Role of Integrin Subunits in Osteoblast Maturation on Microstructured Surfaces Varies with Surface Chemistries

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Introduction: Surface roughness, topography, and energy promote mesenchymal stem cell differentiation, osteoblast maturation, and increase production of osteogenic local factors in vitro and increase bone-to-implant contact in vivo. Cells on rougher titanium (Ti) surfaces express a different integrin profile, notably increased levels of α1, α2, αV, and β1, than cells on smoother substrates, which mediates the effects of the surface on osteoblasts and mesenchymal stem cells. Studies using surfaces with complex microstructured topography formed by grit blasting and acid etching (SLA) and SLA surfaces that have retained their hydrophilic character (modSLA) indicate that osteoblast response depends not only on surface topography but also on surface energy. Although modSLA substrates are more hydrophilic than SLA, both substrates are TiO2 and cells on these surfaces undergo differentiation mediated by α2β1. Surface chemistry also affects osteoblast behavior, but the mechanism is less understood. The aim of this study was to decouple the effects of surface chemistry and structure on osteoblast maturation and integrin expression using smooth Ti (PT) and SLA substrates coated with nanometer films of graphitic carbon, thereby retaining the surface roughness and topography but altering surface chemistry.

Methods: Ti surfaces (PT [Ra<0.4µm], SLA [Ra≥3.4µm]) were magnetron sputter-coated with an ultrapure graphite target, producing a thin graphitic carbon film (aC), and characterized by SEM and water contact angle. MG63 cells were grown to confluence on tissue culture polystyrene (TCPS), PT, aC-PT, SLA, or aC-SLA surfaces. Real-time qPCR was performed to evaluate integrin expression. In a second experiment, wild type MG63 cells, integrin β1-silenced MG63 cells (shITGB1), integrin α1-silenced MG63 cells (shITGA1), integrin α2-silenced MG63 cells (shITGA2), or integrin αV-silenced MG63 cells (shITGAV) were cultured on TCPS or surfaces. Cell number, alkaline phosphatase specific activity (ALP), osteocalcin (OCN), osteoprotegerin (OPG), vascular endothelial growth factor (VEGF), and transforming growth factor beta 1 (TGF-β1) levels were determined at confluence. Data are mean ± SEM of n=6 independent cultures/variable. Statistical significance was determined by ANOVA with post-hoc Bonferroni’s Student’s t-test.

Results: Graphitic carbon films were deposited for 5 min at a target current of 0.4 A, yielding a film thickness of approximately 150 nm. Microstructure of PT and SLA surfaces was preserved after graphitic carbon coating. SEM showed films were uniform with no evidence of detachment. Graphitic carbon films increased surface energy, as determined by decreased contact angle. MG63 cells had higher expression of ITGA1, ITGA2, ITGAV, and ITGB1 on SLA and SLA-aC in comparison to TCPS or PT. ITGA1 and ITGAV were higher in cells on SLA-aC than on SLA. Cell number decreased as surface roughness increased on both Ti and Ti-aC surfaces. Markers of osteoblast maturation (ALP in the cell lysate, and secreted OCN) and secreted local factors (OPG and TGF-β1) were higher on all microstructured surfaces than on TCPS, with no significant difference between the surfaces with or without aC.

shITGB1 cells increased cell number on all surfaces in comparison to wild-type MG63 cells. ALP, OCN, OPG, VEGF, and TGF-β1 were lower in shITGB1 cells than in wild-type MG63 cells on all surfaces. shITGA1 cells had higher cell number on Ti surfaces in comparison to wild-type MG63; however, cell number of shITGA1 on Ti-aC surfaces was similar to wild-type MG63 cells. ALP, OCN, OPG, and TGF-β1 were lower on microstructured Ti surfaces in the shITGA1 cells in comparison to wild-type MG63 cells, but on Ti-aC surfaces shITGA1 showed similar behavior as wild-type cells. shITGA2 cells had higher cell number on Ti surfaces and similar cell numbers on Ti-aC surfaces when compared to wild-type MG63 cells. ALP, OCN, OPG, and TGF-β1 in the shITGA2 cells was dramatically lower on microstructured Ti surfaces when compared to wild-type MG63 cells; however, shITGA2 cells demonstrated the same behavior on Ti-aC surfaces as wild-type cells. Finally, shITGAV cells had similar cell number, OCN, OPG, and TGF-β1 levels on Ti surfaces when compared to wild-type MG63 cells. In contrast, shITGAV had lower cell number on PT-aC, but higher in SLA-aC in comparison with the wild-type MG63 cells. Similarly, ALP, OCN, OPG, and TGF-β1 were higher in shITGAV on PT-aC and lower on SLA-aC than wild-type MG63.

Discussion: This study examined the effect of surface chemistry on osteoblast maturation using titanium and graphitic carbon-coated surfaces with the same roughness and topography. Integrin β1-silenced MG63 cells had less differentiation on rough surfaces independent of surface chemistry, suggesting a major role of integrin β1 in roughness recognition. Interestingly, silencing integrin α1 and α2 affected osteoblast maturation on titanium surfaces, but not on carbon-coated surfaces. Integrin αV was the only subunit we examined that affected osteoblast maturation on carbon-coated substrates. Taken together, this study...
suggests that integrin alpha subunits play a major role in surface chemistry recognition.

**Significance:** While rough titanium implants osseointegrate and induce osteogenic differentiation, the mechanisms by which this occurs are unclear. The current study highlights the importance of surface chemistry in differentiation, and demonstrates the contribution of specific integrin subunits in mediating process.

**Acknowledgments:** USPHS Grant AR052102, CONACYT #152995, UNAM, Institut Straumann

**References:**

Figure 1. Effect of silencing integrin beta 1 (A) or integrin alpha v (B) on MG63 response to surface microstructure and surface chemistry. *p<0.05, v. TCPS; %p<0.05, v. PT; $p<0.05$, graphitic-coated v. Ti; # p<0.05, v. WT.

Biomaterials. 2010 Apr;31(10):2728-35.