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Synergetic effect of topological cue and periodic mechanical tension-stress on osteogenic differentiation of rat bone mesenchymal stem cells

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ABSTRACT

Mesenchymal stem cells (MSCs) are able to self-renew and differentiate into tissues of mesenchymal origin, making them to be significant for cell-based therapies, such as metabolic bone diseases and bone repair. Regulating the differentiation of MSCs is significant for bone regeneration. Electrospun fibers mimicking natural extracellular matrix (ECM), is an effective artificial ECM to regulate the behaviors and fates of MSCs. The aligned electrospun fibers can modulate polar cell pattern of bone mesenchymal stem cells, which leads to more obvious osteogenic differentiation. Apart from the topographic effect of electrospun fibers, mechanical cues can also intervene the cell behaviors. In this study, the osteogenic differentiation of rat bone mesenchymal stem cells was evaluated, which were cultured on aligned/random electrospun fiber mats materials under mechanical tension intervention. Scanning electron microscope and immune-fluorescent staining were used to directly observe the polarity changing of cellular morphology and cytoskeleton. The results proved that aligned electrospun fibers could be more conducive to promote osteogenic differentiation of rat bone mesenchymal stem cells and this promotion of osteogenic differentiation was enhanced by tension intervention. These results were correlated to the quantitative real-time PCR assay. In general, culturing rat bone mesenchymal stem cells on electrospun fibers under the intervention of mechanical tension is an effective way to mimic a more real cellular microenvironment.

1. Introduction

Mesenchymal stem cells (MSCs) with the ability to self-renew and differentiate into bone, cartilage, and other tissues of mesenchymal origin, are the most versatile and promising cell source for bone regenerative medicine, such as cell-based therapies for metabolic bone diseases and bone repair [1]. Regulating the differentiation of MSCs is significant for bone regeneration. Several factors, playing important roles in bone regeneration, have been reported, including morphogenetic signals, responsive host cells, an appropriate carrier of signals and an active cellular microenvironment [2,3]. Considering these factors, building an active cellular micro-environment mimicking the extracellular matrix (ECM) is an effective way to regulating the behaviors and fates of MSCs through the biophysical cues such as topological and mechanical cues from the ECM [4–6]. Artificial ECM with the topographic cue, which can affect the cell adhesion, direct cell migration, induce cell polarization and regulate cellular signal transduction and gene expression [7–11], has gained a lot of attention. For example, topographic micropatterned surface [12–15], surface with micro-/nanoroughness [16] and electrospun fibers [17] have been employed to study the interaction between cells and substrates. Among these artificial ECM, electrospun fibers, with fiber diameters from microscale to nanoscale, high porosity and spatial interconnectivity, are most similar to the fibrous structures of native ECM and well-suitable for nutrient and waste transport and cell communication [18–20]. Additionally, compared to other microfabrication techniques, the process of electrospinning is remarkably simple, robust, facile and cheap [21]. Thus, the electrospinning technique has gained tremendous interest in tissue engineering [22], due to the mentioned advantages of electrospun fibers and the availability to a wide range of raw materials [23–28]. Electrospun micro-/nanofibers have been used to induce the polarity and anisotropy

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of cells through the topographic effect of the micro-/nanofibers, which could have greatly effect on the cell adhesion, proliferation, morphology and fates [29–37]. Furthermore, electrospun fibers would be a good mechanical media to mediate mechanical tension-stress, due to its good mechanical properties [23].

However, in most of the studies, cells are interacting with static artificial ECM, which differs from biological systems. According to previous reports, cells are sensitive to various mechanical forces through tactile mechanisms [38–40] and they can convert the mechanical forces into biological signals [30–34], which result in variations of cellular shapes, functions and fates. For example, simulated microgravity has been demonstrated to inhibit population growth of rat bone marrow mesenchymal stem cells (rBMSCs) and their differentiation towards osteoblasts [41]. While, some researchers also found that within a certain range of tension, the proliferation and osteogenic differentiation of BMSCs would be facilitated [42,43]. Fine bone formation needs the work of mechanical tension, which can modulated cell shape and phenotype, and enhances expression of the mRNA for bone matrix proteins (e.g. bone morphogenetic proteins (BMPs), runt-related transcription factor (Runx), osteopontin (OPN), osteocalcin (OCN), et al.) [44,45].

Therefore, to mimic a more real cellular microenvironment (a dynamically microenvironment), both the topological and mechanical force cues should be considered [46,47].

In present study, we cultured rBMSCs onto aligned or random electrospun fibers under the intervention of periodic tension-stress to investigate the synergistic effect of the topological cue and mechanical force cue on the morphology, cytoskeleton and differentiation of the rBMSCs.

2. Materials and methods

2.1. Materials

Poly (ε-caprolactone) (PCL, Mw $\approx$ 100 kDa) was synthesized as previous report [48]. Briefly, the PCL was synthesized by ring-opening polymerization of ε-caprolactone (ε-CL, Aldrich) monomer using stannous octoate as catalyst. Dimethyl formamide (DMF) and dichloromethane (DCM) were purchased from Kelong Chemical Co. (Chengdu, China) and used as received. Anti-β-actin antibody [AC-15] (FITC) and Rhodamine phalloidin purchased from Abcam CO. (America) were used for labeling cytoskeleton. Real-time PCR primers used in this study were obtained from Life Technologies Corporation-ThermoFisher (America). All other chemicals with reagent grade were obtained from commercial sources and used without further purification. Deionized (DI) water was used in all experiments.

2.2. Isolation and culture of rBMSCs

rBMSCs were obtained from juvenile healthy rat femurs. Firstly femurs were taken out from the rats which were sacrificed by anesthesia, and washed with phosphate buffered saline (PBS) three times. Bone marrows dissected with a syringe were placed onto six-well tissue culture plates. The tissues were cultured with α-Dulbecco's modified Eagle medium (α-DMEM), containing 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/L streptomycin, in a humidified 37 °C and 5% CO_2 incubator.

2.3. Preparation and characterization of polycaprolactone (PCL) electrospun fiber mats

Preweighed PCL was dissolved in mixture of DCM and DMF (DCM: DMF = 3: 1, v/v) to fabricated a 20% w/v homogenous solution. The homogeneous PCL solution was loaded in a 5 mL plastic syringe connected to a stainless needle (inner diameter: 0.7 mm). The syringe was fixed horizontally on a microinjection pump (Longer Pump, LSP02-1B, China). The collector, a grounded drum wrapped with aluminum foil, was placed 15 cm away from the tip of the needle, and a potential (20 kV) was applied between the needle and the collector. Speed of fluid-feeding was kept at 1–1.5 mL/h. According to drum rotation speed, we supposed to gain aligned/random electrospun fiber mats (aligned and random fibers were gained under 2000 rpm and 100 rpm, respectively). The fiber mats were cut into round discs (diameter: 10 mm) or rectangles (80 mm × 40 mm). The direction of aligned fibers were parallel to the long side of the rectangles. The mats were firstly soaked with 75% alcohol and streptomycin solutions alternately, then they were kept dry under low temperature after UV light sterilization. All materials were incubated in culture medium for 24 h before use.

Both of the aligned and random fiber mats were observed with scanning electron microscopy (SEM) (FEI, Quanta 200, Philips, Netherlands). Samples were prepared by coating a thin layer of gold onto the fiber mats. Three randomly selected areas of the samples were recorded in images and used to analyze the diameter distribution and the alignments of the fibers with Image J software (1.46 h, NIH, USA).

2.4. Cell proliferation assay

Cell proliferation assay was carried out by culturing rBMSCs on aligned and random fiber mats in 24-well plates with cell density of 2×10^4 cells/well for 1, 4 and 7 days. Then all three pieces of co-cultured discs for each group were washed three times with PBS. 500 μL DMEM with supplement 40 μL 5 mg/ml MTT solution was added and incubated at 37 °C for 4 h to form MTT formazan. After that, the medium was replaced with 420 μL dimethyl sulfoxide (DMSO) and vibrated for 15 min. Finally, the absorbance was measured at 490 nm by ELX Ultra Microplate Reader (Life Technologies Corporation-ThermoFisher, America).

2.5. Intervention of mechanical tension-stress during cell culture on electrospun fibers

As shown in Fig. 1c, rBMSCs were seeded onto the electrospun fiber mats, which were stuck onto a plate in advance. The plate, which is a part of the four-point-bend loading device, can be bent with stable amplitude as shown in Fig. 1a and a stable tension will occur on the top of the bent plate. After the cells attached well, they were stimulated with stable tension by the four-point-bend loading device. (Deformation $\leq$ 1%, displacement = 2 mm, frequency = 0.5 Hz, Fig. 1). rBMSCs seeded onto the electrospun fiber mats (cell density 2×10^4/well) without stimulation of mechanical tension were also investigated as control.

2.6. Morphology of rBMSCs

Morphologies of rBMSCs, both cultured in the condition with or without stimulation of mechanical stress, were investigated. In the condition without stress stimulating, rBMSCs (cell density of 2×10^4/well) were cultured on both aligned and random electrospun fiber mats in 24-well plates for 4 days in osteogenic medium. In the condition with stress stimulating, rBMSCs (cell density of 2×10^4/well) were cultured on aligned or random electrospun fiber mats stuck to plate for 4 days in osteogenic medium. After incubation, samples from each group were fixed in 3% glutaraldehyde overnight at 4 °C. Then they were washed three times with PBS, and dehydrated with ethanol solutions with different gradient concentrations. After dried, the samples were coated with gold and observed by scanning electron microscopy (SEM) to observe the morphologies of rBMSCs on aligned or random electrospun fibers.