In Vitro and *In Vivo* Studies of a Novel System for Cytokine Local Delivery

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Abstract: Orthopaedic implants help millions of Americans stay active and independent. However, like other medical devices, orthopaedic implants are prone to infection, requiring patients to have a second operation for repair or replacement. In the present study, we aim to explore a new application of local delivery of cytokines for prevention of implant-associated infection. A recently developed nanotechnology, i.e., layer-by-layer self-assembly (LBL), has been used to construct polypeptide nanocoatings on commonly-used orthopaedic implants. Interleukin 12 (IL-12) has been successfully incorporated into the nanocoatings. A rat model has been developed, and the IL-12 incorporated nanocoatings may show promise in prevention of implant-associated infection.

Methods: LBL, mainly based on electrostatic attraction, was used to fabricate multilayer nanocoatings on stainless steel Kirschner wires (K-wires) and to incorporate a cytokine, i.e. IL-12, into the nanocoatings. The LBL procedure involves the repetitive sequential "dipping" of a substrate, e.g. a stainless steel K-wire, in solutions of positively-charged and negatively-charged polyelectrolyte solutions. Positively-charged (e.g. poly(ethylene imine), poly(allylamine hydrochloride), poly(L-lysine), and other polypeptides) and negatively-charged (e.g. bovine serum albumin, BSA) polyelectrolytes were used. IL-12 was dissolved in BSA solution at 20 μg/mL; BSA is a protein carrier. The LBL coating process was carried out at neutral pH under sterile laminar airflow conditions.

Results & Discussion: It has been suggested that IL-12 has therapeutic potential as a stimulator of cell-mediated immune response to microbial pathogens, metastatic cancer, and viral infection.[1-4] Sustained local delivery of IL-12 may enhance cell-mediated immunity and prevent orthopaedic implant-associated infection.

Multilayer nanocoatings were deposited on stainless steel K-wires, and IL-12 was incorporated in the nanocoatings. The release of IL-12 was tested by immersing the samples in a phosphate-buffered saline (PBS, pH 7.4). Aliquots of the PBS buffer were taken at certain times and quantified using an enzyme-linked immunosorbent assay (ELISA) kit. Fig. 1 shows that the release of IL-12 was a function of releasing time. In the one-day releasing period, approximately two times more IL-12 was released from the 20-layer sample than from the 2-layer sample.

To evaluate the efficacy of the IL-12 incorporated nanocoatings in prophylaxis of implant-associated infection, a femur fracture rat model was developed (Fig. 2). Infection was introduced by inoculating Staphylococcus aureus (*S. aureus*) at the site of the fracture; *S. aureus* is the predominant organism associated with infected metal implants. A series of in vivo experiments were carried out. Heavy growth of *S. aureus* on control samples without IL-12 while light growth of *S. aureus* on the IL-12 incorporated samples were observed.

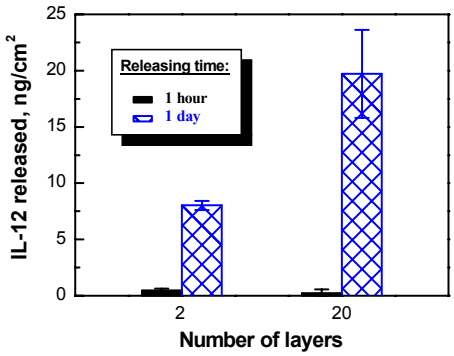

Fig. 1. The release of IL-12 versus number of polypeptide layers. The data shown is an average of three samples.

Acknowledgements: Financial supports from NASA WV EPSCoR and WVU PSCoR, and technical assistance from Nina Clovis and Suzanne Smith, are acknowledged.

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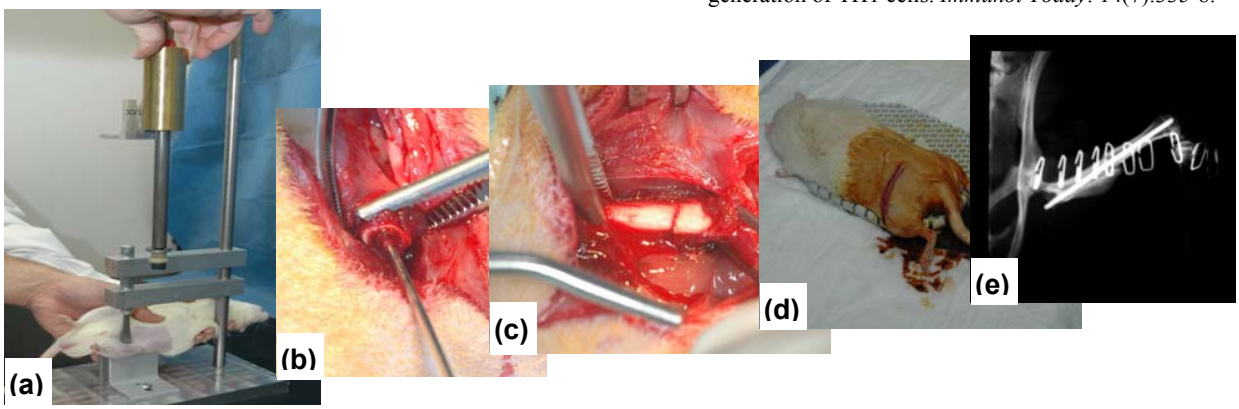

Fig. 2. A custom designed setup for creating a femur fracture (a), and our developed rat model (b-e). (b) and (c) show the fixation by intramedullary nailing of a K-wire. (d) shows the rat after closing the incision using skin staples. (e) represents a post operative X-ray.