Designed Topographies Promote Osteogenic Performance of Medical Devices

Materiomics has identified surface topographies to promote osteogenic performance of medical devices. Titanium-coated substrates were cultured with human mesenchymal stem cells (hMSCs) and ALP expression levels were quantified as measure for their osteogenic potential. ALP expression levels in hMSCs nearly doubled with substrates featuring Materiomics' uniquely designed topographies compared to polished (flat) surfaces, and even more than doubled compared to the laboratory standard. These effects are equal to, or even stronger, than adding the gold standard osteogenic stimulating compound dexamethasone. This effect is not elicited by a change in chemical make-up or a coating/secondary material, but by introducing our unique surface topography (designed roughness) into the surface of the core material. Materiomics' unique and proprietary topographies bring excellent opportunities: incorporation into dental and orthopedic devices improves functionality and performance significantly.

Background

Defined surface topographies can, when featured on the surface of a medical device, steer the osseointegration of that device. To analyze the effect of topographies on such osteogenic properties, the production of alkaline phosphatase (ALP, an early marker of osteogenic differentiation) can be studied. The expression of this marker on the surface of human mesenchymal stromal cells (hMSCs) is quantified using flow cytometry, a technology that is routinely applied for the simultaneous measurement of multiple physical characteristics of cells, as well as the characterization of their surface proteins by fluorescently-labeled specific antibodies.

Materials & Methods

To validate promising osteogenic topographies identified with Materiomics' unique TopoChip platform, a flow cytometric analysis of hMSCs cultivated on selected high- (osteogenesis stimulating) and low-scoring (osteogenesis inhibiting) topographies was performed. This assay was done using Titanium (Ti)-coated Polylactic acid substrates (1.8 cm^2) featuring the individual topographies. hMSCs were seeded at a density of 3500 cells/cm^2 and cultured for 5 days in basic hMSC medium without osteogenesis-stimulating compounds. As a control to the patterned surfaces, cells were also cultivated on nonpatterned (flat) Ti-coated PLA substrates (Low 1) as well as on nonpatterned tissue culture polystyrene (TCPS) surfaces in basic hMSC medium (negative control, TCPS-neg). TCPS supplemented with dexamethasone, the gold standard *in vitro* osteo-stimulant, was included as positive control (TCPS-pos). The medium was refreshed every other day.

Following the cell culture period, the cells were harvested and labeled with an anti-human ALP antibody and a Phycoerythrin (PE)-labeled specific secondary antibody. Flow cytometry was performed immediately after staining using a FACSCalibur and its CellQuest software (BD Biosciences).

Results

Materiomics' topographies strongly influence ALP expression levels. Figure 1 shows the mean percentage of ALP-positive cells, where ALP-positive cells are defined as those cells expressing surface ALP at a higher median fluorescence intensity relative to the specific isotype control. Biological triplicates per surface topography are plotted as individual data points in the graph. The mean percentage of ALP-expressing hMSCs cultured on the positive (high-scoring) surfaces is around 1.6 fold higher compared to those cultured on the negative (low-scoring) surfaces, and up to 2.2 fold higher than those levels observed on nonpatterned TCPS in basic medium without dexamethasone, the laboratory standard material.

The nonpatterned Ti-coated substrate is among the negative surfaces on which the cells show ALP expression levels of about 1.3 fold higher than those levels observed on nonpatterned TCPS in basic medium without dexamethasone. This effect illustrates the importance of using appropriate culture substrates in translational medicine and for medical devices.

Most importantly, the high-scoring surface topographies were able to induce ALP expression levels in hMSCs comparable to those achieved by adding dexamethasone to basic hMSC culture medium, the gold standard in cell culture for inducing osteogenesis.

The stimulation of ALP levels by, and therewith the osteogenic potential of, these uniquely designed topographies are highly significant and are further explored for their *in vivo* performance (see white paper: 'Designed Topographies Equal Osteogenic Performance Clinical Benchmark').

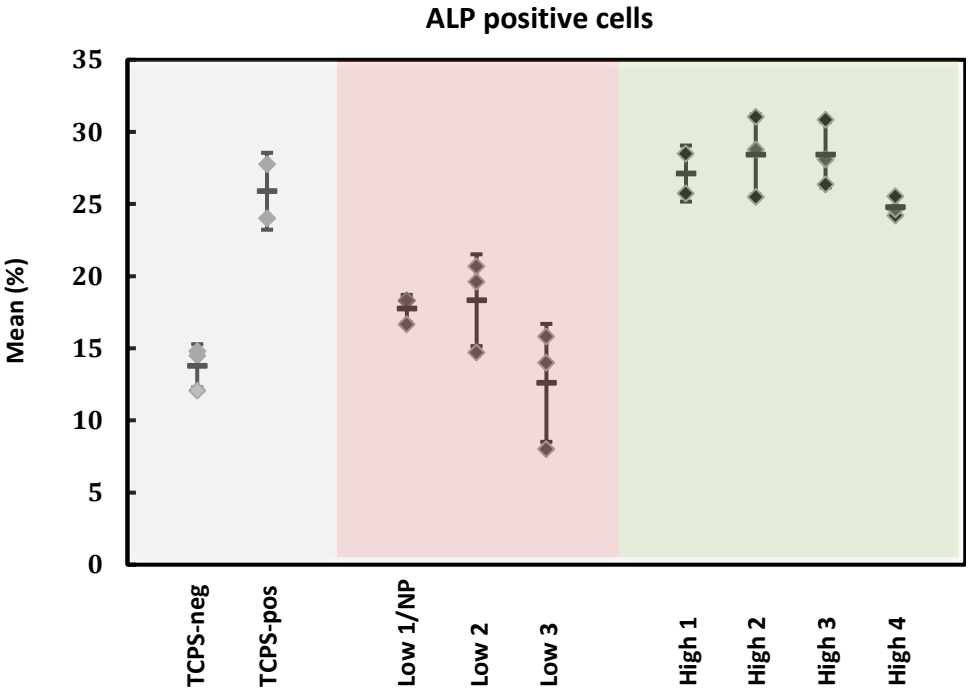

Figure 1. Topography-featuring substrates stimulate osteogenesis equal to and higher than the gold standard compound. Validation of high- and low-scoring surface topographies identified for osteogenesis using flow cytometry.