# Polymer-coated Albumin Nanoparticles for Bone Morphogenetic Protein-2 Delivery: Pharmacokinetics and Osteoinduction Study

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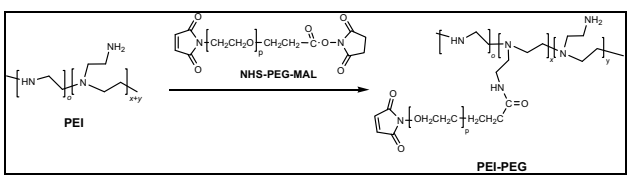
**Introduction:** Bone-inducing growth factor, bone morphogenetic protein-2 (BMP-2), is capable of causing commitment of undifferentiated mesenchymal stem cells into bone-depositing osteoblasts. Currently, BMP-2 has become part of a new treatment modality in orthopaedic practice [1]. Since BMP-2 is able to induce ectopic and orthotopic bone formation, the localization of the delivered growth factor is of great importance. The clinical delivery attempts have relied on adsorption of the protein in collagen-type biomaterials; simple protein desorption from the implanted biomaterial has provided the necessary sustained activity (or concentration) at the local site. A better way to control the BMP-2 delivery kinetics is to encapsulate the protein in nanoparticles (NPs) so that the physicochemical properties of the NPs can control the BMP-2 release profile in a more precise way. This study attempted to develop one such system, where BMP-2 was encapsulated in albumin NPs, which were then coated with cationic polymer by electrostatic interaction in order to control the release process. By subcutaneous implantation of the NPs into rats, the pharmacokinetics and osteoinductivity of the released BMP-2 from different NP formulations were determined.

**Methods:** The BMP-2 (produced in *E. coli* [2]) encapsulated in bovine serum albumin (BSA) NPs was obtained by a coacervation process using ethanol as non-solvent [3]. The NPs were stabilized by coating with polyethylenimine (PEI), or polyethylene glycol (PEG) modified PEI (PEI-PEG, **Scheme 1**). The physiochemical properties of the NPs were characterized, including size, zeta potential and morphology. The toxicity and the bioactivity of the NPs were examined by MTT and Alkaline Phosphatase (ALP) induction assay with human C2C12 cells, respectively. The *in vivo* pharmacokinetics of BMP-2 release from different NP formulations was assessed by using $^{125}$I-labelled BMP-2. The NPs were loaded in collagen sponge, and then subcutaneously implanted into rats. At designated time points, the implants were recovered, and the associated counts were determined. The osteoinductivity of the encapsulated BMP-2 was studied by ectopic bone formation in rat subcutaneous model. The implants were recovered at indicated time points, scanned with micro-CT for the quantification of bone volume (mm$^3$) formed in the implants. The ALP activity and calcium deposition in the implants were also assessed.




**Scheme 1.** Conjugation of PEG to PEI using MAL-PEG-NHS linker

**Results/Discussion:** The coacervation process resulted in spherical BSA NPs with size of 150-200 nm. The PEI-PEG coating produced smaller size NPs than the PEI coating (100-150 nm vs. 200-250 nm). The zeta potential of NPs increased from negative to positive as a result of polymer coating, with the highest zeta potential for the PEI coated NPs. The pharmacokinetics of *in vivo* BMP-2 retention showed that the PEI coated NPs retained significantly higher percentage of BMP-2 than the PEI-PEG coated NPs and the uncoated NPs in a 7-day study period (**Figure 1**). The unstable nature of the uncoated NPs, and the increased surface area as well as the reduced electrostatic interaction between polymer and albumin of the PEI-PEG coated NPs probably contributed to the faster release of BMP-2 from these two NP formulations. The osteoinductivity study of the BMP-2 delivered by different NP formulations showed that the uncoated NPs and the PEI-PEG coated NPs induced more bone formation at earlier stage than the PEI coated NPs, presumably due to the faster release of BMP-2 (**Figure 2**).

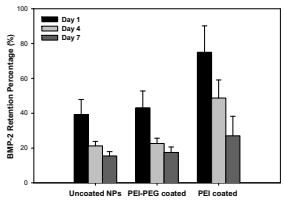



**Figure 1.** *In vivo* pharmacokinetics of BMP-2 released from different NP formulations. The PEI coated NPs demonstrated significantly higher BMP-2 retention than the other two groups at all time points ($p<0.05$).

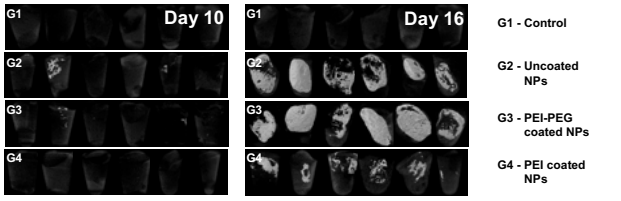



**Figure 2.** Micro-CT images of calcium deposition in the recovered implants for different NP formulations at day 10 and day 16 post-implantation.

**Conclusions:** We conclude that BMP-2 can be encapsulated in BSA NPs in a bioactive form. Coating the NPs with PEI resulted in better control of the BMP-2 release rate, which can be further altered by PEG modified PEI. Future studies will be directed on different PEG substitution on PEI for desired BMP-2 release rate, as well as using targeting-ligands conjugated to PEI for active targeting of NPs to bone.

**References:** [1] Jones, A.L. et al., *J. Bone. Joint Surg.*, 2006, 88:1431-1441. [2] Chatzinikolaidou, M. et al., *Materialwiss. Werkstofftech.*, 2003, 34:1106-1112. [3] Zhang, S. et al., *Biotechnol. Prog.*, 2008, 24:945-956.