

Osseointegration of Silver Treated Titanium Alloy

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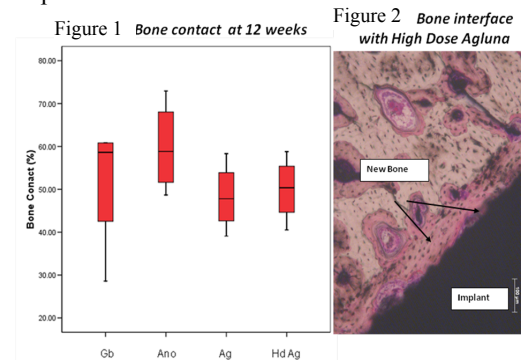
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Statement of Purpose: Infection for total joint replacement and orthopaedic procedures which utilise implants remains a significant problem. A method of incorporating silver into the surface of titanium and its alloys using a modified anodisation technique has been developed and is known as Agluna. The surface is able to release bactericidal concentrations of silver and the aim of this study was to investigate the effect of this surface modification on osteoblast and fibroblast viability and on the osseointegration of a trans cortical pin in an *in vivo* ovine model. Our hypothesis was that silver incorporation had no negative effects on the viability of fibroblasts and osteoblasts *in vitro*, and would have no negative effect on interfacial shear strength, bone turnover or bone contact in an *in vivo* ovine model.

Methods: *In vitro*: Titanium alloy discs were either polished (Ti), anodised (Ano), anodised or Agluna treated (Ag) or anodised and Agluna treated followed by a conditioning step (Ag C). Conditioning of the Agluna surface was achieved by incubating discs with Agluna surfaces in culture fluid (DMEM plus 10% calf serum) for a period of 48 hrs prior to cell seeding. A seeding concentration of 5,000 cells per disc was used and 4 replicates investigated. 14hrs after introducing the cells, live/dead staining was carried out by incubating discs in culture media supplemented with 1.0 μ M calcein and ethidium bromide in phosphate buffered saline. Cells were stained for 1hr and viewed under a fluorescent microscope in the FITC range and the ratio of live to dead cells determined. The level of silver in the culture fluid after conditioning and at the end of the test was measured using atomic emission spectroscopy. *In vivo*: 8 tapered trans-tibial implants were inserted into the mid-shaft of the left and right tibia of female, skeletally mature commercially cross-bred sheep and positions for each treatment rotated assigned (Ethical approval was granted and all procedures were carried out in compliance with UK's Home Office Regulations (Animal Scientific Procedures Act 1986)). A total of 60 implants were inserted in this study (n=5). Grit blasted Titanium alloy (Gb) and Agluna treated grit blasted titanium alloy (Ag) at a silver concentration of 4-6 μ g/cm² were compared at 6 weeks. Gb implants, Ag (at 4-6 μ g/cm²), high dose Agluna implants with silver concentrations at 15-20 μ g/cm² (HdAg) and a grit blasted anodised titanium alloy (Ano) were compared at 12 weeks post implantation. Following retrieval, the pullout strength of implants at both time points were measured (n=5), bone turnover rates calculated and osseointegration determined by measuring the amount of bone in contact with the implant surface. A Mann Whitney U test was used to determine significant differences between groups where p<0.05 was considered significant.

Results: On Ti, Ano and Ag C surfaces the number of live fibroblasts was significantly greater than on Ag (non-conditioned) surfaces. On all surfaces dead cells were

only very occasionally seen. These results indicate that attachment of cells is temporarily reduced on non-conditioned surface, prior to surface conditioning. The average total starting silver inventory on the discs was c 3.4 μ g/cm² (STDEV 0.34 μ g/cm²). An average of 36.4% (3.65 μ g STDEV 0.5 μ g) of the silver was leached from the disc during the initial 48hr conditioning period. A further 7.2% on average (0.74 μ g STDEV 0.37 μ g) was leached during the subsequent 14hr conditioning period. In the ovine model, data from the pull out tests at 6 weeks showed a slightly lower but significant interfacial shear strength in the Ag group (310.4N) when compared with the Gb group (561.2N) (p=0.01). At 12 weeks, the shear strength had significantly increased compared to the pull out strength at 6 weeks. There were no significant differences when each of the 4 treatment groups was compared. Histological analysis showed bone contact to the pin surface in all groups with no difference in the level of osseointegration between Gb and Ag implants at 6 weeks (57.74% and 59.38% respectively) and between all groups at 12 weeks (Gb = 51.68%; Ag = 48.26%; HdAg = 50.01% and Ano= 59.83% ; Figure 1). Figure 2 shows bone in direct contact with the surface of a Hd Ag implant at 12 weeks post implantation. Analysis using light microscopy showed no signs of an adverse tissue response.



Conclusions: *In vitro* tests showed that the initial non-conditioned Agluna surface is cytotoxic but that after a period of conditioning both osteoblasts and fibroblasts are able to attach and remain viable. This is associated with the release kinetics of silver from the Agluna which is high initially but reduces exponentially with time. When investigated *in vivo*, this initial effect does not reduce the extent of osseointegration over the 12 week period in an ovine model. Results from this study have shown that incorporation of silver at a concentration up to 20 μ g/cm² on a grit blasted titanium alloy surface has no adverse toxic effect on osseointegration and the interfacial shear strength of implants and that the antimicrobial effect of silver onto the surface in orthopaedic implants should be considered for use in combating infection. This work was funded by Accentus Medical plc .