Controlled Release of Silver Ions from an Electrochemically Deposited Hydroxyapatite Coating

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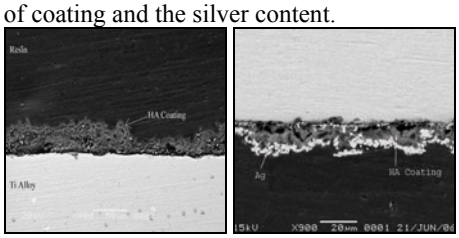
Statement of Purpose: Infections relating to orthopaedic procedures are difficult to treat with systemic antibiotics due to encapsulation of the implant by fibrous tissue, formation of biofilm, antibiotic resistant bacteria and damage to surrounding vascularity due to surgery. Therefore local therapy for controlled drug delivery acting at the implant interface may reduce infection. Silver (Ag) is a potent antibacterial agent with a broad spectrum of activity and has been safely used in medicine for many years. Hydroxyapatite (HA) has been used to promote osseointegration of implants and could act as a suitable carrier for Ag. **We hypothesise that the electrochemical deposition of HA (EHA) can be used to incorporate Ag giving a controlled and sustained release of Ag ions at a bactericidal concentration.**

Methods: Calcium phosphate (CaP) solution was prepared for electro-deposition of HA. A layer of brushite was deposited on the sandblasted 10mm x 3mm Ti6Al4V discs. It was then converted to HA by placing the disc in 0.1M sodium hydroxide for 72 hours. Ag ions were incorporated into the HA coating using two methods. (a)Electrodeposition was carried out after addition of silver nitrate ($AgN0_3$) in the CaP solution. (b)EHA coated disc were immersed in $AgN0_3$ solution for 24 hours. For comparison Plasma sprayed HA (PHA) coated discs were immersed in $AgN0_3$ solution under equivalent conditions. We investigated 6 groups: 1) Electrodeposition of HA and Ag. 2) EHA dipped in $AgN0_3$. 3) PHA dipped in $AgN0_3$. 4) EHA with 2 layers of Ag. 5) EHA. 6) PHA. Isolated Discs were placed in 10ml phosphate buffer solution, pH 7.4, in a water bath at 37˚C to mimic physiological conditions. The phosphate buffer was changed daily. Bacterial inhibition tests were carried out on these discs (*N=6*) at days 0, 1, 6, 10, 15 and 22 using *Staphyloccocus Aureus* (ATC 25923) with zone of inhibition measured from edge of the disc to the edge of the clear zone. Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDAX) and X-Ray Diffraction (XRD) analysis were carried out to measure coating thickness, CaP ratio, the type of coating and the silver content.



(a) EHA thickness (b) EHA dipped in $AgN0_3$

Fig1. SEM micrographs (a) Coating thickness of EHA coated disc. (b) Coating showing Ag layer over EHA coating

Results/Discussion: A coating thickness of 32.98µm (+/-2.50µm) was measured in EHA (Fig 1a) as compared to 76.45µm (+/-2.22µm) in PHA with the CaP ratio of the coatings being 1.71 with EHA as compared to 1.58 with PHA. The Ag content measured using EDAX was 0.38% in group (1), 3.92% in group (2), 0.10% in group (3) and 6.55% in group (4) by atomic %. The XRD analysis of EHA group showed peak corresponding to 100% intensity peak (IP) for HA (black arrow) and secondary IPs for titanium (green arrow) (Fig 2). It also showed peaks corresponding to 100% IPs for silver (blue arrow) and HA in the EHA and Ag group suggestive of incorporation of Ag within the HA coating (Fig 2).

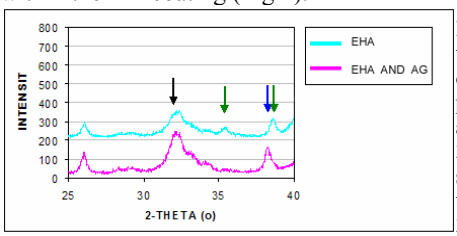


Fig 2. XRD pattern to verify presence of 100% intensity peaks (IP) for HA at 31.8˚, silver at 38.1˚ and secondary IPs for titanium at 35.1˚and 38.4˚.

In the bacterial inhibition test, no zones of inhibition were seen in the EHA and PHA groups. EHA and Ag showed no zone of inhibition at Day 0 but had inhibition zone at Day 6 increasing up to day 15 and maintaining inhibition until 22 days (Fig 3). It was significantly higher than inhibition zones in PHA dipped in $AgN0_3$ at days 15 (p=0.014) and day 22 (p=0.020). The discs in the EHA with 2 layers of Ag and EHA dipped in $AgN0_3$ generally showed significantly higher zones of inhibition over the course of the experiment and were significantly higher than in PHA dipped in $AgN0_3$ at days 6 (p=0.043) (p=0.043), 10 (p=0.004) (p=0.044), 15 (p=0.003) (p=0.006) and day 22(p=0.003) (p=0.003) respectively. The inhibition zones in both these groups were also significantly higher than the EHA and Ag group at days 0, 1, 6, 15 and 22. Inhibition zones in the PHA dipped in $AgN0_3$ group showed a similar inhibition as zones as groups (2) and (4) at days 0 and 1, but reduced significantly by day 6 to 22 (Fig 3).

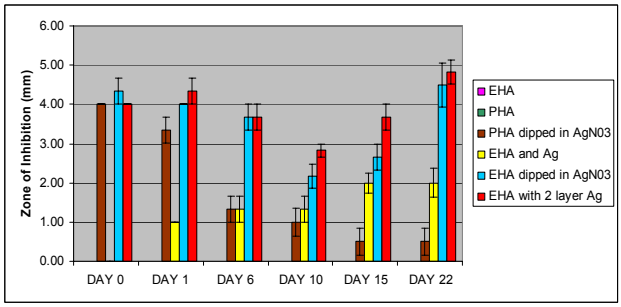


Fig 3. Bacterial inhibition test results.

Conclusions: In the co deposited EHA and Ag group the Ag ions are entrapped between the HA crystals and are released slowly with the dissolution of HA. This would be supported by the results of bacterial inhibition test as in this group bacterial inhibition is prolonged and sustained over the experiment. For the groups where the EHA was immersed in the silver nitrate solution it can be postulated that the increased porosity and better crystallinity of our EHA coatings are able to absorb and entrap more Ag ions, releasing them in a controlled sustained manner over a period of 22 days. This study has demonstrated that it is possible to incorporate silver ions into the HA coating or as layers using an electrochemical technique.