Effect of Bone Remodeling Coupling Factors on Osteogenic Differentiation of Human Mesenchymal Stem Cells Induced by Microstructured Titanium Surfaces

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Statement of Purpose: Osseointegration is a structural and functional contact between a load-bearing implant and living bone, which ensures the success of dental and orthopaedic implants. A critical stage in osseointegration is primary bone remodeling. It requires the balance in the coupling system between bone formation and bone resorption. Understanding the coupling mechanisms underlying bone remodeling and investigating the coupling factors involved will allow us to improve the osseointegration and develop better dental or orthopaedic implants. A new class of molecules has been recently reported to act as coupling factors in remodeling—the semaphorins. This project is focused on Sema4D, which has been reported to inhibit bone formation without affecting osteoclastogenesis during normal bone remodeling, as well as Sema3C, which has been less studies in the context of bone. Our lab previously found that Sema3C mRNA expression is upregulated in osteoblasts. Ti implants with rougher and/or hydrophilic surfaces can induce osteogenic differentiation of human mesenchymal stem cells (hMSCs) or other osteoblast progenitor cells. Therefore, our goal was to determine the effect of semaphorins on osteogenic differentiation of hMSCs induced by microstructured Ti implant surfaces.

Methods: Institut Straumann AG (Basel, Switzerland) provided 15mm diameter grade 2 Ti disks modified to be smooth/hydrophobic (PT), or rough/hydrophilic (modSLA). Modifications create differences in microroughness (PT[SA=1.10μm], modSLA[SA=3.52μM]), contact angles (PT [θCA = 93°], modSLA [θCA = 0°]), and reduced carbon content on modSLA compared to PT. Prior to use, disks were sterilized with 25-kGy gamma irradiation. HMSCs were cultured on PT or modSLA with tissue culture polystyrene (TCPS) as a control in hMSC cell culture media. After seven days, hMSCs were treated with 1μg/ml Sema3C and 1μg/ml Sema4D separately for 24hs. After the treatment, conditioned media were collected for testing the production of osteocalcin (OCN), osteoprotegerin (OPG), vascular endothelial growth factor (VEGF), osteopontin (OPN), and bone morphogenetic protein-2 (BMP2) by ELISA. Endogenous production of SEMA3C was tested by ELISA on media from the non-treated control group. Cell layer lysates were used for quantification of DNA and alkaline phosphatase activity (ALP).

Results: Production of osteoblast differentiation markers was increased in a surface-dependent manner (modSLA>PT>TCPS) and the amount of DNA and ALP activity were decreased. Cells treated with Sema4D and Sema3C for all surfaces showed decreased production of BMP2, OPN, OCN and VEGF. Cells on modSLA treated with both semaphorins exhibited a statistically higher levels of these markers compared to other groups. While less than in untreated cultures, production of osteoblast differentiation markers in semaphorin treated cultures was increased in a surface-dependent manner (modSLA>PT>TCPS). Treatment with Sema3C and Sema4D caused a marked increase in DNA on all surfaces compared to untreated cells, but DNA and ALP activity were decreased with rougher surfaces.

Conclusion: Our results indicate that SEMA3C and SEMA4D inhibit osteogenic differentiation of hMSCs, demonstrated by lower production of most osteoblast differentiation markers. Both SEMA4D and SEMA3C increased DNA and decreased alkaline phosphatase activity compared to control groups, suggesting the cells are retained in a proliferative state. Importantly, neither factor abrogated the influence of the surface, indicating a balance between surface activation and inhibition by Sema4D and 3C. Sema3C is an important molecule in the regulation of osseointegration process.

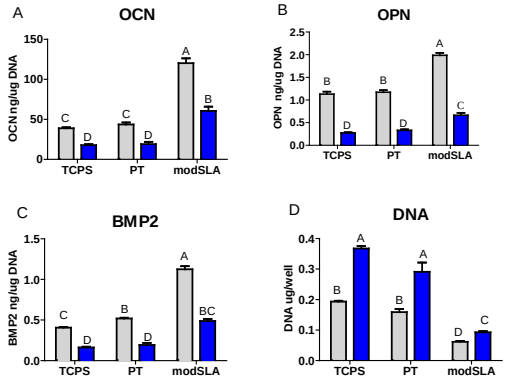

Figure 1. hMSCs response to Sema3C on different surfaces. A. OCN; B. OPN; C. BMP2; D. DNA. Groups not sharing letters are significant at $p<0.05$.