# Integrin Alpha-2 Silencing Alters Osteoblast Response to Titanium Microtopography but Not Surface Energy

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**Statement of Purpose:** Titanium surface microtopography modulates phenotypic maturation of osteoblast-like cells, production of local regulatory factors such as TGF-β1, $PGE_2$, and osteoprotegerin (OPG), and response of osteoblasts to bone anabolic agents such as 1,25 dihydroxyvitamin D3 [1,25] (1,2). Marked surface-dependent changes in cell morphology suggest that integrin-mediated signaling may play a role. Osteoblasts interact with their substrate via integrin binding to proteins absorbed on the surface and to extracellular matrix proteins via several classes of integrin heterodimers including alpha-2/beta-1. Expression of the alpha-2 integrin is increased in osteoblasts grown on Ti surfaces with rough microtopographies and 1,25 modulates expression of its partner beta-1 on these same substrates (3). We previously showed that silencing the beta-1 subunit alters osteoblast response to substrate microtopography and 1,25 (4), but beta-1 can also partner with other integrin subunits, including alpha-5. The goal of our study was to determine if alpha-2/beta-1 was specifically responsible for the surface-dependent changes in osteoblast response. Our hypothesis for this study was that the silencing of the alpha-2 integrin subunit will alter the behavior of MG-63 cells cultured on titanium surfaces in a manner dependent on substrate microtopography but not on surface energy.

**Methods:** MG63 osteoblast-like cells were grown on tissue culture plastic or on Ti substrates with different surface topographies and surface chemistries. Smooth Ti surfaces (PT) had a Ra of 0.5±0.2μm. PT surfaces were grit blasted and acid etched resulting in a mixed microtopography (SLA) with an overall Ra of 4.0±0.1μm. ModSLA surfaces were fabricated using the same procedure to produce the same topography as SLA surfaces. To reduce surface contamination, modSLA surfaces were rinsed under nitrogen, preventing contact with the atmosphere, and then stored in a sealed glass tube containing isotonic NaCl, thereby preserving high surface energy (5).

Alpha-2 siRNA targeted 21 bases starting at base 3406 of the alpha-2 gene (NM-002203.3). The double strand oligonucleotides were constructed in a pSupressorNeo vector containing a U6 promotor (5). MG63 cells were transfected with plasmids containing the alpha-2 siRNA template. Silencing was assessed by Western blot analysis. A permanent cell line exhibiting reduced alpha-2 expression was established. Confluent cultures on the test surfaces were treated with 1,25 for 24 hours.

**Results/Discussion:** siRNA decreased alpha-2 protein by 70%. Cellular alkaline phosphatase specific activity and levels of osteocalcin, $PGE_2$, OPG and TGF-beta-1 in the conditioned media of normal cells were increased on the Ti surfaces with rough microtopographies (modSLA>SLA>PT>plastic). The response to 1,25 also increased the enzyme activity and production of these proteins; these effects were greatest on modSLA. Alpha-2 siRNA had no effect on control cultures grown on plastic or PT, but it blocked the stimulatory effects of 1,25. In contrast, the siRNA decreased alkaline phosphatase and osteocalcin (Figure 1), $PGE_2$, OPG and TGF-beta-1 production on SLA and modSLA and blocked the effects of 1,25. No differences were evident as a function of surface energy.

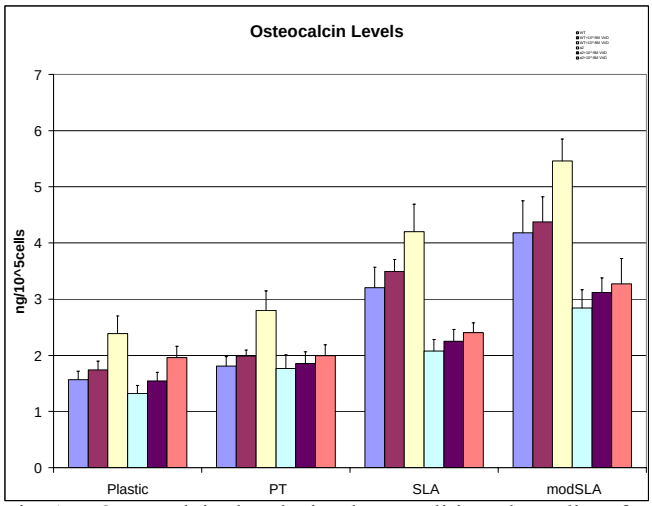


Fig 1. Osteocalcin levels in the conditioned media of MG63 cells grown on tissue culture polystyrene (plastic) or Ti substrates with smooth (PT) or rough surfaces (SLA and modSLA). For each surface: normal MG63 cells (1), MG63 cells treated for 24 h with $10^{-9}$ M $1\alpha,25(OH)_2D_3$ (2), MG63 cells treated with $10^{-8}$ M 1,25 (3), silenced MG63 cells (4), silenced cells treated with $10^{-9}$ M 1,25 (5), and silenced cells treated with $10^{-9}$ M 1,25. Data are means ± SEM for N=6 independent cultures. $P<0.05$, MG63 cells grown on SLA and modSLA v. all other groups; $10^{-8}$ M 1,25 v. control for normal MG63 on all surfaces and for silenced MG63 on plastic.

**Conclusions:** The results indicate that osteoblastic differentiation depends on alpha-2 signaling, and alpha-2 silencing compromised the osteogenic effect of microtopography. Alkaline phosphatase activity, osteocalcin, $PGE_2$, OPG and TGF-beta-1 were reduced, indicating that osteoblastic differentiation and autocrine/paracrine regulation were affected. Although $1\alpha,25(OH)_2D_3$ increased the responses of the silenced cells on rough surfaces, $1\alpha,25(OH)_2D_3$'s effects were not as robust as the effects of surface topography alone on normal cells. This supports our previous studies showing that the synergistic effect of surface roughness and $1\alpha,25(OH)_2D_3$ is mediated by the same signaling pathways. The behavior of silenced cells on SLA and modSLA was comparable, suggesting that the role of alpha-2/beta-1 is to detect surface structure rather than surface chemistry.

**References:** 1. Martin JY et al., J Biomed Mat Res 29:389-401, 1995; 2. Raz P et al., J Biomed Mat Res 71(2):217-25, 2004; 3. Wang L et al., Biomaterials 27: 3716-25, 2006; 4. Paul CP et al. Nature Biotechnol 20:505-8, 2002; 5. Zhao G et al., J Biomed Mater Res 74:49-58, 2005.

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