Regulation of Bone Remodeling by Microstructured Titanium Surfaces via Semaphorin 3C

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Statement of Purpose: Bone remodeling is a critical stage of osseointegration that requires interaction between osteoblasts and osteoclasts and efficient blood vessel formation to deliver nutrients and cells. Previously, our lab demonstrated that microstructured titanium surfaces can promote osteogenesis, regulate osteoclast activity, and influence angiogenesis. However, the mechanisms underneath this regulation remain unclear. In addition, our lab previously showed that the production of semaphorin 3C (sema3C) by bone marrow stromal cells (MSCs) was sensitive to surface properties. Therefore, the purpose of this study is to investigate the role of sema3C in Ti implant surface-mediated bone formation, resorption, and angiogenesis during the bone remodeling stage.

Methods: Institut Straumann AG (Basel, Switzerland) provided 15mm diameter grade 2 Ti disks modified to be smooth/hydrophobic (PT), microrough/hydrophobic by large grit sand-blasting and acid etching (SLA), or microrough/hydrophilic by performing the SLA modification in a N_2 environment with subsequent storage in 0.9% NaCl solution (modSLA). MSCs were cultured on TCPS and titanium surfaces for seven days. On day 7, MSCs were treated with 1 μg/ml sema3C for 24 hours. Then media were collected for testing production of osteocalcin (OCN), osteoprotegerin (OPG), vascular endothelial growth factor (VEGF), and bone morphogenetic protein-2 (BMP2) by ELISA, and cell layer lysates were prepared for DNA quantification. Osteoclasts were differentiated from RAW264.7 cells under 100 ng/ml RANKL treatment from day 1 to day 5. Untreated RAW264.7 cells served as undifferentiated control. On day 3, recombinant sema3C (0.2, 2, 20, 200, and 2000 ng/ml) was added to differentiating RAW264.7 cells for two days. On day 5, the effect of sema3C on osteoclast differentiation was assessed by PCR of NFATc1, TRAP, and CTSK expression. For Ti surfaces' effect on osteoclasts: MSCs were cultured on titanium surfaces for seven days. On day 7, conditioned media were collected and mixed 1:1 with RAW264.7 growth media (DMEM FM 4.5 g/L glucose). Differentiating RAW264.7 cells were treated with the conditioned media supplemented with RANKL for two days, and osteoclast differentiation was assessed by PCR. For sema3C direct treatment on angiogenesis: Human umbilical endothelial cells (HUVECs) were cultured in a Geltrex coated 96 well plate and treated with sema3C (500, 1000, and 2000 ng/ml). The wells were imaged after 12h and 24h of treatment, and ImageJ quantified the number of junctions and total length of tubes. For Ti implant surfaces' effect on HUVECs: Media collected from MSCs on surfaces on day 7 were mixed with HUVECs growth media (2:1). MSCs growth media (CCM) were mixed with HUVECs growth media (2:1) and served as MCSs media control. HUVECs were treated with conditioned media for 12h and 24h. Sema3C production in the MSCs conditioned media was quantified by sema3C ELISA and normalized to DNA in the cell layer lysates.

Results: For the effect of sema3C on surface-regulated bone formation: Sema3C had no effect on MSC osteoblast differentiation on titanium surfaces, as indicated by no change in OCN, BMP2, OPN, OPG, and VEGF production. For the effect of sema3C on surface-modulated bone resorption: After RANKL treatment, RAW264.7 cells differentiated into osteoclasts, as indicated by increased expression of NFATc1, TRAP, and CTSK. To further compare surface effects on osteoclast differentiation, PT, SLA, and modSLA results were normalized to TCPS. SLA and modSLA inhibited NFATc1, TRAP, and CTSK expression more than PT, while there was no difference between SLA and modSLA. Ti surfaces regulated sema3C production in a surface-dependent manner. SLA and modSLA had a higher concentration of sema3C in the conditioned media than PT and TCPS. The results above indicated that sema3C might be involved in the inhibitory effect of microrough Ti surfaces on osteoclasts. When osteoclasts were treated with sema3C, 0.2 ng/ml sema3C decreased NFATc1 expression, while NFATc1 expression was not affected by sema3C at 2, 20, 200, and 2000 ng/ml. Compared to the 0 ng/ml sema3C, 0.2 ng/ml sema3C inhibited TRAP expression while 200 ng/ml sema3C increased it. Sema3C inhibited CTSK expression at 0.2 ng/ml and recovered its inhibitory effect at 200 ng/ml. The findings indicated that sema3C plays a role in osteoclast activity mediated by the surface. For the effect of sema3C in surface-mediated angiogenesis: Conditioned media from TCPS, PT, and SLA increased the number of junctions and total length compared to CCM at 12h. At the same time, modSLA did not affect angiogenesis at 12h. At 24h, TCPS, PT, and SLA did not affect the number of junctions but enhanced the total length. Interestingly, modSLA enhanced angiogenesis by increasing total length at 24h compared to CCM. Sema3C at 500 and 1000 ng/ml inhibited HUVEC angiogenesis, as indicated by fewer junctions and total length at 12h, and 2000 ng/ml sema3C also inhibited total length at 12h. However, sema3C did not affect angiogenesis at 24h at any concentration. Combining these findings, the increased concentration of sema3C in modSLA-conditioned media may compensate for modSLA's effect on early angiogenesis.

Conclusions: Microstructured titanium surfaces regulated MSCs to create a microenvironment inhibiting osteoclasts and promoting angiogenesis to achieve efficient bone remodeling. Additionally, sema3C production was surface-dependent. It directly inhibited osteoclast differentiation and angiogenesis, supporting the hypothesis that sema3C is involved in bone remodeling by affecting osteoclasts and endothelial cells with no effect on bone formation.