# *Ex Vivo* Gene Therapy in Combination with a Novel Tubular Sintered Microsphere Scaffold: The *In Vivo* Delivery of BMP-2 Producing Cells

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**Statement of Purpose:** A tissue engineering approach to bone regeneration requires the use of a scaffold, biological factors, and cells, alone or in combination (1). The delivery of recombinant growth factors using a polymeric matrix has been a major challenge, as these proteins possess a short half-life and are highly sensitive to the solvents and temperatures used during scaffold fabrication. In this study, we investigated an *ex vivo* gene therapy approach for the delivery of human bone morphogenetic protein-2 (BMP-2) in combination with a three-dimensional poly(lactide-*co*-glycolide) (3D-PLAGA) tubular sintered microsphere matrix. We hypothesized transfected cells seeded in the tubular matrix would deliver BMP-2 and promote bone regeneration in a critical size ulnar defect. The results indicated the tubular scaffold design deterred cell migration from the defect site *in vivo* and that *ex vivo* gene therapy may be used in combination with the 3D-PLAGA tubular sintered microsphere matrix to deliver BMP-2 for the enhancement of mineralized tissue formation at a bone defect site.

**Methods:** The 3D-PLAGA tubular sintered microsphere matrix was fabricated as previously reported (Figure 1) (2).

A replication defective human adenovirus serotype 5 encoding human BMP-2 (Ad-BMP2) or β-galactosidase (Ad-β-gal) was constructed. Transgene expression was driven by a CMV promoter.

Primary bone marrow stromal cells (BMSCs) were isolated from New Zealand White rabbits and characterized using FACS (3). BMSCs were infected (MOI = 40) using the Ad-BMP2 or Ad-β-gal vector. Non- and BMP-2-transfected BMSCs were trypsinized and plated in the scaffold at a seeding density of $3.5x10^6$ cells/scaffold. Cell seeded implants were cultured in DMEM at 37°C/5% $CO_2$ for 24 hours prior to implantation in a New Zealand White rabbit ulnar defect model. The ulnar defect surgical procedure (4) was conducted using the implants listed in Table 1.

Acquired X-rays were used for quantitative densitometry analysis (data not shown). The duration and distribution of transgene expression was monitored every 2 weeks following the implantation of 3D-PLAGA scaffold + β-gal transfected BMSCs. The implant, surrounding muscle facia and contralateral limb were stained to detect LacZ expression. An Instron (Canton, MA) was used for quantitative biomechanical testing of the experimental limbs. MicroCT analysis (Scanco Medical AG, Bassersdorf, Switzerland) was used to construct 3D renderings of mineralized tissue formation. Histological processing and analysis are in progress.

Quantitative data was reported as mean ± standard deviation. A single factor ANOVA and Tukey *post hoc* test were used to determine statistical significance between the various groups.

**Results:**

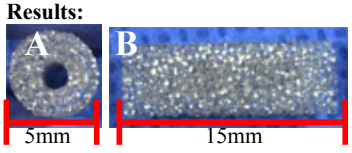


**Figure 1:** Photograph of the 85:15 PLAGA tubular sintered microsphere matrix (**A**) top view and (**B**) side view.

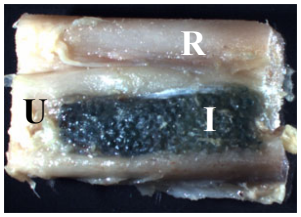


**Figure 2:** Photograph, following staining for LacZ expression, of the experimental limb containing the 3D-PLAGA scaffold + β-gal expressing BMSCs at 8 weeks post-operative. The positive stain (green) indicated long-term transgene expression of the marker gene was achieved.
R = radius; U = ulna; I= implant

**Table 1:** Experimental groups

| Implant | Abbreviation |
|---|---|
| Unoperated contralateral limb | control |
| NZW rabbit ulna segment | allograft |
| 3D-PLAGA scaffold | s |
| 3D-PLAGA scaffold + 5µg rhBMP-2 | s + bmp2gf |
| 3D-PLAGA scaffold + nontransfected BMSCs | s + nontransfected cells |
| 3D-PLAGA scaffold + bmp2 transfected BMSCs | s + bmp2 transfected cells |

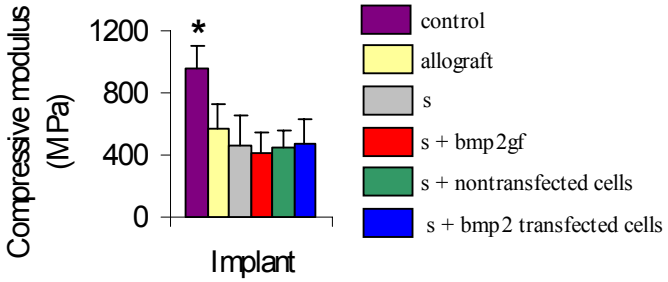


**Figure 3:** At 10 weeks post-implantation, the compressive modulus of the implants was in the range of trabecular bone. The contralateral limb (control), being cortical bone, had a significantly greater compressive modulus as compared to the other experimental groups (*). ($p<0.05$)

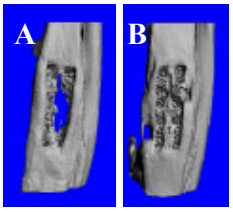


**Figure 4:** MicroCT image of the (**A**) s + bmp2gf and (**B**) s + bmp2 transfected cells at 10 weeks post-implantation. While all experimental implants supported the formation of mineralized tissue, a greater quantity was observed throughout the pores of the matrix seeded with BMP-2 transfected cells.

**Discussion and Conclusions:** Genetically modified BMSCs seeded in a 3D-PLAGA tubular sintered microsphere matrix delivered BMP-2 at a critical size ulnar defect. As endogeneously synthesized proteins have been shown to have a greater biological activity than the recombinant form of the protein (4), the transfected cells were seeded at a density, determined by quantitative *in vitro* release studies (data not shown), to initially deliver $1/10^{th}$ the quantity of factor adsorbed to the s + bmp2gf experimental group. A gene therapy approach to factor delivery ensured the protein was not subject to polymer processing conditions and allowed BMP-2 to be delivered locally for an extended period of time. *In vivo* transgene expression was detected for the full 10 weeks of the study and the distribution of transfected cells was limited to the matrix (Figure 2). We attributed the absence of cell migration to the seeding of cells in the center of the scaffold as opposed to the traditional method of seeding cells on the outer surface of the matrix. Densitometry and microCT analysis indicated mineralized tissue was being formed in all experimental implants (Figure 4). Compression testing (Figure 3) indicated the graft carries the potential to serve as a trabecular bone substitute.

**References:** **(1)** Langer R, *et al.* Science. 1993; 260: 920-6. **(2)** Kofron MD, *et al.* J Biomed Mater Res., 2006, *In Press.* **(3)** Kofron MD, *et al.* Trans. Soc. For Biomat. 2006, 572. **(4)** Borden M, *et al.* J Bone Jt Surg, Br., 2004; 86(8): 1200-8. **(5)** Baltzer AWA, *et al.* Clin Orthop Rel Res. 2000; 379S: S120-5.