Acta Biomaterialia xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Combined chemical and structural signals of biomaterials synergistically activate cell-cell communications for improving tissue regeneration

Yachen Xu [a,c], Jinliang Peng [b], Xin Dong [a,c], Yuhong Xu [b], Haiyan Li [a,c,*], Jiang Chang [c,d,*]

[a] Shanghai Jiao Tong University Affiliated Sixth People's Hospital, School of Biomedical Engineering, Shanghai Jiao Tong University, 1954 Huashan Road, Shanghai 200030, China
[b] School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China
[c] Med-X Research Institute, School of Biomedical Engineering, Shanghai Jiao Tong University, 1954 Huashan Road, Shanghai 200030, China
[d] Shanghai Institute of Ceramics, Chinese Academy of Sciences, 1295 Dingxi Road, Shanghai 200050, China

ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form 24 February 2017
Accepted 31 March 2017
Available online xxxx

Keywords:
Tissue engineering
Chemical signals
Structural signals
Co-cultures
Skin tissue regeneration

ABSTRACT

Biomaterials are only used as carriers of cells in the conventional tissue engineering. Considering the multi-cell environment and active cell-biomaterial interactions in tissue regeneration process, in this study, structural signals of aligned electrospun nanofibers and chemical signals of bioglass (BG) ionic products in cell culture medium are simultaneously applied to activate fibroblast-endothelial co-cultured cells in order to obtain an improved skin tissue engineering construct. Results demonstrate that the combined biomaterial signals synergistically activate fibroblast-endothelial co-culture skin tissue engineering constructs through promotion of paracrine effects and stimulation of gap junctional communication between cells, which results in enhanced vascularization and extracellular matrix protein synthesis in the constructs. Structural signals of aligned electrospun nanofibers play an important role in stimulating both of paracrine and gap junctional communication while chemical signals of BG ionic products mainly enhance paracrine effects. *In vivo* experiments reveal that the activated skin tissue engineering constructs significantly enhance wound healing as compared to control. This study indicates the advantages of synergistic effects between different bioactive signals of biomaterials can be taken to activate communication between different types of cells for obtaining tissue engineering constructs with improved functions.

Statement of Significance

Tissue engineering can regenerate or replace tissue or organs through combining cells, biomaterials and growth factors. Normally, for repairing a specific tissue, only one type of cells, one kind of biomaterials, and specific growth factors are used to support cell growth. In this study, we proposed a novel tissue engineering approach by simply using co-cultured cells and combined biomaterial signals. Using a skin tissue engineering model, we successfully proved that the combined biomaterial signals such as surface nanostructures and bioactive ions could synergistically stimulate the cell-cell communication in co-culture system through paracrine effects and gap junction activation, and regulated expression of growth factors and extracellular matrix proteins, resulting in an activated tissue engineering constructs that significantly enhanced skin regeneration.

1. Introduction

At the beginning of tissue engineering technology, only one type of cells was used to combine with biomaterials in order to reconstruct a specific tissue [1,2]. However, it is known that multiple cell types are often involved in most of tissue regeneration processes, and previous studies have found that cell-cell interactions may significantly affect the tissue regeneration process [3,4]. In addition, another limitation of the conventional tissue engineering approach is that biomaterial scaffolds are only considered as the physical carrier of cells and growth factors, but the potential bioactive effects of biomaterials are ignored. In recent years, the role of biomaterials in tissue engineering has been re-considered, and

* Corresponding authors at: Med-X Research Institute, School of Biomedical Engineering, Shanghai Jiao Tong University, 1954 Huashan Road, Shanghai 200030, China.
E-mail address: jchang@mail.sic.ac.cn (J. Chang).

http://dx.doi.org/10.1016/j.actbio.2017.03.056
1742-7061/

more and more studies have been focusing on the bioactivity of biomaterials to stimulate cell differentiation, growth factor expression and tissue regeneration [5–7]. In particular, some recent studies have demonstrated that both chemical signals such as ions released from biomaterials and structural signals such as surface micro/nano structures have the abilities to stimulate stem cell differentiation and tissue regeneration [8–11]. Among these studies, chemical signals of bioglass (BG), mainly ionic products, have been widely reported to be able to promote matrix synthesis of fibroblasts and angiogenic differentiation of endothelial cells (ECs) [12,13]. In addition, a number of studies have demonstrated that electrospun nanofibers prepared by a mixture of biocompatible polymers, poly (D, L-lactide) (PDLLA) and polycaprolactone (PCL) have shown excellent mechanical strength and stability in cell culture medium [14,15]. Furthermore, the morphology and alignment of PDLLA/PCL electrospun nanofibers, especially aligned arrangement of nanofibers, have profound effects on morphology and behaviors of ECs and fibroblasts, which can significantly stimulate angiogenesis and tissue regeneration [16,17]. However, most studies only investigated the effects of single type of biomaterial signals on behaviors of single type of cells. Although much attention has been paid on cell-cell interaction for tissue engineering, and our previous studies reported the effects of biomaterials as cell carriers on cell-cell interactions in tissue engineering [18,19], the specific roles of different biomaterial signals and the interactions between different biomaterial signals on cell-cell communications during the tissue engineering process has rarely been seriously considered. As angiogenesis is critical for wound healing and interactions between ECs and fibroblasts played important roles in wound healing, in this study, we chose PDLLA/PCL electrospun nanofibers combined with BG to investigate the combinatory effects of the structural and chemical signals on cell-cell interactions of ECs and fibroblasts.

Recent years, significant progress has been made in the development of skin tissue engineering, and the tissue-engineered skin substitutes range from non-cellular polymer scaffolds, such as collagen/chitosan porous scaffolds [20], electrospun poly(lactic acid-co-glycolic acid) scaffolds [21] to non-cellular biological scaffolds, such as small intestinal submucosa [22], extracellular matrix [23] and cellular devices [24]. As for the cellular skin substitutes, cells, including fibroblasts [25], epidermal cells [26], dermal cells [27] and stem cells [28], have been either applied with biodegradable scaffolds or applied as cell sheets without substrates [29]. Although electrospun mats with random nanofibers have been applied as substrates for skin tissue engineering, the effects of electrospun mats with aligned nanofibers have seldom been used for skin regeneration. In addition, the previous studies of skin tissue engineering constructs have rarely discussed the effects of bioactive biomaterials on cell-cell interactions between critical cells involved in wound healing. Furthermore, combination effects of different biomaterial stimulatory effects on cell behaviors or cell-cell interactions involved in wound healing have never been studied, which is important for designing bioactive tissue engineering scaffolds to enhance skin regeneration.

Therefore, in the present study, we proposed a novel skin tissue engineering construct by activating co-cultured cells using combination of two different biomaterial signals. Our hypothesis is that the co-cultured cell system activated by combination of chemical signals such as bioactive ions and structural signals such as aligned nano-fibrous structure may significantly enhance tissue regeneration as compared with traditional tissue engineering approach.

To prove the concept, a co-culture of human dermal fibroblasts (HDFs) and human umbilical vein endothelial cells (HUVECs) was used as the co-culture cell model, and aligned electrospun nanofibers and bioglass ionic products added in cell culture medium were used as structural and chemical stimulatory signals of biomaterials for activating co-cultured cells, respectively. We first proved the effects of combined biomaterial signals on the communications between co-cultured HDFs and HUVECs and analyzed the specific role of each type of signals to elucidate the mechanisms of the activation. Then, we applied HDFs-HUVECs co-cultured skin tissue engineering construct activated by combined stimulatory signals of biomaterials to full-thickness excisions on mouse back and investigated the angiogenesis and protein synthesis in wound area. Our results demonstrated that tissue engineering can be significantly improved through co-cultured cell systems activated by biomaterials, and the design of bioactive materials to optimize synergistic bioactive signals is an effective way to obtain activated tissue engineering constructs for tissue regeneration and wound healing applications.

2. Materials and methods

2.1. Electrospun nanofibrous scaffolds

Poly (D, L-lactide) (PDLLA, Mw = 45 kDa) was purchased from Jinan Daigang Biomaterial Co, Ltd. (Shandong, China). Polycaprolactone (PCL, Mw = 80 kDa) was purchased from Sigma Co. N,N-dimethyl formamide (DMF) and tetrahydrofuran (THF) were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Electrospun nanofibers were prepared according to the literatures [15,30]. Briefly, the blend of PDLLA and PCL with a certain mass ratio (w/w = 50/50) was dissolved in a mixture of DMF and THF (v/v = 4/1), and then stirred for 6 h to obtain a homogeneous and stable solution with polymer concentration of 4.8% (w/v). The flow rate of the solution in the syringe (2 mL) was 0.02 mL/m^{-1} by using a syringe pump (LSP01-1A, Baoding Longer Precision Pump, China). A high voltage power supply (Dongwen, China) was used, and the voltage applied to the needle of the syringe was 8 kV. The distance between the tip of the needle and the collector was 15 cm, and the collecting time was fixed for 1 h. To prepare electrospun scaffolds with random and aligned nanofibers, aluminum foil and high-speed roller (rotating speed = 2000 rpm) were used as collectors, respectively [15,30]. All the experiments were conducted at room temperature and the relative humidity was about 40–60%. All the electrospun scaffolds were vacuum dried for 24 h to completely remove any residual solvent.

Morphology and microstructure of the electrospun scaffolds were observed using a scanning electron microscope (SEM) (S-4800, Hitachi, Japan). Briefly, the scaffolds were cut into 5 mm × 5 mm and attached to the sample stage by conductive adhesive, after gold-plating, the samples were observed and photographed using SEM. Surface wettability of the scaffolds were evaluated by measuring the static water contacting angles (WCA) using a Kruss GmbH DSA 100 Mk 2 goniometer (Hamburg, Germany), followed by image processing of sessile drops using a Data-Physics OCA20 CA system. Water droplets in 3.0 μL were dropped onto the surfaces of the electrospun scaffolds, and the average WCA value was obtained by measuring the water droplets set at 5 randomly distributed positions. Electrospun scaffolds were cut into squares with dimensions of 10 mm × 10 mm and 25 × 25 mm for 24-well plates and 6-well plates, respectively. The obtained scaffold squares were sterilized after being soaked in 75% alcohol for 20 min for further cell culture.

2.2. BG ion extracts

BG powders (over 200 mesh) were kindly provided by Shanghai Institute of Ceramics, Chinese Academy of Science. BG ion extracts were prepared according to the methods reported in literatures adapted from ISO10993-1 procedures [31,32]. Briefly, 1 g of BG

Please cite this article in press as: Y. Xu et al., Combined chemical and structural signals of biomaterials synergistically activate cell-cell communications for improving tissue regeneration, Acta Biomater. (2017), http://dx.doi.org/10.1016/j.actbio.2017.03.056