potential of bioactive glasses [14]. Extracts of the ionic dissolution product of the undoped and Cu-doped glass microfibers were prepared by grinding the microfibers into lengths <38 μm and soaking the particles in serum-free Dulbecco's modified Eagle's medium (DMEM) (GIBCO; Invitrogen Pty Ltd., Australia). A concentration of glass to DMEM of 100 mg/ml was used in accordance with International Standard Organization (ISO/EN) 10993-5 [44]. After incubation for 24 h at 37 °C, the mixture was centrifuged and the supernatant was collected, filtered through a 0.2 μm filter and supplemented with 10% fetal calf serum (FCS) (In Vitro Technologies, Australia) and 1% (v/v) penicillin/streptomycin (P/S; GIBCO; Invitrogen Pty Ltd., Australia).

The human umbilical vein endothelial cells (HUVECs) and fibroblasts used in the present study were approved by the ethical committee of the Shanghai Sixth People's Hospital, Shanghai Jiaotong University School of Medicine. HUVECs were isolated from the human umbilical cord vein as described previously [45] with the written informed consent of the donors. The cell pellets were redispersed in M199 (GIBCO) supplemented with 10% FBS and 1% endothelial cell growth supplement/heparin kit (ECGS/H, Promocell). Primary dermal fibroblasts were isolated and subcultured using a method described previously [46]. Briefly, the skin specimens were cut into small pieces with surgical scissors, placed in a 10 cm tissue culture dish and