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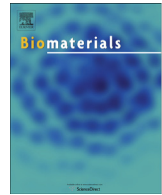

Fibrous scaffolds potentiate the paracrine function of mesenchymal stem cells: A new dimension in cell-material interaction

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ABSTRACT

While the studies on the material interaction with mesenchymal stem cells (MSCs) have been mainly focused on the ability of materials to provide environment to regulate cell viability, proliferation or differentiation, the therapeutic effects of MSC-material constructs may result from the secretion of immunomodulatory and angiogenic cytokines from MSCs. Here, electrospun scaffolds composed of fibers in random, aligned and mesh-like patterns were fabricated, and the paracrine behavior of adipose-derived MSCs (Ad-MSCs) on the scaffolds were investigated in comparison to the cell culture via conventional microplates. It was found that the Ad-MSCs on the electrospun fibers produced significantly higher levels of anti-inflammatory and pro-angiogenic cytokines compared to those cultured on microplates. The enhanced modulatory effects of the secreted products of Ad-MSCs on fibrous electrospun scaffolds were also proven in the cultures of endothelial cells and the LPS-stimulated macrophages, with three types of scaffolds showing distinct influences on the paracrine function of Ad-MSCs. In a skin excisional wound-healing model in rat, the conditioned medium collected from the MSC-scaffold system accelerated the wound closure, promoted the macrophage recruitment and enhanced the polarization of macrophages toward the pro-healing phenotype in the wound bed. Our study demonstrates that the fibrous topography of scaffolds is a key material property that modulates the paracrine function of cells. The discovery elucidates a new aspect of material functions, laying the foundation for developing scaffold materials to promote tissue regeneration/repair through guiding the paracrine signaling network.

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1. Introduction

Mesenchymal stem cells (MSCs) are multipotent adult stem cells capable of differentiating into multiple lineages, including osteoblasts, adipocytes, chondrocytes and myoblasts, under different stimuli and culture conditions [1]. In recent decades, MSCs are one of the most widely investigated cell types in regenerative medicine and have achieved promising therapeutic results in treating graft-versus-host disease, myocardial infarction, cerebral stroke and wound healing etc. [2–4] However, studies have shown that the multi-lineage potential of MSCs may contribute little to their therapeutic effects. Instead, the paracrine products of MSCs could exhibit multifaceted functions including immunomodulation, angiogenesis, anti-apoptosis, anti-scarring, chemoattraction and modulating local stem and progenitor cells [1,5]. In particular, the trophic factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factors (HGF) and basic fibroblast growth factor (bFGF), secreted by MSCs could promote vascularization in the wound area [6,7]. The anti-inflammatory molecules, including indoleamine-2, 3-dioxygenase (IDO), prostaglandin E2 (PGE2) and tumor necrosis factor α (TNF-α)-stimulated gene 6 (TSG-6), would modulate both innate and adaptive immune responses away from scarring but towards regeneration [5,8,9]. Taken together, MSCs hold promise to serve as a vital cellular modulator by sensing the environment and creating an orchestrated network of molecules to promote the tissue repair/regeneration process.

Scaffold is a key component in the concept of tissue engineering, which works as a substrate for cells to attach and grow [10]. With the microstructures, surface morphology/chemistry and mechanical properties, the intriguing function of a scaffold lies in its ability to generate tailored microenvironment that may guide the cell behavior through specific cell-material interactions. Under the assumption that scaffolds may be designed to promote the differentiation of MSCs during cell transplantation to supplement the

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injured tissue with functional cells, numerous studies have been carried out in this aspect to understand/engineer the bioactivity of scaffolds [11–15]. Yet little is known about how scaffolds and the material topographical properties might affect the paracrine function of MSCs, despite the pivotal role of the secretory products of MSCs in mediating the local signaling processes *in vivo*. In this study, we thereby are interested in designing experiments to probe two fundamental questions: 1) how the secretory behavior of MSCs would be altered upon the contact with a fibrous scaffold and 2) whether this new aspect of cell-material interaction has implication for tissue repair/regeneration settings. In particular, we intend to investigate the profile of the pro-angiogenesis and anti-inflammatory factors secreted by MSCs, as these two categories of cytokines may participate in key tissue repair/healing processes and are potent factors that may improve the therapeutic effects of MSCs *in vivo*.

To this end, we here selected electrospun fibers (EFs) to probe the relationship between fibrous microenvironment and the MSC paracrine activity. Because of the advantages such as the convenient fabrication process, the wide choice of materials and precision control over fiber parameters, EFs constitute an important category of scaffold materials that haven been intensively studied in tissue engineering [16–18]. In particular, by mimicking the natural extracellular matrix, the micro-/nano-fibers have been found to provide distinct contact cues to modulate cell activities, including supporting cell proliferation, promoting the stemness and maintaining pluripotency of stem cells [19,20]. In addition, the orientation/alignment of fibers has also been shown to generate topography-induced cues to promote Schwann cell maturation, vascular endothelial cell growth, myotube formation and MSC differentiation [13–15,21,22].

Here, polycaprolactone (PCL) EFs with three different alignment characteristics were fabricated as model scaffold materials. The effects of the fibers on rat adipose-derived MSCs (Ad-MSCs) to produce pro-angiogenic and anti-inflammatory paracrine factors were investigated. The function of these paracrine factors was further studied through collecting conditioned-medium (CM) from different culture systems and applying it to cultures of endothelial cells and macrophages in vitro and a skin wound-healing model *in vivo*. Our study suggests that the fibrous topographical structure has profound effects on the secretory behavior of Ad-MSCs and the cells tend to produce cytokines that are capable of promoting angiogenesis, immunomodulation and tissue healing processes. The discovery on the relationship between cell paracrine function and materials reveals a new aspect of material properties that has not been systematically studied before. The study also suggests the potential approach to modulating of the paracrine signaling network through design of functional scaffolds to improve cell-based regenerative therapy.

2. Materials and methods

2.1. Materials and reagents

Ultrapure water $\geq$18 MΩ was derived from deionized water though a Milli-Q system (Millipore, Billerica, MA). Polycaprolactone (PCL) and lipopolysaccharide (LPS) were obtained from Sigma-Aldrich (Milwaukee, WI). QuantiT™ PicoGreen® dsDNA Assay Kit, TRIzol kit, Dulbecco's phosphate buffered saline (DPBS) and cell culture reagents were purchased from Invitrogen (Carlsbad, CA, USA) unless otherwise specified. Solution Cell Activity Assay (MTS) systems were from Promega (Madison, WI). Matrigel was from BD Biosciences (San Jose, CA, USA). 4′,6-diamidino-2-phenylindole (DAPI) and Rhodamine-Phalloidin were obtained from the Thermo Fisher Scientific (MA, USA). Human umbilical vein endothelial cells (HUVECs), Endothelial cell medium (ECM), endothelial cell growth supplement (ECGS) were purchased from SciEnCell (Carlsbad, CA). Rat adipose-derived mesenchymal stem cells (Ad-MSCs) were purchased from Cyagen Biosciences Inc (Santa Clara, CA, USA). The murine-macrophage cell-line RAW 264.7 were obtained from the Cell Culture Center of the Institute of Basic Medical Sciences (Beijing, China). Suppliers of other chemicals, biological reagents, and equipment were specified below.

2.2. Cell culture

All cells were cultured at 37 °C under humidified atmosphere with 5% CO_2. The Ad-MSCs were cultured in minimum essential medium α (MEM-α) supplemented with 10% fetal bovine serum (FBS), 0.4% penicillin/streptomycin (P/S), without further supplement of growth factors. For the collection of conditioned media, the MSCs were cultured in FBS-free MEM-α for a prescribed period of time. The Ad-MSCs between passage 3 and 5 were used for the following experiments. The human umbilical vein endothelial cells (HUVECs) were maintained in endothelial cell medium (ECM) supplemented with 5% FBS, 1% (P/S) solution and 1% ECGS. RAW264.7 macrophages (MΦ) were cultured in DMEM supplemented with 10% FBS and 1% P/S. To detach cells from the culture plates, Ad-MSCs and HUVECs were treated with trypsin/EDTA solutions, while macrophages were pipetted repeatedly to induce the detachment.

2.3. Fabrication of fibrous scaffolds

Electrospun fibrous scaffolds were prepared from polycaprolactone (PCL) solution. 15% w/v PCL was dissolved in a mixture of $CHCl_3$ and dimethylformamide at the volume ratio of 9:1. After stirring for 3 h until PCL was completely dissolved, 1 mL of the solution was loaded into a syringe with a blunt-ended stainless 21-gauge steel needle. The feeding rate and the applied voltage were controlled at 0.5 mL/h, 12 kV, respectively. The collection distance for random EF (REF) and mesh-like EF (MEF) was 14 cm and for aligned EF (AEF) 10 cm. The REF was collected on a flat plate, the MEF on a copper mesh, and AEF through a rotating cylinder at speed ~3000r/min. The EF membranes were cut into predefined sizes and shapes manually. For sterilization, EF membranes were rinsed with 75% ethanol solution for 30 min and then washed with sterilized DI water for three times and air dried in bio-safety cabinet (Thermo, Germany). The EF scaffolds were exposed under ultraviolet radiation for 30 min on each side in the bio-safety cabinet for further sterilization and were kept in sterile dishes at 4 °C until being used.

2.4. SEM and confocal imaging

Topographical features of the scaffolds were examined by scanning electron microscopy (SEM) (S-4800, Hitachi, Japan). For the measurement of fiber diameter, 300 fibers were randomly selected in the SEM pictures and the diameter was calculated by ImageJ software. To examine the cell morphology on the scaffolds, 2×10^4 Ad-MSCs were seeded on the scaffolds for 24 h. The scaffolds were washed with PBS, and the attached cells were fixed in 2% glutaraldehyde overnight at 4 °C. Samples were then dehydrated by rinsing in ethanol solution with series of concentration (from 30% to 100%, v/v) for 15 min at each step. Once dried, the samples were examined via SEM. For fluorescent confocal imaging, scaffolds seeded with Ad-MSCs were fixed overnight by 2% paraformaldehyde (PFA), and the cells were stained with Rhodamine Phalloidin for 1 h and then DAPI for 5 min. The stained cells were pictured by A1R-si confocal microscope (Nikon, Japan.)

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