

Controlling hard tissue integration at the bone-implant interface.

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Introduction: Complications and costs involved in internal fracture fixation implant removal may outweigh potential risks of device retention¹. However, bacteriological, immunological, oncological and metabolic risks associated with long term implantation necessitate revision of this concept. In paediatric fracture fixation, removal is advocated to prevent plate migration and stress shielding in the fast growing skeleton. Approximately 10 % of all complications relate to difficulty in removing the implant due to the device becoming engulfed by bone (Fig. 1). Often this results in destruction of the device and further trauma to the patient. It is known that surface topography can be manipulated to enhance osseointegration at the bone-implant interface². In this study, we investigated three forms of clinically available implant materials with varying micro-topographies with respect to osteoblast behaviour (proliferation, differentiation and gene expression)



Fig. 1 Image of a fracture fixation plate engulfed by bone after 2 years implantation within a sheep.

Methods: Three clinically available materials were used (commercially pure titanium (cpTi); titanium6%Aluminium7%Niobium (TAN); titanium15%molybdenum (Ti15MO). Each had three distinct surface finishes (electropolished, paste polished, standard micro-rough). All surfaces were characterized using SEM, contact angle, XPS and non contact profilometry. Rat calvarial (RC) cells, originally isolated by sequential enzyme digestion from 6 day old Swiss Wister rats were cultured on 13mm sample discs in DMEM with 15% FCS, 50µg/ml of ascorbic acid, 1% penicillin-streptomycin and 5mM beta-glycerophosphate at 37°C 5% CO₂. Cell growth and morphology was assessed both qualitatively using SEM analysis, and quantitatively using tritiated thymidine incorporation (10µCi/ml) at 7, 14, and 21 day culture periods. Changes in relative gene expression for osteocalcin and collagen type I were

investigated using quantitative real-time PCR normalised to the housekeeping gene 18S.

Results: Profilometry results showed that all micro-rough surfaces had comparable average roughness (Ra). All polished surfaces were comparable to the control (Ss) with the exception of NE, NP and TP which due to manufacturing problems were slightly rougher. Contact angle surface wettability showed all samples were hydrophilic as expected (Fig.2). Results for XPS showed that the surface chemistry was very similar after polishing as all samples underwent anodisation masking any possible changes (Fig. 3)

	TE	NE	ME	TP	NP	MP	TS	NS	MS	Ss
Mean Ra (µm)	0.142	0.409	0.293	0.410	0.243	0.207	0.86	0.75	0.82	0.112
CA °	70	69	74	68	83	75	68	72	78	49

Figure 2 Mean average roughness (µm) and contact angle (CA; degrees) of samples. Ss-stainless steel (control), T-cpTi, N-TAN, M-Ti15Mo, E-Electropolished,P-paste polished, S-standard micro-rough.

	Al 2p	C 1s	N 1s	Na KLL	Nb 3d	O 1s	P 2p	Ti 2p
TE	37.9	4.1	1.4			41.3	2.6	12.2
NE	1.4	28.2	1.1	0.3	0.2	49.2	2.3	17.1
ME		23.9	1.8	1.3		53.1	3.0	16.9
TP		30.4	0.8	0.4		49.2	1.7	17.5
NP	2.4	27.6	1.1	0.6	0.1	49.0	2.5	16.5
MP		35.7	0.6	45.3			2.5	15.4
TS		26.6	0.7	0.9		51.2	2.8	17.6
NS	2.1	26.0	0.9	0.5	0.2	50.9	2.0	17.2
MS		42.8	0.3	40.6			1.7	12.5

	C 1s	Cr 2p	Fe 2p	Mo 3d	N 1s	Na 1s	Ni 2p	O 1s	P 2p	Si 2p
Ss	28.0	7.3	2.3	0.4	1.9	2.6	0.4	49.5	2.1	5.5

Fig. 3 XPS results for samples as expressed as percentage of atomic concentration.

SEM analysis showed that despite the comparable Ra's for the standard surfaces, the micro-topographical features differed greatly (Fig.4).

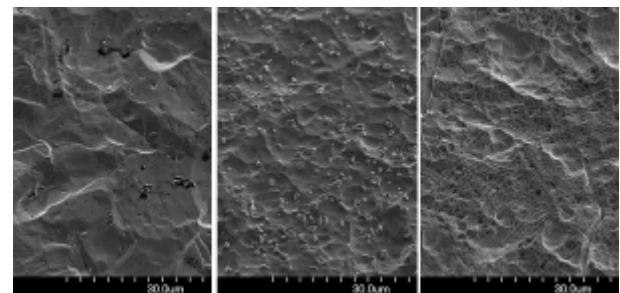


Figure 4 SEM images in secondary electron mode showing that despite similar average roughness, topographical features of the surfaces varied. A) TS B) NS C) MS

The control surface (Ss) had an almost flawless surface and was extremely smooth. Polishing enhanced the grain boundaries' contrast for both TE and MP. The slightly rougher Ra's for NE and NP may be due to the niobium rich inclusions seen on the surface. Independent of material type, micro-rough standard surfaces showed a significant increase for both osteocalcin (Fig 5) and collagen I gene expression over both the polished variants. The highest increase for both genes was exhibited on the Ti15MO surfaces. A significant increase was also found between cpTi and TAN polished variants but not Ti15MO for osteocalcin gene expression. In contrast, for col I expression, a significant increase was observed between ME & MP. This increase for col I expression was not found however for cpTi or TAN. Incorporation of tritiated thymidine was highest on the polished surfaces and decreased in a time dependent manner.

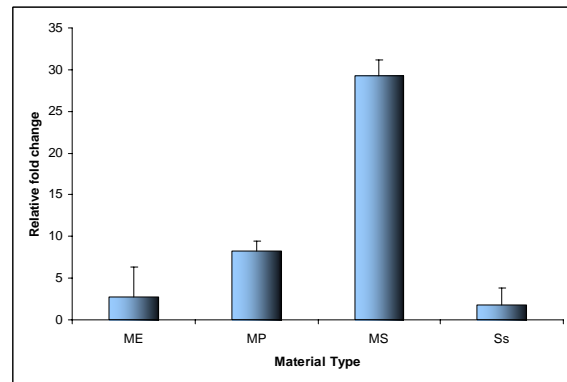
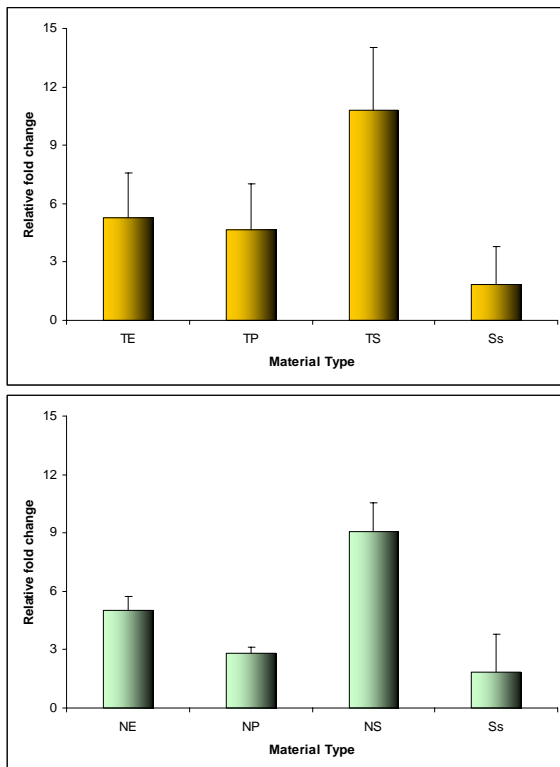


Figure 5 Representative real time qPCR results for osteocalcin gene expression at 21 days relative to 7 day expression (delta-delta Ct) (Gold – CpTi; Green – TAN; Blue – Ti15MO).

Discussion and Conclusion: Previous work in our lab has shown that micro-rough steel induces bone growth at the interface *in vivo*, becoming fully integrated at the implant surface. This has been supported by other studies for a variety of metal fixation devices^{3,4}. In this study, we have shown that surfaces with a micro-roughness of approximately $0.7\mu\text{m}$ support a differentiated osteoblast-like phenotype pertaining to gene expression, in keeping with other recent studies^{5,6}. The superior bone bonding property of titanium⁷ over steel may contribute to the slightly higher expression observed for polished samples compared to the control. The data shows that surface polishing reduces osteoblast cell ability to differentiate and produce mature matrix compared to micro-rough surfaces. We propose that the polishing of clinically used metal implants instead serve to support a cell proliferative state as is suggested by the initial high rate of thymidine incorporation uptake observed for polished samples compared to micro-rough counterparts, and as documented elsewhere^{6,10}. Regardless of time, thymidine uptake was markedly reduced on micro-rough surfaces compared to polished, thus supporting the notion that surface polishing advocates a proliferative cell state whereas micro-rough surfaces support a differentiated cell phenotype. It is possible that surface polishing may allow for either fibrous tissue formation or lower bony adhesion to occur on specific areas of implant devices to allow for easier retrieval. This study highlights the promise of surface polishing in reducing extraosseous formation on internal fixation devices that require removal.

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