Antibacterial efficacy of nano-enhanced titanium alloy medical implants against Streptococcus sanguinis

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Introduction
Titanium and its alloy (Ti6Al4V) are the most common materials used in medical and dental implants, because of their good biocompatibility, mechanical strength and corrosion resistance. Despite the clinical success of dental implants (> 95% survival rates after 10–15 years), the development of peri-implant and dental implant infections can be as high as 14% (Norowali and Bumgardner, 2009). In case of infection, intensive interventional medical and antibiotic treatment is usually required, and if not treated successfully it may lead to loss of the implant, impaired oral function, and even death.

Current strategies to inhibit infection and biofilm formation on dental implants focus on coating the implant surface with antibiotics, antigens (e.g. CRH/ornithine) or other anti-bacterial agents containing metals with known antibacterial activity (e.g. silver, zinc and copper). However, these approaches mostly suffer from short-term antibacterial protection and may also affect the biocompatibility of the implant. It seems likely that these limitations can be overcome with the advances in nanotechnology. Certain metal and metal oxide nanoparticles (NPs) have been suggested for infection control in dentistry and the management of the oral biofilm (AIhmer, 2010). Among metal NPs, Ag NPs are the most antibacterial against oral pathogens (Besinis et al., 2014). The precise mechanism(s) for bacterial toxicity of metal-containing NPs is still being elucidated, but for Ag NPs the possibilities include free metal ion toxicity and oxidative stress. The necessity of using metal NPs as an alternative antibacterial strategy is justified by the acquired resistance of bacteria to the currently available antibiotics (Pallasch, 2003).

Aim
The primary aim of this study was to investigate the antibacterial properties of suitable nanocoatings applied to Ti6Al4V medical implants. A second objective was to examine the stability of the coatings and their ability to maintain their integrity in biological fluids.

Methods & Materials
Medical grade Ti6Al4V discs (15 mm) were coated with TiO2, Ag and hydroxyapatite (HA) NPs following the anodisation, silver electropolishing and sintering techniques respectively. In total, nine different groups were prepared: uncoated Ti6Al4V control (T), Ti6Al4V coated with nano-HA (nHA) particles (Ti+nHA), Ti6Al4V coated with micro-HA (mHA) particles (Ti+mHA), anodised Ti6Al4V (TiO2), anodised Ti6Al4V coated with HA (TiO2+mHA), anodised Ti6Al4V coated with nHA (TiO2+nHA), uncoated Ti6Al4V coated with Ag (Ti+Ag), silver-plated Ti6Al4V coated with nHA (Ag+mHA). All nanocoatings were fully characterised (Fig.1) using scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS) and by measuring the surface roughness (Table 1) with a confocal laser scanning microscope (Lyra XL ALS 3000). The antibacterial efficacy of the nanocoatings was tested against Streptococcus sanguinis (NCTC 10944), which is considered to play a key role in the biofilm formation process, and was compared to the uncoated control. The tests were performed in 24-well plates (n = 6 discs/well). One disc was placed at the bottom of each well, which was then inoculated with 150 µl of bacterial suspension (10 cfu ml-1) and 1 ml of physiological saline (modified Krebs saline). Following a 24 h exposure, the bacterial growth and cell viability in the media were quantitatively assessed by measuring the turbidity, lactate production and proportion of live and dead cells (Backlund et al., 2006). The morphology of developing biofilms was examined with SEM (Fig. 2). Specimens were then sonicated for 60 s to detach the adherent biofilms without damaging the cells, and the bacterial adhesion on the coated implants was investigated employing the same three bio-assays (Fig. 3A, E, F). The integrity of the antibacterial coatings was investigated using inductively coupled plasma mass spectrometry (ICP-MS), Table 2.

Results

Conclusions

1. Application of the biocompatible coating increased the surface roughness of Ti6Al4V discs.
2. Both live and dead bacterial cells were found to be very stable ± 15% and ± 20% respectively, maintaining their integrity in biological fluids.
3. All three silver nanocoatings (Ag, Ag+mHA, Ag+nHA) were found to be highly bactericidal in 24 h. The medium for growth of Ag-coated discs was the Ti6Al4V blank-bacterial adhesion 13% when compared to the uncoated control.
4. The TiO2 and HA-coatings applied to the silver-plated implants did not reduce the strong antibacterial activity of the underlying silver nano-coating.
5. The remaining groups did not demonstrate any antibacterial or biofilm activity suggesting that coated Ti6Al4V surfaces were not antibacterial coatings or did not add additional properties to the uncoated implant surfaces.
6. This study shows that application of a dual anodised silver–nHA coating to the surface of Ti6Al4V implants may be a viable alternative to the currently available antibiotics and a useful strategy against dental biofilm and infection control. Further studies were performed to determine bactericidal substrate required for novel bone formation and regeneration. The samples were then tested to further evaluate:
7. The anti-adherent properties of these coatings were assessed in biofilm and other biomaterial applications.

References