Osseointegration of Silver Treated Titanium Alloy

Introduction:

Infection for total joint replacement and orthopaedic procedures which utilise implants remains a significant problem. Silver has known antibacterial properties and has been included in a number of orthopaedic and non-orthopaedic implants. However silver can also have a cytotoxic effect on fibroblasts. 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 osteointegration 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 10 µM calcine 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. Ethical approval was granted and all procedures 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 µg/cm² were compared at 6 weeks. Gb implants, Ag (at 4-6 µg/cm²), high dose Agluna implants with silver concentrations at 15-20µg/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 osteointegration 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.(Figure 1).

Discussion

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 osteointegration 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 µg/cm² on a grit blasted titanium alloy surface has no adverse toxic effect on osteointegration 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.

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