Osteogenesis, adipogenesis, and myogenesis of rat mesenchymal stem cells on the nanogrooved surfaces

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Statement of Purpose: Interfaces between engineered materials and stem cells play a critical role in biomedical applications where the interaction between cells and material surface dictates cell performance and the success of an implanted device. The extracellular matrix (ECM) imparts a wealth of biochemical and biomechanical cues, of which the latter can be presented in the form of nanotopography. The effects by which nanotopography with different feature sizes and surface chemistry can direct stem cell differentiation remains unclear. Previous studies have showed that nanogrooved surfaces changed the cell morphology, cytoskeletal organization, integrin expression, cellular mechanical properties, and differentiation of stem cells [1-3]. In this study, the osteogenesis and adipogenesis of rat bone marrow-derived mesenchymal stem cells, rbMSCs, on the nanogrooved surface was investigated. Interactive effects of various feature sizes and surface chemistry of culturing matrix on the MSCs differentiation was studied. The interactive effect of surface topography and surface chemistry was also examined. Various hydrophilicity, functional groups, and bioadhesive peptides were conjugated on the grooved surface and flat control. Understanding the effects of how these biochemical and biomechanical cues influence cell behaviours would be valuable for optimization of stem cell differentiation and the design of tissue engineering scaffolds.

Methods: The rectangular nanogrooves/ridges patterned Si wafer was fabricated by both electron beam lithography and dry etching techniques. PDMS replicate was obtained via soft lithography. Patterned polystyrene (PS) substrates were imprinted by PDMS master on PET slide. Regarding the surface chemistry effects, the surfaces of PS were modified by covalent-binding with bioactive peptide such as RGD. Rat mesenchymal stem cells (MSCs) were harvested from femur and tibia. Passage 3 MSCs were used in this study. Cell numbers were determined by a lactate dehydrogenase (LDH) assay at each time point. Alkaline phosphatase (ALP) activity was assayed by determining the release of PNP from *p*-nitrophenyl phosphate at pH 10. Gene expression of ALP was analyzed and GAPDH was used as a normalized gene. The samples were stained by Alizarin Red and Oil Red for osteogenic and adipogenic samples, respectively. The samples were examined for calcium deposition by use of a calcium kit assay. Arsenazo III reacts with calcium in an acid solution to form a blue-purple complex. The color developed has a maximum absorbance at 650 nm and is proportional to the calcium concentration in the sample. Triacylglycerols was quantified using isopropanol dissolved oil drop under OD 490 nm.

Results: Apparently, the orientation and morphology of MSCs was affected by the nanogrooved topography. MSCs aligned with the direction of the nanogrooves which is dominated by the depth of grooves and appeared random distribution on the flat control and TCPS. Due to the topography confined the laterally migration of MSCs, there were a direction of bone nodules-like colonies which is roughly perpendicular to the nanogrooves rather than the normal circular shape. On the other hand, cell migration may not crucial for adpogenesis. Adipogenic MSCs did not form the specific shape and produced lip droplet around the single cells on the patterned and flat surface. ALP staining and activity showed that osteogenic MSCs on the flat surface have the highest ALP activity. However, gene expression of ALP showed that ALP on the grooved surfaces expressed earlier than that on the flat surface. Calcium deposition of osteogenic MSCs showed that cells produced more calcium on the deep groove surfaces than shallow and flat surface after 3 weeks. Taken together, the results indicated that topography feature trigger the ALP expression of osteogenic MSCs earlier than those cells on the flat surface. It might be also the reason why the calcium deposition on the deep groove surfaces was higher than that on the shallow groove surfaces, while the ALP activity was no significant differences. For adipogenesis, triacylglycerols of adipogenic MSCs showed that cells produced more triacylglycerols on wide groove and flat surfaces than narrow groove surfaces after 3 weeks. The cellular behaviours was not only changed by topographic features, but also affected by surface chemistry. The collective effects were both important for MSCs differentiation.

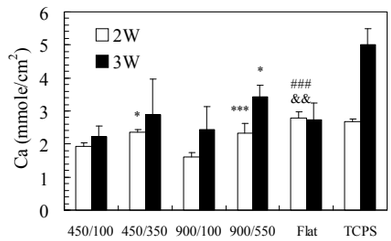

Fig 1. Calcium deposition of osteogenic MSCs after 3 wk. * compared with the same width surface. # compared with the shallow surfaces. & compared with the deep surfaces. (n = 4)

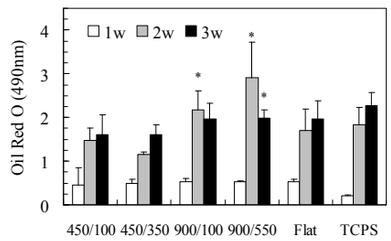

Fig 2. Triacylglycerols of adipogenic MSCs after 3 wk. * compared with the same depth surfaces at the same time point. (n = 4)

Conclusions: Both topography and surface chemistry affected stem cell alignment, migration, and differentiation. Surface topography mainly guided cells migration and morphology, while surface chemistry mainly affected cell differentiation. This study provides the fundamental information for the biomaterials and tissue engineered scaffolds design.

References: [1] EKF Yim, Biomaterials. 2010, 31, 1299. [2] E Martinez, Ann Anat. 2009, 191, 126. [3] MJ Dalby, Biomaterials. 2006, 27, 1306.