

Control of osteoblast genotype with implant surface microtopography.

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INTRODUCTION: Complications relating to excessive bony overgrowth of an internal fixation device account for approximately 13% of all removal-related morbidity problems. We have previously shown *in vivo*, that surface polishing can significantly reduce the percentage of bone contact to an implant^{1,2}. Consequently, a lower removal force is required for extraction¹. However, we do not believe this effect to be exclusively mechanical. Specifically, we hypothesise that both material type and surface micro-topography will influence the temporal expression of genes 'specific' to an osteoblast genotype, as well as reducing expression of factors essential for differentiation, matrix production and mineralisation, and therefore, bone formation. Thus, to elucidate the effect of surface polishing on bone over-growth from a cellular view point, here we have investigated *in vitro*, alterations in osteoblast genotypic expression due to surface polishing.

METHODS: 50mm diameter samples were fabricated from 'standard' orthopaedic grade commercially pure titanium (cpTi), titanium6%-aluminium-7%niobium (TAN) & titanium-15% molybdenum (Ti15Mo) (positive controls for osseointegration). Experimental surfaces were prepared via electro- & paste polishing the above materials. Stainless steel was included as a negative control. Surface characterisation involved SEM, AFM, XPS, contact angle & non-contact profilometry. Cellular response was assessed using primary rat calvarial (RC; 100,000 cells/sample) cells pertaining to the relative fold change in mRNA (osteocalcin (OCN), Alkaline phosphatase (ALP), bone sialoprotein (BSP), Cbfa1, collagen I and osterix) after 7, 14 and 21 days culturing.

RESULTS: No significant difference was observed for Cbfa-1 mRNA levels on TE (p=1.000) or TP (p=1.000) compared to TS, nor for NE (p=1.000) or NP (1.000) compared to NS, nor ME (p=1.000) or MP (0.619) compared to MS. Osx up-regulation on TS was significantly different to Osx up-regulation on TP (p=0.48), but not TE (p=0.086). No significant differences for osx regulation were observed for NE (1.000) or NP (p=0.794) compared to NS. No significant differences

were reported for osx expression on ME (p=1.000) or MP (p=1.000) compared to MS. ALP expression profiles for TE (p=1.000) and TP (p=1.000), did not significantly differ from those accrued for TS or for NE (p=0.257) or NP (p=1.000) compared to NS. ALP expression for ME (p=1.000) and MP (p=0.506) samples was not found to be significantly different to that observed for MS. No significant difference in COL1 regulation was observed for TE (p=1.000) or TP (p=0.672) samples compared to TS. A significant difference in COL1 expression was observed for NE (p=0.000) and NP (p=0.000) compared to NS samples. Cells cultured on MP had a significantly lower COL1 expression profile compared to ME (p=0.000), and MS (p=0.018). No significant difference was observed for BSP expression on TE (p=1.000), or TP (p=1.000) compared to TS, or for NE (p=0.465) or NP (p=0.556) compared to NS samples, or for ME (p=1.000) or MP(p=1.000) compared to MS. OCN expression for TS was noted to be significantly higher compared to TE (p=0.001) and TP (p=0.000) samples. No significant difference in OCN regulation was observed for cells cultured on NS samples compared to NE (p=0.419), NP (p=0.488). However, MS samples compared to ME (p=0.005), MP (p=0.049) showed significant difference in OCN expression.

DISCUSSION: Previous *in vivo*^{1,2} studies from our laboratory have highlighted the promise of surface polishing for ease of removal of temporary fixation devices. This influence will inadvertently improve surgical related complications associated with removal, as well as reducing the economic costs associated with removal related morbidity. To help elucidate the cellular regulatory mechanisms of this influence, in this study, we have investigated *in vitro* the influence of surface polishing on osteoblast genotype, as we believe the effect of polishing not to be purely mechanical. As hypothesised, both material type and surface polishing significantly affected osteoblast genotype. In this study, compared to standard samples, generally, no significant difference was observed for Cbfa-1, osx, ALP, COL1 or BSP expression compared to standard counterparts. Thus it is reasonable to speculate

that from the initial signalling of Cbfa-1, cascades involved down stream of this factor to the point of initial crystal nucleation and apatite growth, remain engaged for cells cultured on polished samples. The exception to this was polished TAN samples, which had significantly lower COL1 mRNA levels, but no difference in OCN expression. The observation in this study that BSP expression is similar for both polished and standard micro-rough samples, but OCN is significantly decreased on polished samples is very interesting. This may be due to the relatively high concentration of BSP, or altered secondary structure on polished samples, which can inhibit apatite formation³. If this were the case, perhaps more time would be required for polished samples for sufficient apatite nucleation and crystal growth to occur, thus OCN expression would be subsequently reduced and/or delayed to allow for this phase of mineralisation to occur. Alternatively, BSP induction may be sufficient polished samples to induce initial phases of mineralisation but at a slower rate compared to standard samples, thus through an alternative signalling mechanism independent of Cbfa-1, or through Cbfa-1 binding with a co-factor specific for OCN repression⁴, OCN is decreased, therefore, terminal differentiation would occur at a later time on polished samples. Since decreasing OCN allows mineralisation to occur, these results, regardless of the events involved in their occurrence, would suggest that terminal differentiation occurs at delayed rate on polished samples compared to micro-rough counterparts. This is an extremely poignant point, as this may elucidate the mechanism by which surface polishing effectively reduces bone over-growth compared to standard devices of similar material.

CONCLUSIONS: These findings indicate that the influence of surface polishing for reduced bone over-growth is in part attributable to alterations in genotype on a cell level. Ongoing studies are now focusing on alterations in cytoskeletal organisation and cell shape on polished surfaces as a potential cause for these distinct genotypic profiles.

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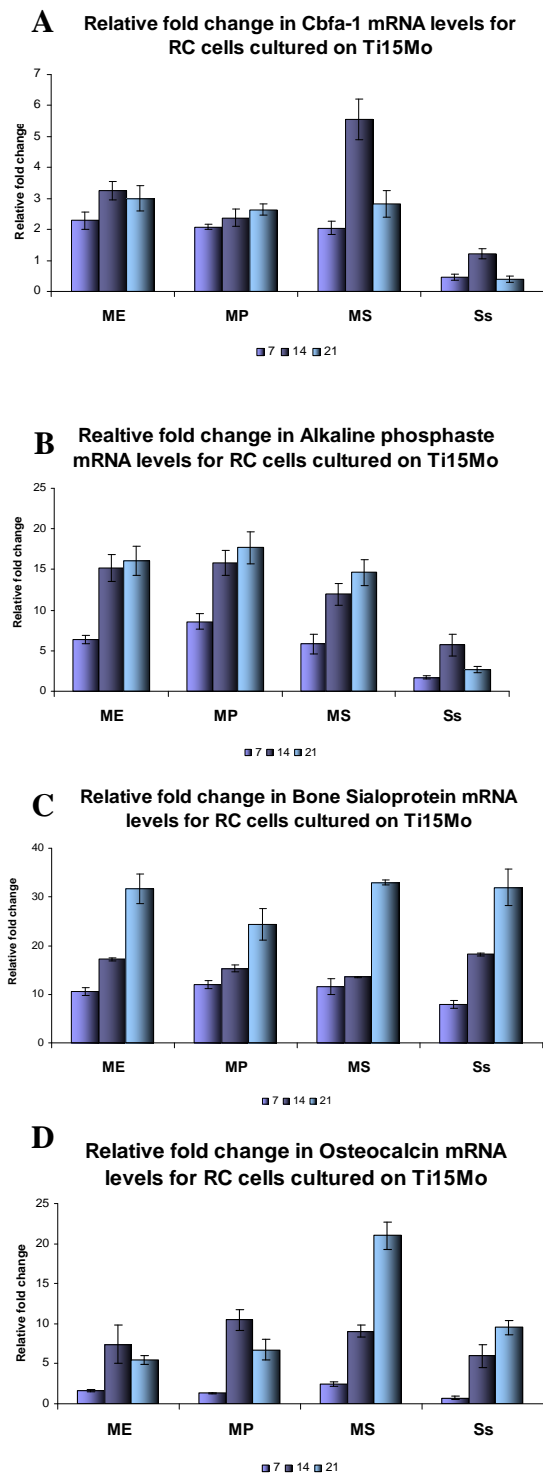


Fig. 1. Representative results for changes in mRNA levels of (A) Cbfa1, (B) ALP, (C) BSP and (D) OCN mRNA levels for RC cells cultured on electropolished (ME), paste polished (MP), and standard Ti15Mo (MS), with stainless steel (SS) as a control.

ACKNOWLEDGEMENTS:

The authors wish to thank Dr. DM Devine & Dr S Lyons for AFM assistance, P Furlong for her invaluable technical support & Dr. V Frauchiger for the XPS analysis.