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Highly efficient local delivery of endothelial progenitor cells significantly potentiates angiogenesis and full-thickness wound healing

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ABSTRACT

Wound therapy with a rapid healing performance remains a critical clinical challenge. Cellular delivery is considered to be a promising approach to improve the efficiency of healing, yet problems such as compromised cell viability and functionality arise due to the inefficient delivery. Here, we report the efficient delivery of endothelial progenitor cells (EPCs) with a bioactive nanofibrous scaffold (composed of collagen and polycaprolactone and bioactive glass nanoparticles, CPB) for enhancing wound healing. Under the stimulation of CPB nanofibrous system, the viability and angiogenic ability of EPCs were significantly enhanced through the activation of Hif-1α/VEGF/SDF-1α signaling. *In vivo,* CPB/EPC constructs significantly enhanced the formation of high-density blood vessels by greatly upregulating the expressions of Hif-1α, VEGF, and SDF-1α. Moreover, owing to the increased local delivery of cells and fast neovascularization within the wound site, cell proliferative activity, granulation tissue formation, and collagen synthesis and deposition were greatly promoted by CPB/EPC constructs resulting in rapid re-epithelialization and regeneration of skin appendages. As a result, the synergistic enhancement of wound healing was observed from CPB/EPC constructs, which suggests the highly efficient delivery of EPCs. CPB/EPC constructs may become highly competitive cell-based therapeutic products for efficient impaired wound healing application. This study may also provide a novel strategy to develop bioactive cell therapy constructs for angiogenesis-related regenerative medicine.

Statement of Significance

This paper reported a highly efficient local delivery of EPCs using bioactive glass-based CPB nanofibrous scaffold for enhancing angiogenesis and wound regeneration. In vitro study showed that CPB can promote the proliferation, migration, and tube formation of EPCs through upregulation of the Hif-1α/VEGF/SDF-1α signaling pathway, indicating that the bioactivity and angiogenic ability of EPCs can be highly maintained and promoted by the CPB scaffold. Moreover, CPB/EPC constructs effectively stimulated the regeneration of diabetic wounds with satisfactory vascularization and better healing outcomes in a full-thickness wound model, suggesting that the highly efficient delivery of EPCs to wound site facilitates angiogenesis and further leads to wound healing. The high angiogenic capacity and excellent healing ability make CPB/EPC constructs highly competitive in cell-based therapeutic products for efficient wound repair application.

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1. Introduction

Skin wound caused by trauma, burns, and chronic diseases remains a great clinical challenge worldwide [1,2]. In general, wound healing is a complex process that is classically divided into three stages including inflammation, proliferation, and remodeling,

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which requires the efforts of multiple cells, growth factors, and extracellular signals [3]. However, owing to several reasons such as ischemia, diabetes, and pressure, chronic wounds usually occur in combination with complications of continuous inflammation, cell dysfunction, and impaired angiogenesis, leading to long-term medical care, high cost, and compromised quality of life in patients with these diseases [4]. The conventional treatment for wounds would be surgical debridement and assistant wound care methods including antibiotics and wound dressings [5]. However, standard surgical treatment with skin substitutes such as autograft, xenograft, or tissue engineered skin has its own disadvantages including limited availability, secondary surgeries, high cost, delayed healing, and fibrosis problem [6]. To overcome these difficulties, cell therapy can be a potential alteration for wound healing [7,8]. Stem cells such as mesenchymal stem cells, endothelial progenitor cells, and epithelial stem cells have been investigated for wound healing applications because of their ability to secrete bioactive factors that can enhance granulation tissue formation, angiogenesis, and reduce inflammation [9–12]. Although problems such as poor engraftment and high rates of cell death often compromise the efficiency of cell-based therapies, stem cells remain as promising approaches for wound healing as they can integrate environmental signals and transduce them into biological factors in wound bed [13]. The endothelial progenitor cell (EPC) is a type of bone marrow mononuclear progenitor cell that acts as endothelial precursor that increase angiogenesis and vascularization by secreting growth factors and cytokines in damaged tissues [14]. EPCs play an important role in vascular regeneration when compared with other stem cells [15]; this is associated with their characteristics such as differentiation into vascular endothelial cells (ECs) and their existence in the vascular wall [16]. Transplantation of EPCs has demonstrated promising results in wound healing [17]. However, the mode of EPC transplantation, such as in situ injection or intravenous injection, cannot achieve the on-site local delivery and will compromise the survival and function of EPCs [8,18]. Therefore, it will be of great significance to fabricate an ideal skin tissue engineering scaffold that is highly bioactive and can provide a temporary residence for maintaining the viability and activating the angiogenic function of EPCs.

To achieve this goal, electrospinning nanofibrous scaffolds with good biocompatibility and high bioactivity can be used as an efficient therapeutic approach for the delivery of EPCs into the wound site [19]. For skin tissue engineering, collagen (Col) fibers are commonly used because they are the main constituent of skin extracellular matrix (ECM), which could create a biomimetic microenvironment for cells to attach and proliferate during wound healing [20]. However, owing to their poor mechanical properties and rapid degradation, collagen fibers should be combined with other materials to obtain a stable and useful scaffold. Various polymer-blended electrospun fibers such as Col/polycaprolactone (PCL), chitosan/PCL, polylactic-co-glycolic acid (PLGA)/Col have been examined for promoting wound healing, yet results are still unsatisfactory owing to their compromised bioactivities and delayed angiogenesis [21–23]. Bioactive glasses (BGs) have shown successful applications both in hard and soft tissue repair because of their high biocompatibility and bioactivity [24–26]. Recently, bioactive glass nanoparticles (BGNs) featured with regular size, large specific surface area, and high bioactivity have presented enhanced cell responses by releasing Si, Ca, and P ions [27–30]. Previous studies showed that BGNs can stimulate the angiogenic ability of human umbilical vein endothelial cells (HUVECs) by upregulating specific angiogenic growth factors such as VEGF and bFGF [31,32] and can efficiently enhance wound healing [27,28]. Moreover, mature bone ECM composed of inorganic apatite nanocrystals and collagen nanofibers has shown a strong capacity for angiogenesis by efficiently mediating cellular differentiation and secreting growth factor [33]. Therefore, it is reasonable to speculate that a BGN-based collagen nanofibrous structure could enhance the delivery efficiency of EPCs and accelerate wound healing.

In this study, we investigated the local delivery performance of EPCs and the mechanism of Col-PCL-BGN (CPB) nanofibrous scaffolds in wound healing. It is hypothesized that the combination of BGNs and collagen could exert a synergistic positive effect as a cell-delivery system on maintaining the viability and activating the angiogenic ability of EPCs and therefore could accelerate wound healing. The regulation and mechanism of CPB scaffolds on EPC behaviors in vitro and wound healing efficiency of the CPB/EPC delivery system *in vivo* were investigated.

2. Materials and methods

2.1. Characterizations of CPB-EPC constructs

The fabrication and characterization of the CPB and CP nanofibrous scaffolds were described in our previous study [34]. The bone marrow-derived EPCs from Sprague Dawley (SD) rats were isolated as described in Supporting Information, and after 1 week of culture, EPCs were characterized by DiI-Ac-LDL/FITC-UEA staining, immunostaining of CD31 and KDR, and the tube formation assay. All EPCs used in this study were cultured in EGM-2MV and in passages 3–5. The CPB/EPC and CP/EPC constructs were obtained by seeding 2×10^4 EPCs on CPB and CP scaffolds and cultured for 24 h. To evaluate the biocompatibility of CPB nanofibrous scaffolds, cell attachment and proliferation morphology were analyzed by SEM and laser scanning confocal microscopy (Olympus, BX61W1-FV1000, Japan). For SEM observation, specimens were fixed using 4% paraformaldehyde for 30 min after 48 h of culture and subsequently dehydrated in increasing concentrations of ethanol (15%-–100% v/v). After drying, the specimens were mounted and coated with gold and observed by SEM (Hitachi H-7500, Japan) at an accelerating voltage of 15 kV. For laser scanning confocal microscopy, F-actin and cell nucleus were stained by phalloidin-FITC and 4′-6-diamidino-2-phenylindole (DAPI), respectively. Briefly, samples were fixed in 4% paraformaldehyde for 30 min and permeabilized with 0.5% Triton X-100 in phosphate buffered saline (PBS) for 10 min, and then incubated with phalloidin-FITC (1:500; Cytoskeleton, Acoma St., Denver, USA) in dark for 1 h. After washing, cell nuclei were stained with DAPI. Slides were maintained in a humidified dark box at 4 °C before observation.

The proliferation of EPCs on different nanofibrous scaffolds was measured by the Cell Counting Kit-8 (CCK-8, Dojindo). Briefly, 100 μL of suspended EPCs at a density of 1×10^5 cells/ml were seeded on the CPB and CP scaffolds, which were previously placed in a 24-well plate (Corning), while cells seeded on the blank cell culture cover slips (Solarbio) were used as the control. After 1 day, 2 days, and 3 days of culture, samples were washed using PBS and added with 200 μL of medium supplemented with 20 μL of CCK-8 reagent, followed by incubating for 1 h. The mixed medium was then transferred into a 96-well plate, and the absorbance was measured at 450 nm wavelength using a microplate reader (Multiskan GO; Thermo Scientific).

A tube formation assay with growth factor-reduced Matrigel (BD Biosciences, US) was also performed to evaluate the effects of CPB scaffolds on angiogenic morphogenesis and tube formation capacity of EPCs. Briefly, the Matrigel solution was thawed at 4 °C overnight and then placed in a μ-Slide (10 μL per well; IBIDI, Germany) in a cell incubator for 30 min for solidification. After co-culturing with CP/CPB for 48 h, a total of 5000 cells per well were detached from the scaffolds using trypsin for 3 min and then seeded in the Matrigel-precoated μ-Slide. EPCs detached from cell

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