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

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Statement of Purpose: Surface properties such as roughness, topography, and energy promote osteoblast differentiation and increase osteogenic local factor production in vitro and bone-to-implant contact in vivo. Cells on rougher titanium (Ti) surfaces express a different integrin profile than cells on smoother substrates, notably exhibiting increased levels of α1, α2, αV, and β1. Surface chemistry also affects osteoblast behavior, but the mechanism is less understood. Studies using complex grit blasted/acid etched surfaces (SLA) and SLA surfaces, which have retained their hydrophilic character, indicate that osteoblast response is sensitive to chemistry in addition to surface topography. Although modified SLA is more hydrophilic than SLA, both substrates are TiO2 and increased differentiation on both substrates is mediated by α2β1. The purpose of the present study was to separate the effects of surface chemistry and surface structure on integrin expression by coating machined Ti substrates (PT) and SLA with amorphous carbon, thereby retaining the surface morphology but altering surface chemistry.

Methods: Ti surfaces (PT [Ra<0.4μm], SLA [Ra≥3.4μm]) were sputter-coated using a magnetron sputtering system with an ultrapure graphite target, producing a graphitic carbon thin film (aC), modifying surface chemistry but not roughness. MG63 cells were grown until confluence on Ti or Ti-aC surfaces. RNA extraction and real-time qPCR was performed to evaluate integrin subunit 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 tissue culture polystyrene (TCPS), Ti, or Ti-aC surfaces. At confluence, cells were harvested and cell number, alkaline phosphatase specific activity (ALP), osteocalcin (OCN), osteoprotegerin (OPG), VEGF, and TGF-β1 levels were determined. Data represent mean±SEM for n=6 cultures per variable. Statistical significance was determined using ANOVA followed by Bonferroni's modification of Student's t-test.

Results: The aC films were deposited at a target current of 0.4 A for 5 min, yielding a film thickness around 100 nm. Graphitic carbon films had no significant effect on surface energy, as determined by contact angle. PT and SLA surfaces conserved the original microstructure after graphitic carbon coating (Ti-aC). The thin film was uniform and no evidence of film detachment was found using SEM. MG63 cells upregulated expression of ITGA1, ITGA2, ITGAV, and ITGB1 on SLA and SLA-aC in comparison to TCPS or PT. ITGA1 and ITGAV were upregulated in SLA-aC in comparison to SLA. Cell number decreased as surface roughness increased on Ti and Ti-aC surfaces in MG63 cells. Markers of osteoblast maturation including ALP in the cell lysate, and secreted OCN, OPG, and TGF-β1 increased on all microstructured surfaces with no significant difference between Ti and Ti-aC. shITGB1 cells increased cell number on all surfaces in comparison to wild-type MG63 cells. ALP, OCN, OPG, VEGF, and TGF-β1 levels were lower in shITGB1 cells than in wild-type MG63 cells on all surfaces. shITGA1 cells showed higher cell number on Ti surfaces in comparison to wild-type MG63; however, cell number 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 increased cell number on Ti surfaces and had 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 showed the same behavior as wild-type cells on Ti-aC surfaces. Finally, shITGAV cells showed similar cell number, OCN, OPG, and TGF-β1 levels on Ti surfaces when compared to wild-type MG63 cells. In contrast, shITGAV cultured on Ti-aC showed 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 increased in PT-aC, and decreased in SLA-aC in comparison with wild-type MG63.

Conclusions: This study examined the effect of surface chemistry on osteoblast maturation using substrates with the same roughness. Lack of osteoblast differentiation in integrin β1-silenced MG63 cells on surfaces with similar roughness but different chemistries suggests a major role of the integrin β1 subunit in roughness recognition. Interestingly, integrin α1 and α2 silencing affected osteoblast maturation on titanium surfaces, but failed to affect osteoblast behavior on carbon-coated surfaces. Integrin αv silencing was the only subunit that affected osteoblast maturation on carbon-coated substrates. These results suggest that integrin alpha subunits play a major role in the surface chemistry recognition.

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