Decreasing bacterial colonization around the Intraosseous Transcutaneous Amputation Prosthesis without inducing cytotoxicity to fibroblasts using hydroxyapatite, silver and fibronectin

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Statement of Purpose: The Intraosseous Transcutaneous Amputation Prosthesis (ITAP) is an osseointegrated titanium alloy skin-penetrating implant which provides attachment for artificial limbs directly to the skeleton. ITAP improves the quality of life for amputees by avoiding the soft tissue complications associated with wearing traditional socket prostheses, whilst enabling improved function due to increased sensory feedback. In order to prevent infection, a tight seal between the soft tissue and the implant is necessary and the cells must win the 'race for the surface' against bacteria to reduce biofilm formation[1]. Silver (Ag) has a broad spectrum of antimicrobial activity. Ag is known to have cytotoxic effects but these effects are dose dependent[2]. Hydroxyapatite (HA) with adsorbed fibronectin (Fn) has been shown to promote fibroblast attachment[3]. **Aims:** This study aimed to decrease bacterial colonization without inducing cytotoxicity to fibroblasts by coating titanium alloy with HA, Ag and Fn.

Methods: The following coatings were tested: HA with Ag (HAAg), HA with Fn (HAFn), HA with Ag and Fn (HAAgFn) and HA as a control (as the current model of ITAP used in clinical trials has an HA coated flange). HA and Ag were electrochemically deposited onto 10 x 2mm titanium alloy discs using a modification of the technique described by Ghani et al[4]. For HAAg surfaces, 50mg (HAAg50) or 10mg (HAAg10) $AgNO_3$ was added to 1 litre of a 0.13M solution of monobasic calcium phosphate. Deposition was conducted using the discs as a cathode and a platinum anode in 200ml of the electrolyte solution. A current of 70mA for 4 min was used to apply the coatings. Fn was adsorbed onto surfaces as a 20µl droplet containing 500ng. Surfaces were preconditioned for 24 hours (P24) in fetal calf serum to simulate the *in vivo* environment. Bacterial colonization was assessed by direct colony counting (n=3). 1ml of 10^6 Staphylococcus aureus 29213 was placed in well-trays containing the coated discs and cultured in a shaking incubator at 5rpm at 37°C. After 24 hours, bacteria within the biofilm were counted by ultrasonicating discs for 2min to remove adherent bacteria, followed by vortexing the solution to separate the colonies. The resultant bacterial solution was then serially diluted and plated in triplicate onto Columbia blood agar plates. The planktonic bacterial solution within the well trays was also serially diluted and plated onto agar plates. The agar plates were incubated for 24 hours at 37°C and the number of colony forming units counted. Fibroblast viability was assessed using a live:dead assay with calcein and ethidium homodimer stains (n=3, 6 areas per disc).

Results: There were reduced numbers of bacteria within the biofilm and the planktonic solution for HAAg surfaces with or without Fn compared to HA and HAFn surfaces, whether the surfaces were preconditioned or not ($p<0.05$). (Figure 1). HAAg surfaces were cytotoxic to fibroblasts when not preconditioned compared to HA and HAFn ($p<0.05$). However, after a 24 hour period of preconditioning there was no difference in the viability of fibroblasts on HAAg surfaces compared to those on HA and HAFn surfaces ($p>0.05$).

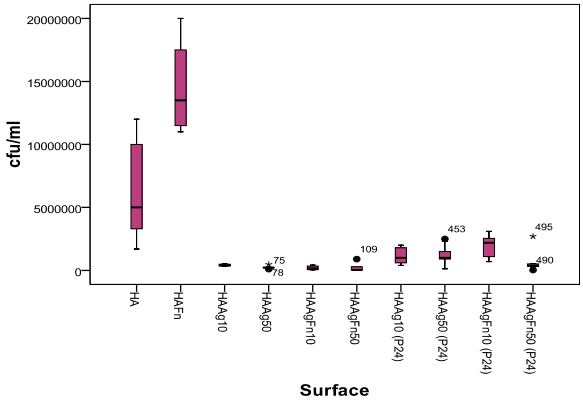

Figure 1. Direct colony counts for biofilm bacteria

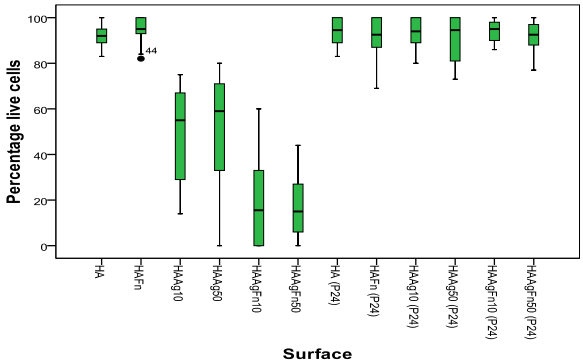

Figure 2. Percentage live cells

Conclusions: The HAAg surfaces tested in this study have antibacterial activity compared to HA controls before and after preconditioning in fetal calf serum. Preconditioning removes the detrimental effect of Ag on fibroblast viability. The clinical significance of the presence of cytotoxicity before preconditioning is unknown, but it is likely there would be a delay in soft tissue attachment. A study of the effect of preconditioning for longer time periods would be useful to determine whether the antibacterial activity of these HAAg surfaces is maintained longterm. Challenges with other bacteria, including Pseudomonas Aeruginosa and bacteria from a clinical pin site infection will be carried out. Additionally, an *in vivo* study of antibacterial activity and soft tissue attachment using an established transcutaneous ovine model is planned.

References: 1. Subbiahdoss G. Eur Cell Mater 2010;19:205-13 **2.** Agarwal. Biomaterials 2010; 31:680-90 **3.** Chimutengwende-Gordon. J Biomed Mater 2011:6:0250008**4.** Ghani Y. J Orthop Res 2012; 30:356-63