

Effect of standard orthopaedic metal implant surfaces on cell behaviour

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INTRODUCTION: The variance in surface chemistry and topography of orthopaedic materials makes for difficult characterisation and comparison. Their utilisation is widespread, but 'interface' properties relative to soft tissue cells have not been studied extensively. We present an investigation on the cytoskeletal, adhesive, morphological and molecular interaction of human fibroblasts (hTERT, Infinity™ Telomerase-Immortalized) with a set of standard implant materials: (Stainless Steel (SS), Titanium (CpTi) and titanium alloy, Ti-6Al-7Nb (TAN)). The aim was to analyse the materials and to provide extensive in vitro characterisation of human fibroblast cells on these frequently used materials.

METHODS: Atomic Force Microscopy (AFM), Laser Profilometry (LP), Scanning Electron Microscopy (SEM) (secondary and backscattered electron modes) and Contact Angle (CA) were used to characterise the surfaces. hTERT fibroblasts were cultured on implant quality EPSS, CpTi and TAN. Thermanox (polyethylene terephthalate) coverslips (Therm) were included as a control substrate. Cultured cells were fixed for SEM and Light Microscopy (LM) evaluation. The cells fixed for LM were fluorescently triple stained for F-actin and DNA as well as either Tubulin or Vinculin. Cell morphology was assessed qualitatively with SEM and quantitatively by image analysis of fluorescence images. For cDNA microarray studies 50,000 cells were seeded on the materials and cultured for 10 days before RNA was extracted. The transcribed DNA incorporated either Cy3 or Cy5 fluorescent markers and were hybridised on Human 1.7K chips (University Health Network, Toronto). The control RNA was obtained from cells cultured on Therm.

RESULTS: CA and the Roughness Average measurements describe CpTi and TAN as being similar surfaces. CpTi has an RA of 1.15µm and a CA of 80.2° while TAN returned 0.99µm and 85.2°. EPSS was considerably smoother with an RA of 0.21µm and CA of 73.1°. This indicates a positive correlation between surface roughness and contact angle. AFM and SEM demonstrate that CpTi and TAN have significantly different micro-topographies; CpTi having a rugged irregular surface while TAN displays a 'micro-spiked'

topography consisting of 'electron dense' particles. EPSS appears smooth and unblemished.

Qualitatively, the cells cultured on both CpTi and TAN were restricted in spreading by the topography. Focal adhesion (FA) size was also limited for cells grown on CpTi and TAN; this is currently being measured quantitatively. The FA of cells cultured on TAN actively avoided the microspiked particles. Cells cultured on EPSS spread freely. Cells were sufficiently spread on EPSS such that we could distinguish 'dot' adhesion sites at the cell periphery, and mature 'dash' adhesions with attached actin cytoskeleton further inside the cell. None of the surfaces visibly disrupted actin filament but tubulin polymerisation was disrupted by TAN. Total RNA amounts extracted from cells cultured for 10 days on CpTi, SS and Therm substrates were between 3-4.5µg while only 0.6µg was extracted from cell cultured on TAN. RNA expression can vary due to the level of cell activation but this low number for TAN was correlated to a low cell count. Microarray studies of cells cultured on CpTi had significant up or down regulation of 213 genes from the 1700 arrayed. SS had significant up-regulations for 16 genes. 6 of these genes were commonly up-regulated in cells cultured on both substrates, in comparison with the Therm control. The most notable change was the upregulation of Matrix Metalloproteinase 7, an enzyme used to remodel extracellular matrix and associated with wound healing.

DISCUSSION & CONCLUSIONS: Difference in surface roughness correlates with differences in cell behaviour. However, CpTi and TAN elicited varying cell reactivity: CpTi topography visibly dictated cell spreading and FA development. Of the six common genes, all were upregulated more on CpTi than EPSS. Cells cultured on TAN had statistically decreased spreading, the FA formation was affected and most notably there was an apparent lack of cell proliferation. EPSS and CpTi indicated topographical variance as the mode for cell reactivity, but TAN indicated that physio-chemical material properties cannot be overlooked. The notable cell variance warrants further investigation into the cell compatibility of these 'standard' materials.