Osteoblasts Require Both Micron Scale and Submicron Scale Surface Structure for Synergy with Surface Energy

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Statement of Purpose: Biomaterial surfaces with complicated micron and submicron topography have been shown to increase osteoblast differentiation and result in better osseointegration both in animal studies and clinically. Recent studies show that high surface energy promotes osteoblast differentiation and expedites bone growth when cells are cultured on Ti substrates with complex micron scale and submicron scale surface structures.[1] However, it is not known whether the synergy in response is primarily a function of high surface energy or it is also depends on specific architectural element. It is important to understand the relationship between surface roughness and surface energy to develop better biomaterials for implantation.

Titanium (Ti) is a widely used model material for studying cell substrate interaction because of its good biocompatibility and clinical relevance. Ti spontaneously forms an outmost layer of TiO_2, which presents high surface energy. However, during production, transportation and storage, the biomaterial surfaces are immediately contaminated with hydrocarbons and CO_2 through contact with atmosphere. These adsorbed carbon contaminations lower the surface energy and potentially impair cell substrate interactions. By reducing the exposure of the surface to atmospheric contamination, the high surface energy can be preserved. This has been accomplished by a new method of production that maintains Ti implants in a hydrated state.

The object of this study was to investigate the relationship between surface roughness and surface energy in determining osteoblast response to Ti. The experiments tested the hypothesis is that the synergistic effects of Ti microtopography and surface energy are due to the micron-scale features of the substrate surface.

Methods: Ti disks (15 mm diameter) were supplied by Institut Straumann AG (Basel, Switzerland). Three different surface roughnesses were prepared. The smooth pretreatment (PT) surface had an R_a of 0.60 ± 0.02 μm; the acid etched (A) surface had a submicron R_a of 0.83 ± 0.05 μm; the coarse grit blasted and acid etched (SLA) surface had an overall R_a of 3.97 ± 0.04 μm and consisted of 100 μm diameter craters with an overlay of 1-3 μm diameter pits. The surface free energy of PT, A and SLA are 28.41, 10.33 and 4.28mN/m, respectively, calculated using the equation of state approach.[2] Modified A (modA) and modified SLA (modSLA) surfaces were manufactured with the same methods as A and SLA. To reduce surface contamination, modA and modSLA surfaces were rinsed under nitrogen during production, preventing contact with the atmosphere, and then stored in a sealed glass tube containing isotonic NaCl solution. These sealed disks were sterilized by gamma irradiation at 25 kGy overnight. The modification significantly lowered carbon contamination without changing surface topography. Free energy of both surfaces were above 72.8mN/m.

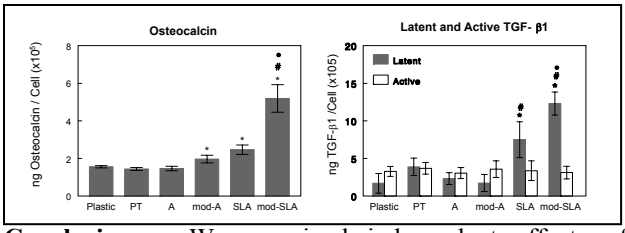
MG63 human osteoblast-like cells were cultured on tissue culture polystyrene (plastic), as well as Ti surfaces mentioned above. Cells were cultured in 10% FBS-DMEM until confluence; and then cell morphology, cell number, differentiation and local factor production were analyzed.

Results: MG63 cells cultured on smooth PT surfaces were flat and spread out. On submicron rough A surfaces, cells were elongated with spindle-like morphology. On SLA surfaces, cells were wider and polygonal with large amounts of filopodia extended to sense the surface. Modification treatment of Ti surfaces with high energy did not change cell morphology.

Cell numbers were the same on plastic, PT and A surfaces. With higher surface energy on modA, cell number was reduced 25%. On rough SLA, cell number decreased 37%. The combination of high surface energy and micron-scale roughness on modSLA decreased cell number by more than 66%.

Osteocalcin is a differentiation marker of osteoblasts. MG63 cells cultured on modA produced 30% more osteocalcin than on A (Fig. 1, left). On SLA, osteocalcin increased 60% compared to A. However, high surface energy and micron-scale roughness of the modSLA surface synergistically improved osteocalcin levels by 250%.

Local factor production was also affected by surface properties. Surface energy and roughness did not affect levels of active TGF-β1 but latent TGF-β1 was increased on rough SLA, and synergistically amplified on modSLA (Fig. 1, right). Similar effects were also observed with respect to osteoprotegerin and PGE_2 levels.



Conclusions: We examined independent effects of micron-scale and submicron-scale topography and surface energy by applying a modification technique to eliminate contamination and retain a high surface energy. Osteoblast-like cells cultured on higher energy surfaces exhibit a more differentiated phenotype that on surfaces with identical architecture. However, synergistic increases in osteoblast differentiation required both micron-scale and submicron scale structural features. In addition, micron scale architecture was needed for high surface energy to effect an increase in production of local factors that promote osteogenesis and inhibit osteoclast activity. The results suggest that surface energy is an important factor in mediating cell-substrate interactions, and higher surface energy should be incorporated in biomaterial design to improve the host tissue response. In addition, they show that beneficial effects of high surface energy may depend on other structural elements on the substrate surface.

References: 1. Zhao et al., J Biomed Mater Res, 74, 2005; 2. Rupp et al., J Biomed Mater Res, 76, 2006

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