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Identifying and mimicking features of unfavourable topography and investigating cellular reactions

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INTRODUCTION: Orthopaedic implant manufacturers offer a considerable range of metals. The finish varies from electropolishing of stainless steel (SS) to micro-rough titanium (TS) and Ti-6Al-7Nb (NS). In the context of soft tissue, represented *in vitro* by fibroblasts, rough versus smooth on TS and SS did not significantly affect cell adhesion or subsequent growth. However, fibroblast spreading and cell growth were seriously compromised on micro-rough NS[1]. This led to the question; is the contributory cause chemistry or topography or a combination of both? Here we investigate the influence of chemistry and topography by means of surface coating and microfabrication.

METHODS: Material coating was conducted utilising an e-beam evaporator to deposit 50nm of either gold or titanium on NS, TS and SS. Surface characterisation was performed using Atomic Force Microscopy (AFM), Profilometry and Scanning Electron Microscopy (SEM). Qualitative assessment utilised SEM for cell growth and morphology at 24h, 5 and 10 day timepoints and Fluorescence Microscope (FM) for quantitative cell counts. Intracellular components, vinculin, tubulin, actin and DNA, were fluorescently labelled and imaged using the FM at 48h. A silicon die with inverted micropyramids was produced by photolithography and wet etching methods. Replicas were produced by embossing in polycaprolactone and evaporated with 50nm titanium.

RESULTS & DISCUSSION: Surface coating of NS produced a homogenous layer of either gold or titanium. Cell growth demonstrated no statistically significant differences per sample type at 24h, 5 or 10 days – cell growth was typically depressed on all sample types of NS. Cell morphology and cytoskeletal staining, examined using SEM and FM, demonstrated no differences between uncoated NS and its coated counterparts. To safeguard against coating compatibility issues, SS and TS were also coated and cell growth was demonstrated to be normal for these surfaces in comparison with the uncoated versions – confluent monolayers were observed at 10 days on all. These results indicate that for NS, the surface topography and not the underlying chemistry was the primary cause of inhibited cell growth. This finding was confirmed with coated SS and TS surface models.

Numerically the roughness average of NS (0.77μm) was similar to TS (0.90μm), however when examined utilising SEM and AFM, the surfaces were markedly different with NS displaying a rough microspiked topography. AFM analysis demonstrated that these microspikes had surprisingly uniform dimensions and demonstrated with FM to interfere with cellular processes of adhesion and microtubule formation. This cellular impairment made the microspikes a primary suspect in suppressing cell growth.

A metallic topography inspired by the dimensions and general morphology of the spikes was microfabricated. The topography produced was a uniformly spaced pyramid topography. Cells on these topographies were demonstrated to display low cell growth, low spreading, and their adhesion sites and microtubule networks were visibly influenced by the presence of the pyramids (Fig 1).

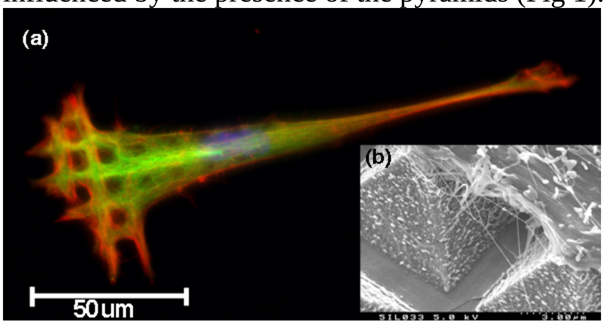

Fig 1 (a)Tubulin, Actin, DNA stain of a cell cultured on the pyramid microtopography. (b) SEM of cell elevated on the pyramid topography.

CONCLUSION: In mimicking such dimensions and eliciting similar cellular reactions, the importance of roughness morphology is illustrated. A reciprocal trend could be developed, as metal topographies could provide inspiration for fabricated microtopographies that in turn could be utilised to produce optimal metallic implant topographies, and not only with regard to fibroblasts.

REFERENCES: [1] D.O.Meredith *et al J Biomed Mat Res A (in press).*

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