INTRODUCTION:

Cells (in particular osteoprogenitor and bone cells) are highly responsive to mechanical stimuli for the renewal and remodeling of bone. As such, the employment of mechanical stimulations (e.g. uniaxial and biaxial stretch, fluid shear, and hydrostatic compression) has been applied to cells to enhance osteogenic differentiation for bone repair and remodeling. It is well-accepted that the magnitudes of applied strain, frequency, cell cycle number and duration of applied strain have an influence in cell response. Several material matrices have been investigated as potential cell carriers for proliferation and differentiation for bone applications. For instance, nanofibers are popular options as they resemble native extracellular matrix (ECM) and its high area-to-volume ratio promotes protein adsorption, thereby enhancing cell adhesion. The objective of this study is to investigate the effects of mechanical stimulation (cyclic strain) on human bone marrow derived mesenchymal stem cells (BM-MSCs) differentiation cultured on nanofibrous scaffolds and cast films. The hypothesis is that the degree of strain experienced by the BM-MSCs is dependent on the material surface-cell adhesion, where the substrate affects the transmission of strain to the adhered cells.

METHODS:

Poly(L-lactic) acid (PLLA, 300,000Da) (Polysciences Inc, U.S.) and Type I collagen (Col) (Koken, Japan), with a weight ratio of 80:20 were dissolved in 1,1,1,3,3-hexafluoro-2-propanol (HFP) solvent, with a weight-to-volume ratio of 3% and magnetically stirred overnight. Electrospinning was employed at room temperature (RT) and relative humidity of 65% where the electrospinning parameters were as follows: voltage-15 kV, feed rate-1mL/hr, and tip-to-collector distance-12cm. Fibers were collected and aligned along the rotating direction of the wheel, at 1000 RPM. For solvent casting, PLLA/Col (80:20) was dissolved in HFP, with a weight-to-volume ratio of 1.5% and stirred overnight. Cast films were prepared by casting the polymer blend onto a flat Teflon sheet and kept for 48 hrs at RT. The films were then peeled off. All substrates (40x10mm) were clamped to the stretching device where the effective area was 20x10mm. BM-MSCs (Lanza Walkersville Inc.) were cultured in expansion media consisting of low glucose Dulbecco’s modified Eagle’s medium (DMEM), 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (PS) (all from Invitrogen, U.S) and 2.5x10^6 cells were seeded on the substrates and incubated to constant uniaxial cyclic strain of 1%, at a frequency of 1Hz for 1 day in media consisting of minimum essential medium (αMEM) (Invitrogen, U.S), 10% FBS, 1% PS and 10^-8 mol/L dexamethasone (Sigma-Aldrich, U.S.). Media was changed every 4-5 days. Alkaline phosphatase (ALP), activity was assessed using the Phosphatase Substrate Kit (PIERCE, U.S), and absorbance at 405nm was measured in a FLUO Star Optima microplate reader (BMG Labtech GmbH, Germany).

RESULTS:

ALP, an early osteogenic differentiation marker, was significantly lowered in strained cast films than unstrained cast films in week 4. ALP was significantly enhanced when strain was applied to aligned nanofibers in week 1 but decreased in week 4. Moreover, ALP activity was significantly increased in stained aligned nanofibers than strained cast films in week 1 but was lower in week 4.

Fig. 1: ALP expression of BM-MSC on (a) strained and unstrained cast films and (b) strained and unstrained aligned * denotes significant difference (p<0.05) between material groups

Discussion:

Osteopontin, another medium-late osteogenic differentiation marker, was detected on all substrates after week 4 as seen in Fig. 2, but under strain conditions, greater osteopontin expression (higher intensity of staining) was seen on stained cast films and aligned nanofibers.

Fig. 2: Osteopontin expression after 4 weeks of culture. (A): Unstrained cast film, (B): Strained cast films, (C): Unstrained aligned nanofibers, and (D): Strained aligned nanofibers. Blue stains denote cell nuclei and red stains denote osteopontin expression

DISCUSSION:

Cyclic strain conditions enhanced ALP expression in the short term but inhibited the activity in the long run on aligned nanofibers. This was in good attainment with some reports, where ALP was elevated in the short term (a few days to a week) under mechanical stimulation, although long term characterization was not done. The variation in ALP activity could be seen in various reports (whether mechanical stimulations had a positive or negative modulation on osteogenic differentiation) could be due to several factors such as the duration of mechanical stimulus (continuous or intermittent), type of substrates (2D or 3D matrices) and material surface-cell adhesion. Strained aligned nanofibers had greater ALP activity than stained cast films in week 1, suggestive that better cell adhesion was on aligned nanofibers than cast films. Thus substrates play a role in strain transmission to the cells, thereby affecting ALP. Lastly, mechanical stimulation had a positive influence on osteopontin expression, which was consistent with other reports. In summary, when there was better adhesion on MSCs on the material surfaces, the strain transmission improved owing to the uniqueness of nanotopography of aligned nanofibers, stimulating osteogenic differentiation in the short term.