CELL-BASED OPG GENE MODIFICATION ON ASEPTIC KNEE IMPLANT LOOSENING IN A MOUSE MODEL

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STATEMENT OF PURPOSE: Wear debris-associated aseptic loosening remains the most common long-term complication following total joint replacement. Exogenous OPG gene modification at the osteolytic site appears to be a therapeutic strategy for periprosthetic bone resorption and aseptic loosening. The objective of the current study was to evaluate the feasibility and efficacy of a cell-based OPG gene delivery approach in halting and reversing the periprosthetic osteolysis and the implant loosening using a murine model of the knee prosthesis failure.

METHODS: Mouse fibroblast-like synoviocytes (FLS) were harvested from the knee joints of donor Balb/c mice and then cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin in a humidified 5% CO2 atmosphere. At confluence of 30-40%, the cells were co-cultured with $2x10^7$ particles/ml titer of AAV-eGFP-OPG and AAV-LacZ (adeno-associated virus encoding OPG or LacZ). The transduction efficacy was determined by fluorescent microscopy (for emission of GFP) and X-gal staining (for LacZ gene transduction).

All the surgical procedures on mice have been approved by the Institutional Animal Care and Use Committee. Under aseptic condition, the proximal tibia condyle of Balb/c mouse was surgically exposed; and a special-made titanium pin (Stryker Orthopedics, Inc) was press-fitted into the tibial plateau after intramedullary reaming to make the surface of the pin-head flush with the cartilaginous surface of the tibial plateau and contiguous with the joint surface. 20 μl of a titanium particle suspension was transferred into the tibia canal before the insertion of the pin, followed by 40 μl of titanium particle injected into prosthetic knee 4 weeks later. The mice were divided into 3 groups after surgery. Mouse FLS cells pre-transduced with either human OPG gene or β-galactosidase (LacZ) gene were transfused into the prosthetic joints of the mice in OPG or LacZ groups, respectively, at 3 weeks post surgery. The virus-free control group received intra-articular injection of the wide-type mouse FLS cells. The mice were sacrificed at 4 weeks after cell-based gene therapy for mechanical and histological evaluation.

Pull-out test: Soft tissue around proximal tibia bearing the Ti-pin implant was carefully removed to expose the pin surface while the distal tibia was cemented into a custom designed holding jig with dental cement. The head of the pin was secured between the blade holders of a custom-made fixture apparatus. The apparatus was then mounted on MTS 858 Bionix material testing system actuator for biomechanical pin-implant pullout test at 2.0 mm/min. Histology: Prosthetic joint tissues were stained with hematoxylin - eosin to examine new bone formation or erosion, interfacial inflammatory membrane thickness and cellular infiltration. TRAP staining was performed to localize mature osteoclast, and immunohistochemical stains for pro-inflammatory cytokines and osteoclast markers.

RESULTS: in vitro gene transfers mediated by AAV indicated more than 90% of transduction efficiency confirmed by GFP fluorescent signals and positive X-gal stains. The mice tolerated the surgical procedure well and ambulated with the implanted limb within 3 days after surgery. Transgene Engineered FLS cell transfusion to the prosthetic joint did not change mouse activity. Pull-out test indicated that the FLS-AAV-OPG treatment significantly increased the implant stability, with 16.3±2.7 N of pulling force to dissociate the implant from the bone (Fig 1, $p<0.05$); while The average of the peak interfacial shear strength against pulling was 7.3±1.4 N and 8.1±1.2 N on virus-free wild-type FLS group and the FLS-AAV-LacZ treated group, respectively. Histological evaluation revealed the development of extensive inflammatory peri-implant membranes in either FLS-AAV-LacZ or virus-free group, while the interfacial membranes in OPG-treated group was significantly thinner. Mature osteoclasts within the peri-implant tissues were evaluated by TRAP staining. There were few TRAP-positive cells present at the bone surfaces in OPG gene-transferred prosthetic joint. In contrast, ubiquitous TRAP+ osteoclasts were observed within periprosthetic tissues in the LacZ-treated group and virus-free controls. Immunohistochemical stains also showed a decreased accumulation of TNFα, IL-1β and CD68+ expressing cells in the OPG gene-treated particle stimulated periprosthetic tissue sections.

DISCUSSION: The data suggest that the exogenous OPG gene was stably transduced into FLS, and the transgene-engineered cells survived in the prosthetic joints to subsequently secrete OPG protein. The cell-based gene modification obviously inhibited Ti particle-induced local osteolysis by blockage of the osteoclastogenesis and inflammatory cell infiltration. The murine model of the knee prosthesis appears an excellent tool to examine the implant stability by biomechanical pull-out test. Long-term study is warrant to evaluate the fate of the engineered cells and the safety issues

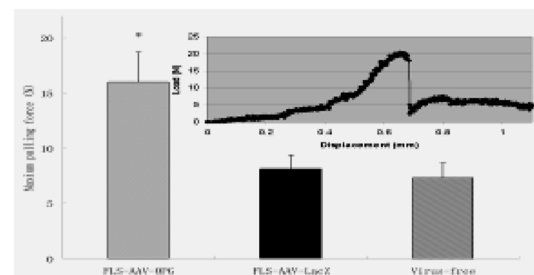

Fig 1: The average peak extract forces required to pull out the implants from proximal tibiae among groups