Antimicrobial Hybrid Coatings for External Fixation Pins

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Statement of Purpose: External fixation pins are the standard of care to stabilize complex fractures. However, like other percutaneous devices, these suffer from limited soft tissue integration, resulting in skin invagination (epithelial downgrowth), inflammation, infection, device loosening and failure. While superficial infections are more common than deep sepsis, both cause patient pain and interfere with rehabilitation. We have created a series of metal oxide and polymer hybrid materials which influence human cellular and bacterial bioactivity in a drug-like fashion. These materials been screened *in vitro* for cellular proliferation, attachment, elution of bioactive components, photoactivity and antimicrobial potential and applied to several small animal models. In this study we use rapid screening *in vitro* techniques to select optimal chemical composition of nanotechnology hybrid coatings. The application method was then optimized for use on stainless steel external fixation pins, which were surgically implanted and explanted from pig long bones to determine durability of coatings. This durability study was in anticipation of a live pig study comparing coated and control pins inoculated with drug-resistant *Staph. aureus.* The porcine model allows for use of FDA approved human medical devices for the evaluations and is the preferred species for similarity to human skin. Developments made toward improving soft-tissue sealing around these devices and reducing infection, have carryover value with other percutanous implants and for the creation of anti-infective orthopaedic devices.

Methods: *Cell growth assays:* To measure proliferation of human fibroblasts and keratyocytes, seeded microplates were incubated at 37 °C with 10 % CO_2 for 48 hours, after which 10 µl of WST-1 (Roche Applied Science, Indianapolis, IN) was added into each well and incubated for 3 hours at 37 °C. The optical density within each well, resulting from the cellular metabolism of the tetrazolium salt, was quantified using a microplate reader for absorbance at 440 nm and plotted. *Bacterial growth assay:* To determine the antimicrobial properties of the coatings, the change in optical density (OD) within microplates filled with 200 µl of Luria-Bertani broth inoculated with *Staph. aureus* ($1.2x10^4$ CFU/ml) was measured every 15 minutes for 20 hrs to establish growth curves. Onset time was defined within the exponential growth phase as 0.2 OD at 578nm. Polystyrene, titanium oxide, pure silver coatings and Degussa P25 titanium dioxide photocatalyst were used as controls. *Durability:* Coated and control implants were percutaneously placed in the femurs and tibia of 45 kg pigs (Fig 2, left). Insertion and extraction torques were measured with a digital gauge before SEM analysis. *Surface Analysis*: Coatings were inspected under optical microscopy. Self drilling/tapping, stainless steel pins were coated with optimized hybrid for scanning electron microscopy (SEM) and durability testing.

Results: The influence of the hybrid composition was dependent on cell type, with osseoblasts and chondracytes being less sensitive to noble metal concentration than fibroblasts and keratyocytes. No bacterial growth was seen on hybrids with noble metal concentrations greater than 0.2 wt% (Fig 1). The hybrid coatings had a sub-micron scale phase separated microstructure. Air-dried implants had a reticular surface with micro porous texture, compared to the control surface. The coating topography changed from the shearing action of insertion into and extraction from the bone. Little change was noted on the portions of the pins which penetrated the soft-tissues (Fig 2, right).

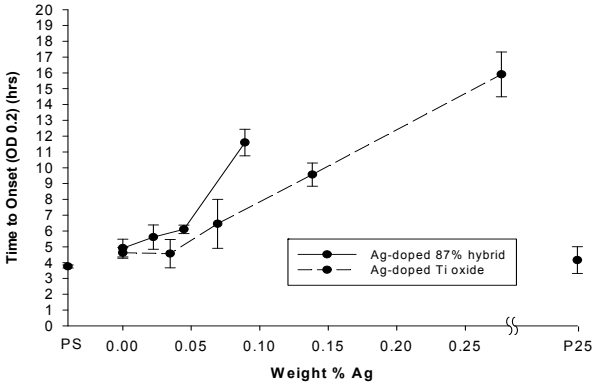

Fig 1. Bacterial growth stopped by doping hybrid coatings with greater than 0.2 wt% noble metals

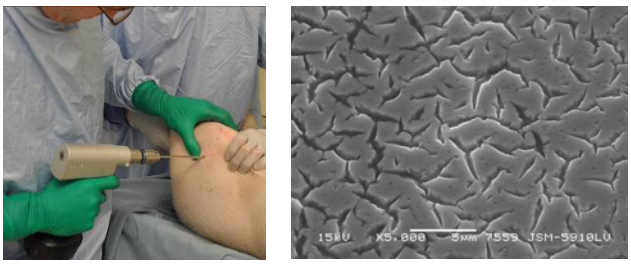

Fig 2. Insertion of pin (left), SEM of coating after insertion through soft-tissue of skin and muscle (right).

Conclusions: Metal oxide and polymer hybrid coatings may be rapidly screened for optimal antimicrobial and mammalian cell proliferation and adhesion properties using a coated microplate approach. By this *in vitro* method, we identified an optimized coating to reduce bacterial growth, while supporting cell growth for healing. Hybrid coating on external fixation pins experienced deformation during insertion and extraction from bone, but remain unchanged from contact with muscle and skin.

References: (1) Jarrell JD. J Biomed Mater Res A, 2009;90A(1):272-281.