Magnetic field triggered osteogenic differentiation of human mesenchymal stem cells on ferromagnetic substrates

Sunil Kumar Boda[1], Greeshma Thrivikraman[3], Bikramjit Basu[1,2]
[1]Laboratory for Biomaterials, Materials Research Centre, Indian Institute of Science, Bangalore - 560012, India
[2]Interdisciplinary Bio-Engineering Program, Indian Institute of Science, Bangalore - 560012, India
[3]Centre for Nanoscience and Engineering, Indian Institute of Science, Bangalore - 560012, India

Statement of Purpose:
The modulation of cell response in terms of cell proliferation and differentiation by the application of physical and mechanical forces, with minimal presence or absence of growth factors is one of the major objectives of stem cell based tissue engineering [1]. Among the multiple physical cues for manipulating stem cell behavior, the potential of magnetic stimuli in eliciting a desired cell behavior is still unperceived. In the present study, the fabrication, characterization and *in vitro* response of human mesenchymal stem cells (hMSCs) on hydroxyapatite (HA) - magnetite (Fe_3O_4) composites is presented. Further, the cell cultures on the magnetic substrates were periodically stimulated with 100 mT static magnetic field (SMF) and the cell viability, proliferation and differentiation were recorded.

Methods:
For the study, a graded series of magnetic composites as HA-xFe_3O_4 composites (x = 0, 5, 10, 20 and 40 wt%) were prepared by hot-pressing at 950°C in argon atmosphere. The composites were characterized for phase assemblage rietveld refinement of X-ray diffraction data, Mössbauer spectroscopy and X-ray photoelectron spectroscopy (XPS) while the magnetic properties were quantified by vibrating sample magnetometry (VSM). *In vitro* cell culture studies with hMSCs were performed on such graded series of magnetic samples using hMSCs along with regular application of SMF for 30 min on alternate days. The cell behaviours were characterized at 7, 14, 21 and 28 days time intervals.

Results:
The phase analysis revealed a major retention of magnetite (Fe_3O_4) in all the composites with minor amounts of maghemite (γ-Fe_2O_3), wustite ($Fe_{1-x}O$) and Fe doped HA. The saturation and remnant magnetizations of the composites were proportionate to the amount of Fe_3O_4 retained. In the *in vitro* cell culture studies, SMF stimulation moderately decreased hMSC viability on less magnetic substrates. However, flow cytometry analysis suggested the proliferation arrest of hMSCs on ferromagnetic substrates triggering their differentiation. Further, SMF exposure led to higher intracellular calcium levels in cells cultured on substrates with higher magnetization. The early osteogenic markers (Runx2, ALP and Col IA) evaluated after 7 and 14 days of SMF stimulated culture were upregulated w.r.t control. Fig. 1 corresponds to Runx2 expression, a nuclear transcriptional factor after immunofluorescent staining of the cells on HA-40% Fe3O4 composite under SMF culture. Furthermore, a marked increase in the gene expression of late osteogenic markers (OCN and OPN) after 14 and 21 day of SMF culture was observed only in the ferromagnetic compositions. The mineralization of hMSCs at 28 days time interval was in commensurate with the earlier recorded ALP activity and Osteocalcin (ELISA) levels. In summary, the osteogenic differentiation of hMSCs was enhanced by SMF stimulation on weak and strongly ferromagnetic composites. Such non-invasive stimulation protocols can be adopted to promote stem cell differentiation in the absence of growth factors/ chemical inducers.

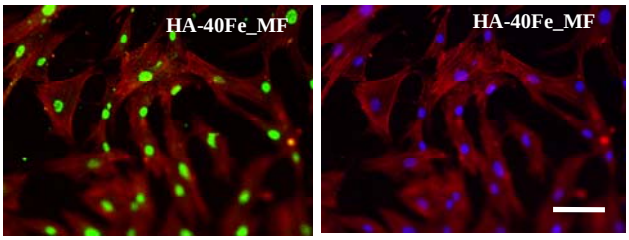

Fig. 1: Runx2 expression in the HA-40% Fe_3O_4 composite after 5 days of SMF stimulated culture. The green nuclei (left image) correspond to Runx2 expression detected by indirect immunofluorescence (2°Ab labeled with FITC). The blue nuclei (right image) are due to DAPI staining.

Conclusions:
The doping of iron into hydroxyapatite (HA) to few hundred ppm, similar to that of teeth/ bone [2] presents the HA-Fe_3O_4 composites as potential orthopaedic materials. The application of periodic magnetic stimuli to trigger the osteogenesis of hMSCs on ferromagnetic bone replacement materials can be promising as tissue engineering based strategy to enhance bone healing/repair.

References:
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[2] Jiang M, Terra J, Rossi AM, Morales MA, Baggio Saitovitch EM, Ellis DE, *Physical Review B*, 2002, **66**, 224107.