Biomaterials 117 (2017) 1–9

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Mutant CCL2 protein coating mitigates wear particle-induced bone loss in a murine continuous polyethylene infusion model

Akira Nabeshima [a], Jukka Pajarinen [a], Tzu-hua Lin [a], Xinyi Jiang [a], Emmanuel Gibon [a], Luis A. Córdova [a, c], Florence Loi [a], Laura Lu [a], Eemeli Jämsen [a], Kensuke Egashira [d], Fan Yang [a, b], Zhenyu Yao [a], Stuart B. Goodman [a, b, *]

[a] Department of Orthopaedic Surgery, Stanford University, Stanford, CA, USA
[b] Department of Bioengineering, Stanford University, Stanford, CA, USA
[c] Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Chile, Santiago, Chile
[d] Department of Cardiovascular Research, Development, and Translational Medicine, Kyushu University, Fukuoka, Japan

ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form
10 November 2016
Accepted 24 November 2016
Available online 24 November 2016

Keywords:
Osteolysis
Mutant CCL2 protein
Implant coating
Macrophage
Total joint replacement

ABSTRACT

Wear particle-induced osteolysis limits the long-term survivorship of total joint replacement (TJR). Monocyte/macrophages are the key cells of this adverse reaction. Monocyte Chemoattractant Protein-1 (MCP-1/CCL2) is the most important chemokine regulating trafficking of monocyte/macrophages in particle-induced inflammation. 7ND recombinant protein is a mutant of CCL2 that inhibits CCL2 signaling. We have recently developed a layer-by-layer (LBL) coating platform on implant surfaces that can release biologically active 7ND. In this study, we investigated the effect of 7ND on wear particle-induced bone loss using the murine continuous polyethylene (PE) particle infusion model with 7ND coating of a titanium rod as a local drug delivery device. PE particles were infused into hollow titanium rods with or without 7ND coating implanted in the distal femur for 4 weeks. Specific groups were also injected with RAW 264.7 as the reporter macrophages. Wear particle-induced bone loss and the effects of 7ND were evaluated by microCT, immunohistochemical staining, and bioluminescence imaging. Local delivery of 7ND using the LBL coating decreased systemic macrophage recruitment, the number of osteoclasts and wear particle-induced bone loss. The development of a novel orthopaedic implant coating with anti-CCL2 protein may be a promising strategy to mitigate peri-prosthetic osteolysis.

1. Introduction

Total joint replacement (TJR) is a highly successful and widely used surgical procedure for patients with end-stage degenerative and inflammatory arthritis. However the long-term survival of replacements is limited by wear of their load bearing surfaces [1–3]. Wear particles are inevitable byproducts of all TJR procedures; they stimulate chronic inflammation that delays osseointegration, leading to peri-prosthetic bone loss (osteolysis) and eventual loosening of the implant [4]. Currently, no clinically successful non-surgical treatments for wear particle-induced osteolysis exist. Revision surgical procedures are technically more difficult than primary procedures and carry high rates of complication. Therefore, new strategies are required for reducing wear particle-induced osteolysis to prolong the lifespan of TJRs.

Monocyte/macrophage lineage cells (macrophages, foreign body giant cells and osteoclasts) are the key players of chronic inflammation, the foreign body reaction to wear particles, and peri-prosthetic osteolysis [1,5,6]. In the peri-prosthetic tissues, macrophages are activated by wear particles to release pro-inflammatory cytokines and chemokines which induce systemic macrophage recruitment, thereby accelerating inflammation and subsequent bone resorption by osteoclasts [4,5]. Monocyte Chemoattractant Protein-1 (MCP-1, also known as CCL2) is one of the main chemokines regulating systemic and local trafficking of monocyte/macrophages in particle-induced inflammation. Osteoblasts and fibroblasts in the peri-implant tissue also release CCL2 in the presence of wear particles, however macrophages, especially M1 (pro-inflammatory) macrophages, are the main source of CCL2 secretion [7,8]. Retrieval studies have shown that the peri-implant

* Corresponding author. Department of Orthopaedic Surgery, Stanford University School of Medicine, 450 Broadway Street, Redwood City, CA, 94063, USA.
E-mail address: goodbone@stanford.edu (S.B. Goodman).

http://dx.doi.org/10.1016/j.biomaterials.2016.11.039
0142-9612/

tissue expresses high levels of CCL2 [9]. Furthermore, both wear particle-induced macrophage recruitment and bone loss were mitigated by inhibition of the interaction between CCL2 and its receptor CCR2 by systemic administration of a CCR2 antagonist in vivo [10]. 7ND recombinant protein is a mutant CCL2 protein, which lacks the amino acids 2 through 8 on the N-terminal, and acts as a dominant negative inhibitor of CCL2 [11–13]. Anti-CCL2 therapy has been successfully applied following drug-eluting coronary stents, which attenuated monocyte/macrophage infiltration and unwanted neointimal formation [14,15]. A previous in vitro study suggested that 7ND may be used as an inhibitor to reduce wear particle-induced macrophage migration and cytokine release [16]. Moreover, 7ND locally injected onto the calvaria reduced wear particle-induced osteolysis in a murine calvarial osteolysis model [17]. We recently developed a layer-by-layer (LBL) platform for coating implant surfaces, which can accomplish controlled release of biologically active 7ND protein [18]. We hypothesized that 7ND locally released from LBL coated implants can mitigate wear particle-induced macrophage recruitment and subsequent bone loss. In this study, we investigated the effect of 7ND protein coating on wear particle-induced bone loss using the clinically relevant murine continuous femoral intramedullary polyethylene (PE) particle infusion model with 7ND coating of a titanium rod as a local drug delivery device.

2. Materials and methods

2.1. 7ND coated rod

Hollow A-40 titanium rods (6 mm, 21 G, New England Small Tube, Litchfield, NH) were coated with a 7ND-releasing coating following a LBL method previously established [18]. End-modified poly (β-amino ester) (C32-130) was synthesized as described [18], and used as the positively charged polyelectrolyte (10 mg/ml). Polystyrene sulfonate (3 mg/ml) was used as the negatively charged polyelectrolyte. 7ND was suspended in 0.1% bovine serum albumin at 30 μg/ml. To aid the attachment of the coating, titanium rods were surface etched with concentrated hydrochloric acid for 2 h. Rods were then washed with H_2O and ethanol. After air drying, LBL deposition was performed with polyelectrolyte layers on the bottom deposited for 5 min, and the 7ND layer on top deposited for 10 min. Rods were washed in H_2O between layer depositions. The sustained release of 7ND from the surface coating was confirmed in vitro using a CCL2 ELISA Kit (Peprotech, Rocky Hill, NJ) and bioactivity was confirmed by macrophage migration assay as previously described [18].

2.2. Isolation of polyethylene particles and osmotic pumps

Clinically relevant conventional ultrahigh molecular weight polyethylene (UHMWPE) particles were a kind gift from Dr. Timothy Wright, Hospital for Special Surgery, New York. Particles were obtained from joint simulator tests and isolated according to an established centrifugation protocol [19]. Frozen aliquots of particle-containing serum were lyophilized for 4–7 days. The dried material was digested in 5 M sodium hydroxide at 60 °C for 1 h, followed by ultrasonication for 10 min. The digested particle suspension was centrifuged through a 5% sucrose gradient at 40,000 rpm at 10 °C for 3 h. The collected particles at the surface of the sucrose solution were incubated at 80 °C for 1 h before ultrasonication, and centrifugation through an isopropanol gradient (0.96 and 0.90 g/cm^3) at 40,000 rpm at 10 °C for 1 h. The purified particles at the interface between the two layers of isopropanol were collected. Isopropanol was evaporated from the particle mixture which was then lyophilized. After lyophilization, the particles were suspended in phosphate buffered saline (PBS) and kept in a −80 °C freezer. The particles tested negative for endotoxin by a Limulus Amebocyte Lysate Kit (Lonza, Allendale, NJ). The mean diameter of the particles was 0.48 ± 0.10 μm (mean ± standard error, averaged from 125 scanned particles) measured by electron microscopy. An Alzet mini osmotic pump model 2006 (Durect Corporation, Cupertino, CA) was loaded with PE particles (15 mg/ml) or PBS. The pumps and the titanium rods were connected via a 6 cm vinyl catheter tubing (Durect Corporation) which was prefilled with PE particle solution or PBS.

2.3. Mouse model of particle-induced osteolysis and experimental design

The murine continuous femoral intramedullary PE particle infusion model was performed as previously described [20]. Six experimental groups were established (Table 1): Group 1, PBS + control rod; Group 2, PBS + 7ND coated rod; Group 3, PE particles + control rod; Group 4, PE particles + 7ND coated rod; Group 5, PE particles + control rod + RAW 264.7 cell injection via the tail vein; Group 6, PE particles + 7ND coated rod + RAW 264.7 cell injection. For each group, twelve athymic nude male mice (Crl:NU(NCr)-*Foxn1*^{nu}, 11–12 weeks old, Charles River Laboratory Inc., Wilmington, MA) were used. The experimental design was approved by the Institutional Administration Panel for Laboratory Animal Care at Stanford University. Under inhalation anesthesia, we approached the right distal femur via a lateral parapatellar incision, and pierced through the intercondylar notch to access the medullary cavity by a series of needles (25-21 gauge). The titanium rod with or without 7ND coating was press-fit into the distal femur. An osmotic pump containing PE particles or PBS was implanted into dorsal side of the mouse subcutaneously through a second incision and connected to the implanted rod via subcutaneous vinyl catheter tubing. Skin incisions (knee and dorsal) were closed with 5-0 Ethilon sutures. Buprenorphine (0.1 mg/kg s.c.) was used for postoperative analgesia.

2.4. MicroCT

MicroCT scans were performed immediately pre-operation and 4 weeks after operation for Group 1 to 4 using eXplore Locus RS150 microCT (GE Healthcare, Fairfield, CT) with 49 μm resolution. At the post-operation scanning, mice were euthanized and the titanium rod removed from the distal femur before microCT scan was performed. A phantom that mimics hydroxyapatite and contains water and air inclusions was used for image calibration. A 3D region of interest (ROI, 4 mm × 4 mm × 3 mm) was created which contained only the diaphysis proceeding proximally, beginning 3 mm from the distal end of the femur. The thresholded bone mineral density (BMD) was quantified and analyzed by using GEMS MicroView software (threshold: 700 HU). BMD was normalized by the value of pre-operation scan (postop − preop value).

Table 1
Experimental design of murine model.

Group (n)	1 (9)	2 (9)	3 (9)	4 (9)	5 (11)	6 (12)
Rod	Control	7ND	Control	7ND	Control	7ND
PE particle			✓	✓	✓	✓
RAW cell					✓	✓

7ND, 7ND protein coating; PE, polyethylene; RAW cell, tail vein injection of RAW264.7 cells which expressed Green Fluorescent Protein and firefly luciferase as a reporter cell.