A Drug Eluting, Osseointegrative Phospholipid Coating for Titanium Implants

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Statement of Purpose: Phospholipid coatings, especially 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS), on titanium implants have been shown to enhance osteoblast activity, promote mineralization, and facilitate implant osseointegration *in vivo*.[1,2] In addition, numerous studies have shown phospholipids to be effective drug delivery agents.[3]

Electrostatic Spray Deposition (ESD) is a method of spraying a source material at a controlled rate onto a target material under the influence of a high voltage differential between source and target. ESD is able to create thin, conformal coatings with good control of coating morphology[4].

The goals of this work were to: (1) use ESD to apply thin coatings of onto titanium (Ti); (2) demonstrate controllable drug delivery capabilities of e-sprayed DOPS coatings by measuring the effects of ESD voltage used to apply the DOPS coatings on elution of gentamicin sulfate (GS), and; (3) demonstrate non-cytotoxicity of the coatings, typical osteoblast morphology, adhesion and spreading, along with positive effect of DOPS coatings on cell viability.

Methods: DOPS was dissolved in chloroform, to a concentration of 1.3% w/vol and e-sprayed onto Ti slabs (flat, commercially pure, 5 mm square, 0.016in. thickness). ESD parameters were: 14ml/hour pump rate; 8cm distance; 10, 12 and 14kV.

400 µg of GS were loaded onto titanium samples, sandwiched between two layers of e-sprayed DOPS. The OPA colorimetric assay[5] was used to detect GS that eluted over a period of 8 hours from the DOPS coatings.

Rat marrow stromal cells were seeded ($0.5E^6$/well) onto test coatings, successfully cultured and differentiated into osteoblasts. Calcein-AM stain was applied and live cells were visualized on a fluorescent microscope (470 nm) to verify non-cytotoxicity of the coatings. Standard, commercial assays were used to assess cell viability (MTT—BioAssay Systems, Hayward, CA), and intracellular protein content (BCA—BioAssay Systems). Cell morphology, adhesion and spreading were evaluated via scanning electron microscopy (SEM).

Results: DOPS coatings e-sprayed at 12kV exhibit 18% lower GS elution at 8 hours, and slower initial release (coefficients 0.18 vs. 0.24) than coatings e-sprayed at 10kV or 14kV (Fig. 1, 2). At 8 hours, 12kV DOPS coatings are eluting approximately 7µg of GS per hour.

E-sprayed DOPS coatings are not cytotoxic (Fig. 3, 4). Cells on DOPS coatings exhibit higher cell viability (results not shown), larger and more clustered populations (Fig. 4), increased adhesion (Fig. 5) and higher intracellular protein content (results not shown) than Plain Ti. SEM revealed osteoblast morphology and spreading on DOPS-coated surfaces (Fig. 5) and Plain Ti (results not shown) which is typical at the week 1 time point, but rounded morphology on GS-loaded surfaces (Fig. 6).

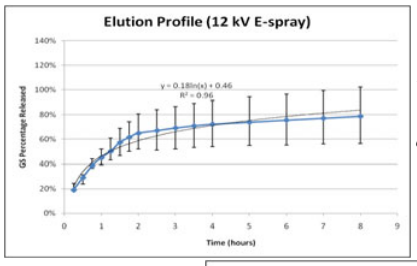
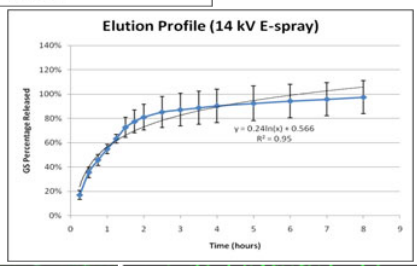


***Fig. 1**: GS elution from DOPS coatings applied at 12kV shows 18% lower GS elution at 8 hours and lower initial release than 14kV (Fig. 2)*

***Fig. 2**: GS elution from DOPS coatings applied at 14kV shows 18% higher GS elution at 8 hours and higher initial release than 12kV (Fig. 1)*



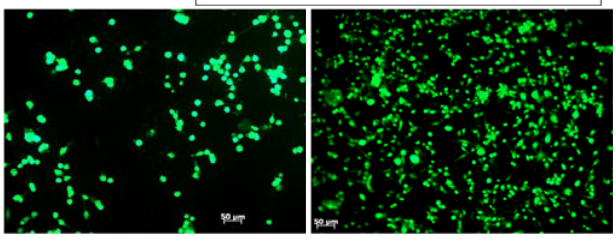


***Fig. 3,4**: Live cell stain (Calcein-AM) on Plain Ti (left) and DOPS coating (right), both images day 7 post-seeding*

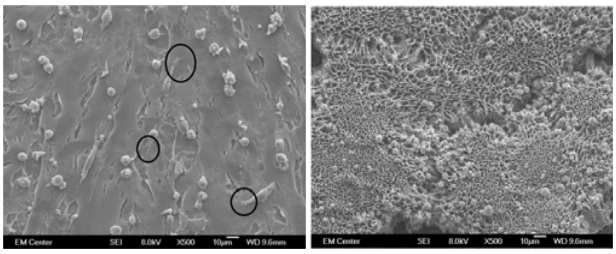


***Fig. 5,6**: Cells spreading on DOPS (circled, left) and rounded morphology on GS-loaded coatings (right); both images week 1 post-seeding*

Conclusion: We demonstrate that ESD is an effective technique for creating thin phospholipid coatings on Ti. Coatings applied with varying ESD voltages result in changes in GS elution, probably due to changes in the degree of packing and crystallization of the polar DOPS molecule. DOPS coatings exhibit higher cell viability, population spatial arrangement and density, protein content, and more typical cell morphologies than GS or Plain Ti coatings.

References: (1)Merolli, A. *et al*. *J Mater Sci Mater Med* 17, 789-794 (2006); (2)Santin, M. *et al*. *J R Soc Interface* 3, 277-281 (2006); (3)Cajal, Y. *et al. J Liposome Research*, 2(1), 11-22 (1992); (4)Prawel, DA. *et al. Society for Biomaterials. 31st Annual Meeting*, Seattle, WA. 712; (5) Cabanillas, PF. *et al. Intl. J Pharm.* 209, 15-26 (2000).

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