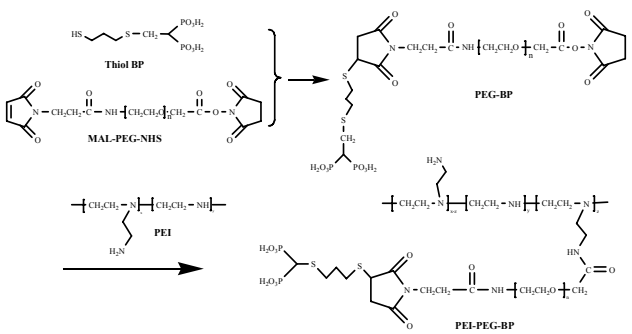
Preparation of PEI-PEG-BP Coated Albumin Nanoparticles as Delivery System for BMP-2

Guilin Wang[1], Sufeng Zhang[1], Xiaoyue Lin[1], Cezary kucharski[1], Hasan Uludag[1-3]
[1]Department of Chemical & Materials Engineering, Faculty of Engineering, [2] Faculty of Pharmacy & Pharmaceutical Sciences, [3]Department of Biomedical Engineering, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada T6G 2G6

Introduction: Bone morphogenetic protein-2 (BMP-2) has been identified as a potential therapeutic agent for bone regeneration because it can induce the differentiation of mesenchymal stem cells into bone-depositing osteoblasts. However, the short *in vivo* half-life of BMP-2 made it necessary for the development of a carrier system for efficient BMP-2 delivery. We recently reported the polyethylenimine (PEI) coated bovine serum albumin (BSA) nanoparticles (NPs) as an approach to control the release rate of encapsulated BMP-2 and protect its bioactivity [1]. The present study was aimed to optimize the BSA NPs with respect to their physicochemical, biological properties and bone-seeking ability by coating the NPs with PEI modified by polyethylene glycol (PEG) and bisphosphonate (BP).

Methods: PEG was conjugated to PEI by the linker N-hydroxysuccinimidyl-PEG-maleimide (MAL-PEG-NHS). BP with a thiol group [2] was then linked to PEI-PEG via the maleimide end of MAL-PEG-NHS to form the PEI-PEG-BP (**Scheme 1**). The conjugates were characterized by ^{1}H NMR, phosphate assay and copper/PEI complex assay for BP conjugation efficiency (i.e. the average number of BP per PEI). BSA NPs were prepared by a coacervation method [3] with acetone as the non-solvent. For the polymer-coated NPs, an aliquot of BSA NP suspension was mixed with the same volume of PEI-PEG or PEI-PEG-BP solution dissolved in phosphate buffer (pH=7.4). The coated NPs were purified by dialysis and characterized by size, zeta potential and morphology. BMP-2 was encapsulated into the polymer-coated BSA NPs during the particle formation. The toxicity of the NPs and the activity of the encapsulated BMP-2 were examined by MTT and alkaline phosphatase induction (ALP) assay with human C2C12 cells, respectively.

Results/Discussion: The graft of PEG on PEI backbone was confirmed by ^{1}H NMR. The BP conjugation efficiency was increased linearly as the concentration of the linker MAL-PEG-NHS used for the reaction increased (**Figure 1A**).



Scheme 1. BP Conjugation to PEI via NHS-PEG-MAL

The PEGylation of PEI reduced the bone mineral (hydroxyapatite, HA) affinity of PEI, while BP conjugation recovered it to a level of ~100%. The zeta potential of PEI-PEG coated BSA NPs decreased as compared with the PEI coated NPs. The PEI-PEG-BP coating reduced the zeta potential of NPs more significantly due to the presence of the negatively charged BP (**Figure 1B**). The BP conjugation efficiency influenced the surface charge of NPs, as higher BP substitution on PEI leading to lower zeta potential. The particle size of PEI-PEG or PEI-PEG-BP coated BSA NPs was typically ~100 nm, much smaller than PEI coated NPs.

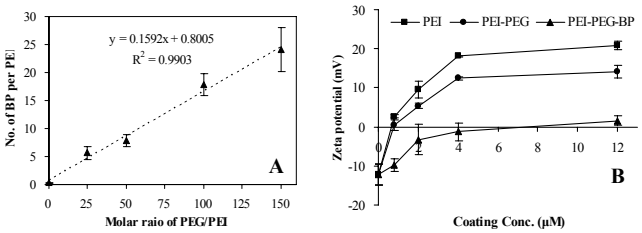


Figure 1. BP conjugation efficiency (**A**) and zeta potential (**B**) of the uncoated and coated NPs with PEI, PEI-PEG or PEI-PEG-BP.

Based on the *in vitro* bioassays, no significant toxicity on human C2C12 cells was observed for the uncoated BSA NPs and coated NPs, except for PEI at the highest concentration tested (**Figure 2A**). This indicated the reduced toxicity of PEI by PEG modification. The ALP results demonstrated that the retention of BMP-2 bioactivity for coated NPs was equivalent to free BMP-2, and significantly higher than the uncoated ones (**Figure 2B**).

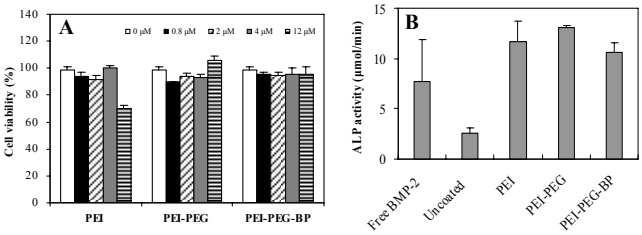


Figure 2. The cytotoxicity determination of different NPs (**A**) and the ALP activity of the encapsulated BMP-2 (**B**).

Conclusions: The PEI-PEG-BP coated BSA NPs provide a potential delivery carrier for BMP-2. They displayed several beneficial features in comparison with PEI coated NPs: smaller particle size, lower surface charge, and decreased toxicity. The bone specific targeting of the described NPs and the efficiency of sustained-release formulations on BMP-2 induced bone formation will be investigated in future study.

References: [1] Zhang S., et al., Biotech Prog. 2008; 24: 945-956. [2] Zhang, S., et al, Biomacromolecules, 2005; 6: 2800-2808. [3] Langer K., et al., Int J Pharm. 2003; 257:169-180.