Peptide-functionalized polymers for bone targeted drug delivery systems

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Statement of Purpose: Osteoporosis results from deficiencies in both bone production and resorption and affects ~10 million Americans, costing the US ~$20 billion in 2005 alone (1). The majority of osteoporosis therapies are anti-resorptive, acting only to inhibit overactive osteoclasts. Development of bone-selective osteoanabolic therapies, therefore, may revolutionize osteoporosis therapies by offering an alternative and/or synergistic mechanism to restore bone health. Bone-specific delivery of drugs can be difficult due to poor bone distribution, rapid clearance, and undesirable off-target effects of the therapeutic agent. Here, we have explored the use of a peptide to target polymers to bone resorption surfaces. The targeting peptide, TPLSYLKGLVTVG, was previously identified to bind to tartrate resistant acid phosphatase (TRAP) with subnanomolar affinity (2). TRAP is a protein deposited by osteoclasts in resorption pits during remodeling, thus is an excellent target for development of drug delivery ssytems to bone. Poly(ethylene glycol) (PEG) polymers were produced with varying degrees of targeting peptide incorporation, and the TRAP binding affinity of the TBP and TBP-containing polymers was examined *in vitro* and *in vivo*.

Methods: Peptides were synthesized using a CEM Liberty 1 automated peptide synthesizer and analyzed with matrix assisted laser desorption/ionization-time of flight (MALDI-TOF). Introduction of a polymerizable methacrylamide group to peptides was accomplished via microwave-assisted reaction with methacrylic anhydride prior to cleavage (3). Polymers with 0-20% of TRAP binding peptide (TBP) were synthesized via RAFT as copolymers of TBP methacrylamide (TBPMA) and poly(ethylene glycol) methyl ether methacrylate (PEGMMA). Synthesized polymers were characterized with gel permeation chromatography (GPC) for polymer molecular weights and polydispersities and absorbance for peptide incorporation (λ=280 nm, tyrosine amino acid). Functionalized polymers were assessed for TRAP binding affinity using a Biacore T200 with a CM5 Series S dextran chips functionalized with TRAP. Finally, polymers labeled with TexasRed were characterized for resorption site-specificity *in vivo*. Briefly, calvaria of mice were exposed to parathyroid hormone, a drug known to increase osteoclast activity, via localized injection. After 1 week to allow for bone resorption, 50 mg/kg of TexasRed-labeled, 10% TBP-functionalized polymer was administered using intraperitoneal injections (IP) and targeting of polymer to the resorption site in calvaria was qualitatively evaluated using live animal fluorescent imaging 4 hours post-injection.

Results: After verification of correct peptide synthesis and modification with methacrylamide using MALDI-TOF, polymers were formed with 0-20% TBPMA (or methacrylamide-functionalized scrambled peptide) in the feed. GPC analysis showed that all polymers had molecular weights of ~30-45 kDa and were well-controlled, with polydispersities of ~1.3 or less. Peptide incorporation was found to be in good agreement with the feed; no peptide was detectable for the 0% TBP polymer and the peptide incorporation increased as the TBP feed percentage increased (Table 1).

Table 1. Analysis of surface plasmon resonance data (Biacore) provided disassociation constants (Kd) of peptide and peptide-functionalized polymers for TRAP.

Polymer	Molecular weight (g/mol)	% Peptide Incorporation	Kd (M)
TBP	1341	100	$6.3x10^{-4}$
Scrambled TBP polymer (10%)	42,000	88.2	Not detectable
0% TBP Polymer	38,000	0.2	0.0020
5% TBP Polymer	30,000	3.1	$6.0x10^{-6}$
10% TBP Polymer	36,000	7.5	$4.7x10^{-9}$
20% TBP Polymer	25,000	12.3	$5.0x10^{-6}$

TBP, a scrambled peptide (which contains same amino acids as TBP but in a random order), and 0%, 5%, 10%, and 20% TBP polymers were analyzed for TRAP affinity using Biacore. Binding data revealed that TBP exhibited specific binding to TRAP. Interestingly, the 10% TBP polymer exhibited a 134,000-fold increase binding affinity than TBP alone. The scrambled peptide showed no detectable binding affinity towards TRAP. The control polymer, 0% TBP, had lower binding than TBP, as expected, demonstrating that the incorporation of multiple TBPs results in high binding affinities is specific to TBP and not nonspecific interactions of PEG alone (Table 1).

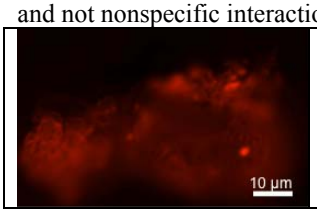

Figure 1. Resorption pit localization of red fluorescent TBP-functionalized polymers injected systemically into mice (50 mg/kg) and imaged after 4 hours in calvarial tissue.

In vivo analyses of targeted polymers showed robust localization of polymer at localized resorption surfaces, as indicated by the red fluorescent area depicted in Figure 1.

Conclusions: RAFT polymerizations were used to controllably produce peptide-functionalized polymers with differing affinity towards TRAP, a useful homing target for drug delivery to bone. Incorporation of multiple TBP moieties provided targeted binding of polymer to TRAP even greater than TBP alone. Finally, preliminary experiments in mice show localization of targeted polymer with resorption surfaces, illustrating the potential for these polymers to be used for highly tunable bone targeted drug delivery in vivo. Future work includes more thorough and sophisticated pharmacokinetics and biodistribution analyses of targeted polymers and inclusion of bone-acting drugs to initiate bone regeneration specifically at the sites of bone resorption.

References: 1. National Osteoporosis Foundation (2010); 2. T.J. Sheu *et al. J Bone Miner Res.* **17**, 915 (2002); 3. A. Van Hove *et al. JoVE*. In press.