Multifunctional Block Copolymer Nanoparticles for Controlled Release of Bone Morphogenetic Protein 2

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Statement of Purpose: Bone morphogenetic protein 2 (BMP-2) is a potent cytokine that enhances the recruitment of mesenchymal progenitors to cartilage condensation, regulates their chondrogenic development and stimulates the synthesis of cartilage matrix.[1] BMP-2's high diffusivity in the cartilage tissue, combined with its susceptibility to enzymatic degradation, necessitates the employment of drug delivery systems[2] to maximize its therapeutic potential. Nanoparticles derived from poly(acrylic acid) (PAA)-based amphiphilic block copolymers are attractive vehicles for the delivery and controlled release of BMP-2 due to the presence of a multifunctional, hydrophilic corona suitable for ligand immobilization (Figure 1). In addition, the segregated core can be utilized as a reservoir for hydrophobic molecules that, when released in a temporal manner, can synergistically enhance the chondrogenic activities of BMP-2. We have synthesized amphiphilic diblock copolymers comprised of a hydrophilic, poly(acrylic acid) (PAA) block and a rubbery, hydrophobic poly(*n*-butyl acrylate) (P*n*BA) (PAA-*b*-P*n*BA)[3] block and multiblock copolymers consisting of a hydrophilic PAA block alternating with glassy, hydrophobic poly(styrene) (PS) segments $(PAA\text{-}b\text{-}PS)_n$. Both polymers self-assembled into defined micellar structures. Partial esterification of the PAA block in both types of copolymers led to the introduction of acrylate groups, through which a heparin-binding peptide derived from antithrombin III (ATIII) was conjugated. BMP-2 was released from these multifunctional nanoparticles in a controlled manner over a prolonged period of time.

Methods: P(AA-*b*-P*n*BA) was synthesized following previously reported procedures.[3] The multiblock copolymer, $(PAA\text{-}b\text{-}PS)_n$, was synthesized by multi-step chemical transformations. Atom transfer radical polymerization was employed for the synthesis of a poly(*t*-butyl acrylate) (P*t*BA) macroinitiator using 3-(1,1,1-trimethylsilyl)-2-propynyl 2-bromo-2-methylpropanoate as the initiator, CuBr as the catalyst and N,N,N',N',N''-pentamethyldiethylenetriamine as the ligand. The macroinitiator was chain extended with PS under similar reaction conditions. The resulting copolymer was treated with sodium azide, followed by tetra-*n*-butylammonium fluoride to afford an α-alkyne, ω-azide P*t*BA-*b*-PS. The multiblock copolymer was synthesized by condensation polymerization of α-alkyne, ω-azide P*t*BA-*b*-PS employing copper-catalyzed azide-alkyne cycloaddition reaction. After the removal of the *tert*-butyl groups by trifluoroacetic acid, the acrylate groups were introduced to the PAA block by partial esterification with 2-hydroxyethyl acrylate (HEA). Nanoparticles were obtained after extensive dialysis against water. Direct mixing of ATIII peptide (CK(βA)FAKLAARLYRKA) with acrylate-decorated nanoparticles led to the coupling of the peptide to the nanoparticles. Particles were subsequently immersed in a BMP-2 loading buffer containing a pre-determined amount of heparin. The BMP-2 loading and release was measured using the BMP-2 ELISA kit.

Results/Discussions: We have synthesized two types of amphiphilic block copolymers capable of forming unique nanoscale assemblies. With an M_n of 11.2 kg/mol, the diblock copolymer had a molecular composition of $(PAA_{100}\text{-g-}HEA_{20})\text{-}b\text{-}PnBA_{16}$. The multiblock copolymer, with an M_n of 38 kg/mol and a PDI of 2.2, had an estimated 10 mol% PAA modified with HEA. While the diblock copolymers assembled into micelles of ~20 nm at a critical micelle concentration (CMC) of 5×10^{-3} mg/mL, the multiblock copolymers aggregated into particles of 40 nm in size with a CMC of 1.6×10^{-4} mg/mL. ATIII peptide was conjugated to the acrylated corona of the micelles through the cysteine residue on the peptide. The self-assembled micellar structures were used as carriers for the controlled release of BMP-2 (Figure 1). Nanoparticles derived from the multiblock copolymers exhibited higher BMP-2 loading compared to those assembled from diblock copolymers. Our preliminary data suggests that the BMP-2 was released from the ATIII decorated block copolymer micelles in a linear fashion over 15 days.

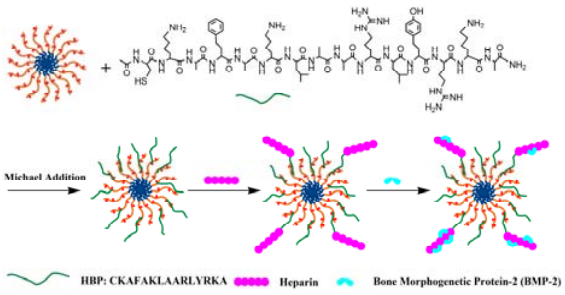

Figure 1. Schematic depiction of peptide conjugation and BMP-2 immobilization to the self-assembled block copolymer nanoparticles.

Conclusions: We have successfully synthesized amphiphilic diblock and multiblock copolymers capable of organizing into defined micellar structures in aqueous media. A BMP-2/heparin complex was immobilized to the particles through a heparin binding peptide conjugated to the hydrophilic shell of the nanoparticles. Controlled release of BMP-2 was achieved through the interplay of peptide/heparin/BMP-2 interactions. Molecular design of block copolymers with defined architectures and assembly characteristics offers the opportunity to modulate the release of BMP-2 in a spatial and temporal manner.

References:

1. Schmitt, B. et al. *Differentiation*, **2003**, *71*, 567.
2. Jha, A. K. et al. *Biomaterials*, **2009**, *30*, 6949.
3. Xiao, L. et al. *Soft Matter*, **2010**, *6*, 5293.