# Osteoblast Response to Surface Microtopography is Modulated by Caveolin-1

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**INTRODUCTION**: Engineering orthopaedic implant surfaces to increase the rate and extent of bone growth requires a better understanding of the underlying mechanisms involved in the response of osteoblasts to surface microtopography and surface chemistry. Anchorage dependent cells like osteoblasts interact with proteins on the surface via receptors called integrins. We and others have found that integrin expression is surface dependent [1], suggesting that changes in integrin signaling mediate osteoblast responses to their substrate.

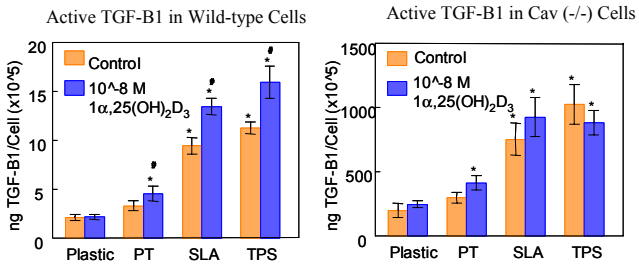
Recent studies indicate that integrins can localize and that $\alpha_2\beta_1$ integrin clustering can activate caveolae-mediated endocytosis in a PKCα-dependent manner [2]. The PKCα-signaling pathway is also used by the vitamin D metabolite $1\alpha,25(OH)_2D_3$, and intact caveolae are required for $1\alpha,25(OH)_2D_3$-dependent activation of PKC [3]. Cross-talk between the two pathways may contribute to synergistic effects of rough surface microarchitecture and $1\alpha,25(OH)_2D_3$ on osteoblastic differentiation.

Caveolae are cholesterol-rich specialized regions of the plasma membrane. Caveolin-1 is the major scaffolding protein present in caveolae, providing structural support for organizing multiple components of membrane-associated signaling pathways. In addition, both the traditional nuclear vitamin D receptor (VDR) [4] and the membrane $1\alpha,25(OH)_2D_3$ receptor ERp60 [3] have been reported to be in caveolae. These studies suggest that caveolae and caveolin-1 might provide a site for synergy between surface dependent integrin signaling and signaling initiated by the vitamin D metabolite. To test this hypothesis, we took advantage of a caveolin-1 knockout mouse model [Cav-1(-/-)] in which the cells lack caveolae. For the experiments described below, osteoblasts were isolated from the calvaria of these mice and their response to surface microarchitecture and $1\alpha,25(OH)_2D_3$ was compared to that of wild-type mice.

**METHODS**: Osteoblasts were isolated enzymatically from the calvaria of nine 8-week old Cav-1(-/-) and wild-type C57BL/6 mice. Cells from the first digestion were discarded, but cells from the second and third digestion were collected and centrifuged to pellet the osteoblasts. Cells were then washed and resuspended in DMEM containing 10% FBS and 1% antibiotics. The osteoblasts were then plated at 20,000 cells/cm$^2$. At second passage, the osteoblasts were plated in 24-well plates containing 15mm diameter Ti disks of different surface roughness (Institut Straumann AG, Basel, Switzerland). Surface profilometry showed that smooth Ti surfaces (PT) had an Ra of 0.5 ± 0.3 μm, grit blasted and acid etched surfaces (SLA) had an Ra of 4.0 ± 0.1 μm, and plasma sprayed (TPS) surfaces had an Ra of 5.2 ± 0.3 μm. Confluent cultures were treated with $10^{-8}$ M $1\alpha,25(OH)_2D_3$ for 24 hours. Cell number and alkaline phosphatase specific activity were measured and media levels of osteocalcin, latent and active TGF-$\beta_1$, and $PGE_2$ were assessed using commercially available immunoassays.

Each variable was tested using six independent cultures. Data were analyzed by ANOVA and significant differences between groups were determined using Bonferroni's modification of Student's t-test ($P<0.05$). All experiments were repeated to ensure validity.

**RESULTS**: Both Cav-1(-/-) and wild-type osteoblasts exhibited a reduction in cell number on SLA and TPS surfaces. $1\alpha,25(OH)_2D_3$ caused a further reduction in cultures of wild-type osteoblasts but had no effect on the Cav-1(-/-) osteoblasts. Both cell types exhibited an increase in cellular alkaline phosphatase specific activity that was surface roughness dependent. $1\alpha,25(OH)_2D_3$ caused a further stimulation in wild-type osteoblasts cultured on SLA and TPS. In contrast, treatment of the Cav-1(-/-) cells with $1\alpha,25(OH)_2D_3$ caused a decrease in enzyme activity in all cultures. Cav-1(-/-) cells had higher levels of both osteocalcin and TGF-β1 in their conditioned media than was found in wild-type cultures. Osteocalcin was increased in both Cav-1(-/-) and wild-type cultures grown on SLA and TPS and $1\alpha,25(OH)_2D_3$ caused a further increase. Wild-type cells exhibited $1\alpha,25(OH)_2D_3$ dependent increases in latent and active TGF-β1 in cultures grown on SLA and TPS. However, $1\alpha,25(OH)_2D_3$ had no effect on TGF-β1 in the Cav-1(-/-) cultures. In contrast to the TGF-β1 levels, $PGE_2$ was higher in conditioned media of wild-type cells. Moreover, $1\alpha,25(OH)_2D_3$ increased $PGE_2$ in all cultures grown on Ti, including those cultures grown on PT.




**DISCUSSION**: The Cav-1(-/-) cells exhibited anticipated surface dependent decreases in cell number and increases in markers of osteoblastic differentiation and local factor production. This suggests that caveolae-dependent integrin signaling is not a requirement. However, levels of both osteocalcin and TGF-β1 were higher in Cav-1(-/-) cells compared to wild-type cells, while levels of $PGE_2$ were lower, suggesting differential regulation of these proteins at a basal level in Cav-1(-/-) cells. Response to $1\alpha,25(OH)_2D_3$ was markedly affected by lack of caveolin-1. While $1\alpha,25(OH)_2D_3$ increased osteocalcin levels and $PGE_2$ levels in the Cav-1(-/-) cells, it had no effect on cell number or TGF-β1 and it inhibited alkaline phosphatase activity. Overall, our results suggest that caveolae are involved in osteoblast response to the surface and these changes alter the response to $1\alpha,25(OH)_2D_3$. In addition, biological responses to $1\alpha,25(OH)_2D_3$ are mediated by multiple pathways, only some of which are dependent on caveolae.

**REFERENCES**: (1) Raz P et al., J Biomed Mater Res A 2004; 71:217-25; (2)Upla P et al., Mol Biol Cell 2004; 15: 625-36; (3) Boyan BD, et al., Steroids 2005, in press; (4) Huhtakangas JA et al., Mol Endocrinol 2004; 11:2660-71.

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