

Osteoblast adhesion and morphology and *Staphylococcus aureus* adhesion on various coated orthopaedic implant surfaces.

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Introduction: Determination of surface cytocompatibility using cells and bacteria helps determine the suitability for *in vivo* use. The ability of *Staphylococcus aureus* to adhere to biomaterials is a significant factor in the pathogenesis of medical-device related infections^{1, 2}. Osteoblast adhesion and morphology along with *S. aureus* adhesion was evaluated for various surfaces, to indicate which are likely to promote osseointegration or infection.

Materials and Methods: A Laser profilometer (LP) and Scanning Electron Microscope (SEM) were used to characterise the surface topographies (Table 1). Primary rat calvarial osteoblasts were cultured³, 20,000 cells on each surface for 48hrs, (sub-confluent). For the morphological analysis cells were fixed then imaged with an SEM using the high emission backscattered electron (BSE) technique⁴ at low voltage (5kV). The area of 30 cells from random positions was calculated. The focal adhesion protein vinculin was immunogold-labelled⁵ and enlarged with gold enhancer to allow imaging of labelled whole cells with the SEM at 6kV⁶. The percentage area of labelling on 20 random cells was measured.

Label	Description	Ra (μm)
NS	Standard anodised TAN (Ti AL Nb)	0.99
NT	Tiodised TAN	0.79
TS	Standard anodised Ti titanium	1.15
TT	Tiodised TS	0.51
TSS	Titanium-101 gold anodized control	0.83
THY	TSS with grafted sodium hyaluronate (THY)	1.09
TAST	TSS with polymer cell promotion (TAST)	1.09
TIG	Nitrogen ion implanted TSS (TIG)	1.05
TLF	Low friction grey anodized TSS (TLF)	1.14

Table 1- Substrates investigated.

S. aureus were cultured on the surfaces in brain heart infusion broth for 1h at 37°C, stained with fluorescent redox dye, 5-cyano,2-ditolyl tetrazolium chloride (CTC)⁷ for 1h, and visualised with a Zeiss Axioplan 2 Epifluorescence microscope fitted with a digital camera. The density of live bacteria adhering to the surface was quantitatively measured using image analysis software.

Results: Cells were generally well spread on all surfaces except THY, to which no cells adhered (Fig. 1). The degree of cell spreading varied slightly between surfaces. Dash adhesion labelling was visible on the TAST, TIG, TS and NT surfaces, suggesting these surfaces may be more cytocompatible. TT, TLF and TSS surfaces showed

no mature dash adhesion labelling (Figure 2). Bright surface artefacts interfered with image analysis of NS samples (✦).

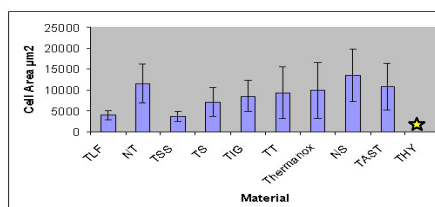


Fig. 1
Average cell area on surfaces.

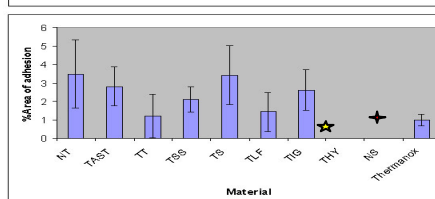


Fig. 2
Average % labelled adhesion area.

Fluorescence microscopy showed *S. aureus* adhered to all the surfaces (Fig. 3), with the exception of THY. The amount of adhesion was low with TIG, and TLF.

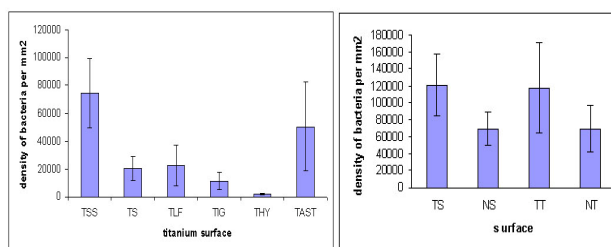


Fig. 3 Density of *S. aureus* adhesion to the surfaces.

Conclusions: The surfaces showed different degrees of cytocompatibility with regards to osteoblast morphology and adhesion. These surfaces (exception sodium hyaluronate coated surface) did not have a significant effect on the adhesion of *S. aureus*. The least cytocompatible surface to osteoblasts and *S. aureus* was THY.

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