**Efficacy of Ex Vivo OPG Gene Therapy in Preventing Wear Debris Induced Osteolysis**

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**Introduction:** Irreversible joint destruction caused by various arthritides leads to total joint arthroplasty. There are currently more than 400,000 arthroplasties performed annually in the United States to treat this condition. Up to 20% of arthroplasties will require revision surgery due to aseptic loosening. This complication of arthritic treatment constitutes a major source of morbidity associated with this disease and represents billions of dollars in health care costs. The current paradigm to explain aseptic loosening is that wear debris generated from the prosthesis are phagocytosed by macrophages, which produce pro-inflammatory cytokines (i.e., TNF-α, IL-1, IL-6) in response to the particles. These cytokines subsequently promote the genesis of an erosive pannus, which stimulates osteoclasts to resorb the bone. It has been shown that osteoprotegerin ligand (OPGL, also called RANKL, ODF, TRANCE) stimulates the differentiation of osteoclast progenitors to mature osteoclasts. Furthermore, it has also been shown that osteoprotegerin, OPGL’s soluble decoy receptor, has the ability to inhibit this stimulation. Using a murine calvaria model that we have developed as a model of periprosthetic osteolysis, our group explored the use of murine fibroblast-like synoviocytes stably transfected with OPG, as a vector for ex vivo gene therapy for aseptic loosening.

**Methods:** Creation of the Stable Cell Lines. The OPG (a gift from Amgen, Inc.) and LacZ cDNA fragments were both cloned into the pLNCX retroviral vector (Clontech). The pClN-OPG and pLCN-LacZ target vectors were transfected along with the pMDG envelope vector, and pRSV-LacZ using calcium phosphate into the 293GP (gag/pol) packaging cell line (Clonetech). The primary fibroblast-like synoviocytes (FLS) were isolated and cultured (DMEM/10% FBS/1% Pen/Strep) from the knees of CBAxB6 mice in as was previously described. Two days post-transfection the supernatant was collected off the transfected 293GP cells and placed on the murine FLS, which were at 40% confluence. After the removal of the retroviral media, the 293GP cells were stained with X-gal (1mg/ml) and analyzed for β-galactosidase staining and ELISA, for OPG concentration in culture. Furthermore, based on soon to be published experiments, we feel that the amount of OPG being produced in culture would be substantial enough, when delivered locally, to alter the degradation of bone associated with this disease. The stably transfected cells were producing the OPG soluble protein in culture was also done using a commercially available ELISA kit. (R&D Systems) The resulting stable cell lines were then verified using X-gal staining and ELISA, for β-galactosidase expression, respectively. OPG ELISA. The measurement of OPG concentration in culture was done using a commercially available ELISA kit. (R&D Systems) The optical density was determined at a wavelength of 450 nm using an ELISA microplate reader (Bio-Rad). A standard curve was constructed (R²=0.93) and the concentration on the OPG in the conditioned media was calculated. In Vivo Experiments. In vivo murine model for aseptic loosening is one in which 30 mg of titanium particles are surgically implanted directly on the calvaria of the mice. These animals display an enormous response to the titanium, with extensive inflammation and bone resorption. The stably transfected cell cultures were harvested, counted, washed once with PBS, and were then injected onto the mouse calvaria, using a 26 gauge syringe, 1 day prior to the surgical implantation of the titanium particles. A SHAM control was also done in which 50 µl of phosphate-buffered saline (PBS) was injected instead of cells and while an incision was made on over the calvaria, no titanium was implanted. A total of 1 x 10⁷ cells, resuspended in 50 µl of PBS, were implanted on each of the LacZ and OPG treatment group calvarias. (Sample size of each group, n=3) The calvarias were subsequently harvested 10 days post-titanium implantation, paraffin-embedded, histological sections were made, and bone loss was calculated via histomorphometry. As per our groups previous experiments using this model, Day 10 was determined to be the time point of maximal bone loss.

**Results:** Using ELISA we were able to determine that the rate at which our stably transfected cells were producing the OPG soluble protein in culture was 0.3 ng/ml/72 hours. Our LacZ stably transfected cell line produced no detectable OPG. The results of the in vivo experiments are shown in Figure 1 below. We show the resolution response, in terms of the sagittal suture area (mm²), in each of our 3 experimental groups: SHAM (32 ± 0.05 mm²), treatment with LacZ stably transfected FLS (57 ± 3 mm²), and treatment with OPG stably transfected FLS (57 ± 2 mm²). [p-values: * p<0.18, ** p<0.08, *** p<0.018]

**Figure 1:**

**Discussion:** The potential for ex vivo gene therapy in musculoskeletal disorders is well documented and has already lead to the first-ever clinical trials investigating the use of such an approach in treating arthritis. Ex vivo gene therapy is a highly invasive and technically demanding treatment option, however these drawbacks ultimately give way the superior safety advantages that can be built into using such an approach in clinical trials. The most significant of which is the ability to conduct all genetic manipulations outside of the body, which allows for the use stringent testing of the cells prior to re-implantation to ensure that no viral particles or other substances are being introduced into the body. We have shown above that it is possible to produce stably transfected cell lines, which are able to produce detectable amounts of OPG in culture. Furthermore, based on soon to be published experiments, we feel that the amount of OPG being produced in culture would be substantial enough, when delivered locally, to alter the degradation of bone associated with the titanium stimulation. Our in vivo results, while not statistically significant, potentially due to sample size, lend cautious optimism for this to be the case and further in vivo experiments are currently underway to fully investigate the potential efficacy of using such a treatment in our model.

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**References:**