Decoy Protein Delivery from Titanium Implants Modulates Inflammatory Response

Michael Keeney[1], Zhenyu Yao[1], Kensuke Egashira[3], Stuart Goodman[1], Fan Yang[1, 2]
[1]Department of Orthopedic Surgery and [2]Department of Bioengineering, Stanford University, CA, USA
[3]Department of Cardiovascular Research, Development, and Translational Medicine, Kyushu University, Japan

Statement of Purpose: Total joint replacement (TJR) is a very successful surgical procedure, however the long-term survivorship is limited by wear of the bearing surfaces. Wear begins during the initial "bedding in" phase and continues during use of the TJR. Wear particles stimulate chronic inflammation leading to peri-prosthetic bone loss (osteolysis), implant loosening and pathologic fractures. Derivatives of the monocyte/ macrophage cell line are among the key cells that perpetuate this adverse biological reaction. Monocyte Chemoattractant Protein-1 (MCP-1) is the most important chemokine regulating systemic and local trafficking of monocyte/macrophages in inflammation.

The goal of this study is to deliver a decoy MCP-1 protein (7ND) from an implant surface to block macrophage receptors, hence reducing local migration and production of inflammatory cytokines.

Methods: *Cell Treatment*: Human acute monocytic leukemia cell (THP-1) were used for all migration assays while mouse macrophages (RAW 264.7) cells were used for the production of inflammatory cytokines. RAW cells were seeded onto 24-well plates in the presence of polymethylmethacrylate (PMMA) particles (0.05 vol/vol), LPS (10μg/ml) or cell culture media alone as a negative control. The supernatants were collected after 48 hours and used for macrophage chemotaxis assays.

Chemotaxis Assay: THP-1 cells were pre-incubated with 7ND (0-100 ng/ml) for 1 hour. For raw cell supernatant studies, THP-1 cells were pre-incubated with 25 ng/ml 7ND. Chemotaxis assays were performed using a 96-well migration plate (NeuroProbe). An inflammatory signal (10 ng/ml MCP-1 or raw cell supernatant) was placed in the bottom chamber while 6×10^4 THP-1 cells were placed in the upper chamber. The chemotaxis assay was performed for 4 hours followed by quantification of migrated cells using a PicoGreen (Invitrogen) DNA quantification assay.

Controlled Deposition and Release: Titanium rods were coated with 7ND using a layer-by-layer technique. The layer-by-layer (LBL) coating was applied by the sequential deposition of positively (poly(β-aminoester)s) and negatively (polystyrene sulfate) charged polyelectrolytes. The 7ND protein was suspended in 1% BSA and attached to the positively charged layer. A total of 14 layers were applied (4 layers containing 7ND). Release studies were performed in PBS containing 0.1% BSA. Immunofluorescence was performed using a biotin labeled secondary antibody against MCP-1. FITC-avidin was used to detect the secondary antibody.

Results: 7ND inhibited macrophage migration in a dose dependant manner. (Fig. 1A) Pre-incubation of THP-1 cells with 25 ng/ml 7ND resulted in a 64% decrease in migration towards MCP-1. The 7ND protein blocked receptors for MCP-1, mitigating macrophage recruitment. A similar effect was observed when THP-1 cells were exposed to an inflammatory cytokine cocktail produced by RAW cells. The addition of 7ND dramatically decreased migration to a level equal to untreated cells (Fig. 1B). Addition of 7ND not only eliminated the chemotactic effects of MCP-1 protein, but also decreased the release of inflammatory cytokines from RAW cells, for example, TNFα, by approximately 30%. It was also shown that 7ND (0-100 ng/ml) did not affect THP-1 viability

Coating of the titanium rod was performed using only 14 layers, an order of magnitude lower than that reported in the literature. [1] Immunofluorescence demonstrated the successful deposition of 7ND on the surface of titanium. (Fig. 2A) A total of 440ng was released over 12 days. (Fig. 2B) The release rate was controlled by the chemical structure of poly(β-aminoester), specifically increased hydrophobicity of the polymer decreased the rate of release.

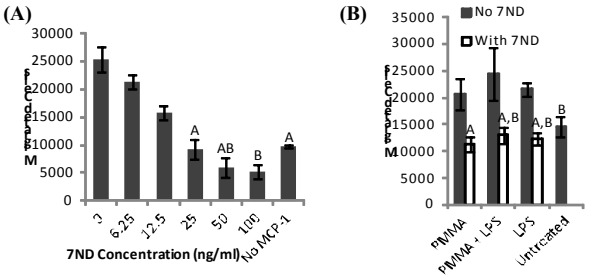

Figure 1. Migration of THP-1 cells to (A) 10 ng/ml MCP-1 and (B) inflammatory cytokines produced by RAW cells. THP-1 cells were pretreated with 7ND. Bars with similar letters are not statistically different. (n=6, $p<0.05$)

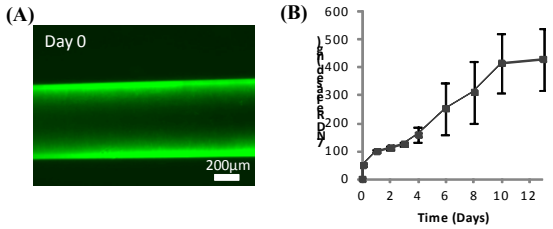

Figure 2. (A) Immunofluorescence showing 7ND coated on titanium rod using a LBL coating technique. (B) 7ND release from titanium rods.

Conclusions: MCP-1 is the most potent regulator of macrophage migration during the "bedding-in" process. We have demonstrated that the delivery of a decoy MCP-1 (7ND) results in decreased migration of THP-1 cells in response to MCP-1 and inflammatory cytokines produced by RAW cells. Furthermore, we have developed a highly efficient coating technique that enables the delivery of a biologically relevant concentration of 7ND from the surface of titanium rods. Taken together, we have demonstrated a novel strategy for the treatment of wear particle induced chronic inflammation.

References

[1] Macdonald et al. Biomaterials, 2011;32(5):1446-53

Acknowledgements: Supported by NIH grants 2R01 AR055650-05 and 1R01AR063717-01.

©2013 Society For Biomaterials