Direct and Indirect Osteogenic Differentiation of Human Mesenchymal Stem Cells by Surface Microtopography and Surface Energy

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Statement of Purpose: Studies examining bone formation around Ti implants in vivo have shown that surfaces with micron and submicron scale roughness support greater bone to implant contact than smooth surfaces. In vitro studies have found that osteoblast-like cells exhibit a more differentiated phenotype on surfaces with these micron and submicron scale topographies. However, in vivo, the cells that initially attach to the implant surface are mesenchymal stem cells and progenitor cells (MSCs), and these cells must then differentiate into osteoblasts. Not only are the direct effects of substrate microstructure on the differentiation of MSCs not well understood, but peri-implant osteogenesis also depends on the osteogenic differentiation of cells in the surrounding bone that are not adherent to the implant surface. The aim of the present study was to determine if MSCs are sensitive to surface roughness and surface free energy and whether MSC differentiation is modulated by factors produced by osteoblasts grown on these surfaces using a co-culture system.

Methods: Human MSCs (Lonza) were seeded at 5000 cells/cm^2 on tissue culture polystyrene (TCPS) or on Ti microstructured substrates with different surface topographies and surface chemistries. Smooth Ti surfaces (PT, Ra=0.5+0.2 μm) were grit blasted and acid etched resulting in a mixed microtopography (SLA, Ra= 4.0+0.1 μm); SLA surfaces fabricated under nitrogen resulting in a surface with identical topography but high surface energy (modSLA). At confluence, cell number was determined. Alkaline phosphatase specific activity and osteocalcin (OCN) were used as measures of osteoblast differentiation and levels of osteoprotegerin (OPG) and vascular endothelial growth factor-A (VEGF-A) were used as indicators of soluble factor production. To determine if factors produced by osteoblasts on the material surface could modulate MSC behavior, a co-culture system was established. Briefly, MG63 cells were grown for 6 days on tissue culture glass, PT, SLA and modSLA disks in 24 well plates, resulting in confluent cultures. On day 6, these disks were transferred to a 6-well culture insert and co-cultured with previously plated MSCs on tissue culture polystyrene (TCPS, plastic) for an additional 12 days. At harvest, MSC number and phenotype were examined. Data were calculated as means $\pm$ SEM for N=6 independent cultures for each variable. Statistical significance was determined using ANOVA followed by Bonferroni's modification of Student's t-test.

Results: MSCs grown directly on the disks exhibited reduced cell number on the surfaces with higher roughness (SLA and modSLA). The greatest decrease in cell number was on the modSLA disks, indicating a role for surface energy. Surface roughness positively correlated with alkaline phosphatase activity and the levels of OCN, OPG and VEGF in the conditioned media. Surface energy was an important variable as the greatest increases in these parameters were in cells cultured on modSLA. The factors produced by osteoblasts on Ti microstructured and hydrophilic surfaces affected MSC differentiation on TCPS. MSCs in the co-culture system exhibited surface dependent decreases in cell number as well as increases in alkaline phosphatase specific activity (Fig 1A) and production of OPG (Fig 1B) and VEGF.

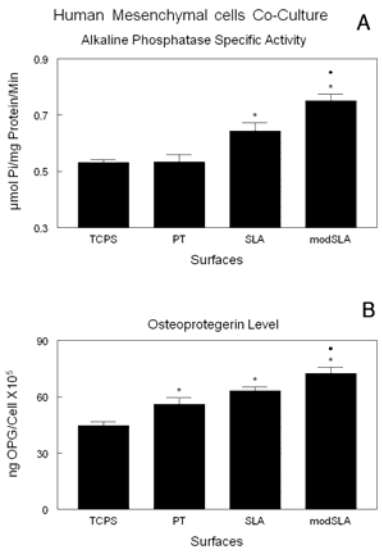

Figure 1: The effect of co-cultured MG63 cells grown on different substrates on alkaline phosphatase specific activity (A) and osteoprotegerin levels (B) in human MSCs.

Discussion: The results indicate that the surface microtopography and surface free energy had a direct effect on MSC growth and differentiation, as well as on the production of soluble factors that act in a paracrine manner. This suggests that the regulation of MSC differentiation into the osteoblast lineage as part of the osseointegration process is modulated by implant surface characteristics. The data also indicate that the peri-implant bone bed is modulated by implant materials, which regulate osteointegration in two ways: by inducing osteoblastic differentiation of MSCs distal to the implant surface and by controlling bone remodeling through specific local factors.

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