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Cyclic strain dominates over microtopography in regulating cytoskeletal and focal adhesion remodeling of human mesenchymal stem cells

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ABSTRACT

Human bone marrow-derived mesenchymal stem cell (hMSCs) function depends on chemical factors and also on the physical cues of the microenvironmental niche. Here, this physical microenvironment is recapitulated with controlled modes of mechanical strain applied to substrata containing three-dimensional features in order to analyze the effects on cell morphology, focal adhesion distribution, and gene expression. Ten percentage of strain at 1 Hz is delivered for 48 h to hMSCs cultured on flat surfaces, or on substrata with 15 μm-high microtopographic posts spaced 75 μm apart. Adding strain to microtopography produced stable semicircular focal adhesions, and actin spanning from post to post. Strain dominated over microtopography for expression of genes for the cytoskeleton (caldesmon-1 and calponin 3), cell adhesion (integrin-α2, vinculin, and paxillin), and extracellular matrix remodeling (MMP13) ($p < 0.05$). Overall, attention to external mechanical stimuli is necessary for optimizing the stem cell niche for regenerative medicine.

1. Introduction

Stem cells are a major focus of regenerative medicine. Among the different types of stem cells, human bone-marrow-derived mesenchymal stem cells (hMSCs) are an attractive cell source since they differentiate into a variety of cell types, such as osteoblasts, adipocytes, chondrocytes, ligament, and smooth muscle cells. This regulation depends not only on chemical factors but also on the physical cues of the microenvironmental niche, which is defined as a combination of structural and cellular components that vary from one tissue to another to control proliferation and differentiation [1]. Nonetheless, how mechanical parameters in the physical microenvironment affect cellular function is not yet well understood.

Cells remodel in response to changing physical stresses and mechanical loads of the external environment that influence gene expression patterns during development [2–4]. Furthermore, tissues present a three-dimensional (3D) environment to cells. Therefore, in addition to mechanical stimuli to cells, it is desirable to mimic the 3D of the tissue by microtopography. Cellular processes affected by engineered microtopography in culture include cell adhesion, the subcellular cytoskeleton, and differentiation [5]. External perturbation of force occurs *in vivo* with shear stress, extension, or compression [6–8]. Forces generated by motor proteins can reorganize the cytoskeleton in response to external stiffness, surface topography, or ligand density [9,10]. In this study, the effects of the combination of both external cyclic strain and the impact of 3D microtopography on cells are studied on cytoskeletal organization, focal adhesions, and gene expression.

2. Materials and methods

2.1. Fabrication of microtopographic substrata

The ratio of curing agent to silicon elastomer base was 1:10, resulting in a Young's modulus of approximately 1.7 MPa. Liquid polydimethyl-siloxane (PDMS) was spread over the BioFlex plates, and the parylene mold was placed on top of the PDMS layer, which was about 1 mm thick, cured, and gently removed, resulting in flat or textured elastomeric membranes in the flex dishes. The fabrication of the parylene microtopography molds has been previously reported [11]. Flat two-dimensional sheets of PDMS were used as a control for the same surface properties. As a result, PDMS 15-μm-high and 15-μm diameter circular posts were created on top of the BioFlex plates in tetragonal array spaced 75-μm center to center [12], then coated with laminin at a concentration of 10 μg/mL for 1 h. In order to assess the uniformity of laminin distribution on the flat and microtopographic substrata, anti-laminin antibody produced in rabbit (Sigma) was incubated over night at a dilution of 1:25, and followed by the secondary antibody Alexa Fluor 568 conjugated goat-anti-rabbit antibody (Invitrogen) at a

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dilution of 1:200 for 30 min. All flat textured surfaces were uniformly coated with laminin as seen by confocal microscopy (Fig. 1A and B).

2.2. Cell culture

Institutional approval was received to obtain and use hMSCs isolated from human bone marrow aspirates (Texas A&M Health Science Center College of Medicine Temple, TX). Microarray analyses indicate that gene expression is consistent for hMSCs from different donors, isolated and expanded as described previously [13]. Experiments were performed on passage three or lower from hMSCs obtained from 3 separate donors. hMSCs were cultured in complete culture media (CCM) consisting of MEM-α supplemented with 16.5% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin, and incubated at 37 °C.

2.3. Cyclic strain

After two days of cell culture, hMSCs were cyclically strained with 10% strain at 1 Hz for 48 h in cell culture media using the Flexcell Strain Unit (Model FX-4000, Flexercell International, McKeesport, PA). The base plate with a diameter of 25 mm was used to produce equibiaxial strain for the majority of the area. The computer system controlled the frequency of deformation and the negative pressure applied to the culture plates. There are four conditions in this study: Flat (control group), flat-strain, post, and post-strain. All the four experiments were done under similar culture conditions.

2.4. Distribution of cells upon plating by time lapse imaging

In order to determine the initial distribution of hMSCs on the flat or microtopographic substrata, time lapse movies were recorded using the Olympus VivaView soon after plating and followed over the next 12 h. A frame is recorded every 5 min and played back 2300 times faster in the movies, see Supplement.

2.5. Actin, focal adhesion, and nuclear staining

In order to analyze subcellular features, cells were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for 10 min at room temperature, rinsed three times with PBS and permeabilized by 0.1% Triton X-100 in PBS for 10 min, and washed 3 times with PBS. Cells were pre-incubated in blocking solution (PBS, 1% bovine serum albumin (BSA)) for 15 min and then incubated with rhodamine conjugated phalloidin (Molecular Probes) at a dilution of 1:400 to stain actin, or paxillin anti-rabbit antibody (Abcam) at a dilution of 1:250 for 1.5 h followed by another incubation with secondary antibody Alexa Fluor 488 conjugated goat-anti-chicken antibody (Invitrogen) at a dilution of 1:1000 for 45 min to stain the focal adhesions of the cells. DAPI (Sigma) was used for nuclear staining. Confocal images of actin and focal adhesions were obtained with Zeiss LSM 510 META and LSM 710 microscopes.

2.6. Actin and nuclear distribution from post

Actin distribution was measured morphometrically and the frequency of distribution calculated as a function of the distance from the post. The location of actin and nuclei as a function of distance was determined at 7.5 μm intervals away from a post, the presence of actin was tallied by detectable phalloidin staining at the intervals. For nuclear distribution, the region less than 37.5 μm from center of the post was considered as the "close" region, and beyond that was called the "far" region. In all, over 60 posts in 3 samples were pooled for frequency distribution and statistical analysis.

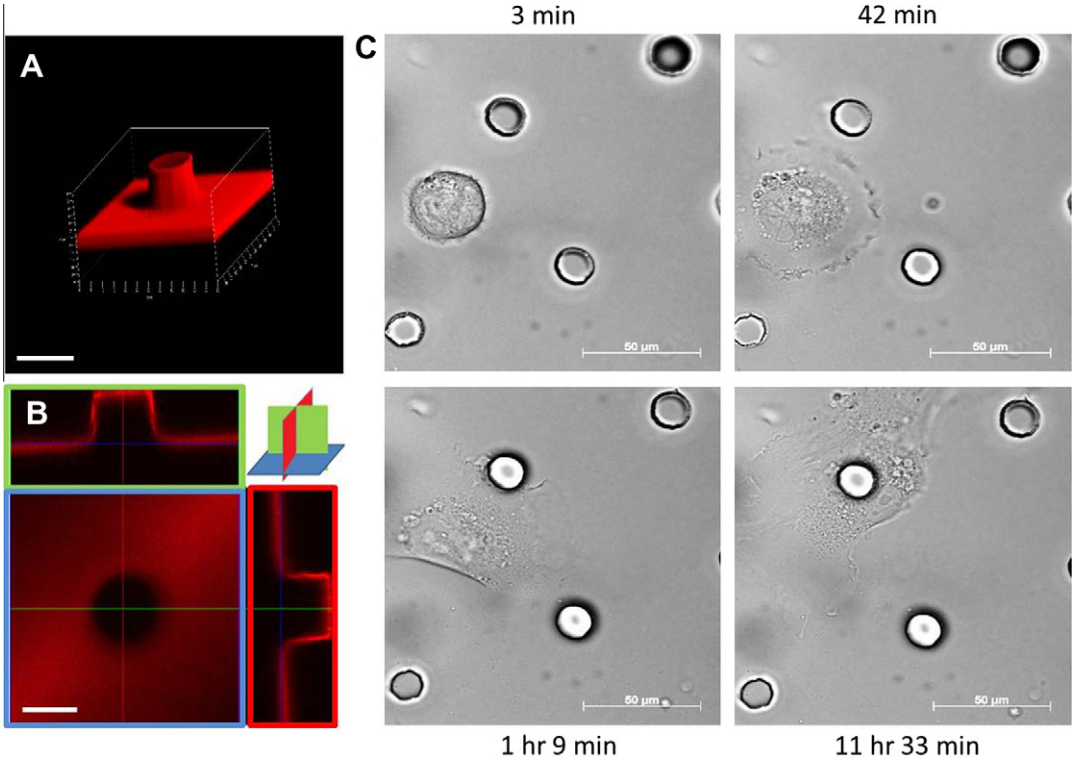

Fig. 1. Confocal images of post and cell interaction: (A) 3D image of a post 15 μm high, and 15 μm diameter with laminin coating. Scale bar, 20 μm. (B) Uniform laminin staining on flat base and sides of the post seen by confocal microscopy. Scale bar, 20 μm. (C) Time lapse images show initial migration and preferential adhesion of a cell to a post during the following 12 h. Scale bar, 50 μm.