INTRODUCTION:
Bone is a composite matrix of organic collagen and inorganic apatite constituents that could potentially serve as site-specific receptors for targeted-delivery vehicles. Small molecules such as bisphosphonates (BP) or aspartic acid (Asp) oligopeptide have been shown to be able to target bone in vitro and in vivo. Targeted drug delivery systems have several benefits including fewer side effects, lower systemic toxicity, lower dosage requirement and increased efficacy. In this current study, we aim to develop a bone-targeted drug delivery system which carries proteosome inhibitors to treat myeloma cancer.

METHODS:

Synthesis of bone targeting nanoparticles: PLGA-PEG polymers were modified to have either bisphosphonate or (Asp) end groups. A water-oil-water double emulsion method was used to synthesize the nanoparticles. Briefly, 100 mg of PLGA and PLGA-PEG polymer (modified or unmodified) were dissolved in 6mL methylene chloride. Drug was incorporated in 100-200µL water phase and emulsified in the above solution and then further dispersed in 1% sodium cholate solution. Subsequently, this double emulsion solution was diluted in 0.5% sodium cholate solution and evaporated for 2 hrs to remove the solvent. The nanoparticles (NPs) were collected by ultracentrifuge at 20,000 rpm. For radio labeling of NPs, BSA-Tc99 conjugate, fresh Tc, or oxidized Tc sodium cholate solution and evaporated for 2 hrs to remove the solvent. Subsequently, this double emulsion solution was diluted in 0.5% sodium cholate solution and evaporated for 2 hrs to remove the solvent. The nanoparticles (NPs) were collected by ultracentrifuge at 20,000 rpm. For radio labeling of NPs, BSA-Tc99 conjugate, fresh Tc, or oxidized Tc was introduced in the water phase and a similar NP synthesis procedure as above was followed.

In vitro binding with hydroxyapatite: Binding of NPs with hydroxyapatite (HA) was quantified by two independent methods: in one method the intensity of radioactive Tc was used to quantify the binding efficiency. In another method, the NPs were modified with a pyrene moiety during synthesis and the binding was quantified by a fluorescence intensity reading.

Biodistribution in mouse: Radiolabeled NPs were injected into the mouse tail vein and imaged was taken by µSPECT and µCT.

Drug release and in vitro efficacy against myeloma cancer cells: 50mg of NPs were suspended in 2mL PBS solution at 37°C at a shaking speed of 1 Hz. At each time point the released drug was collected and fresh PBS was added. Murine 5TGM1 myeloma cells were seeded at 5,000 cells per well in 96-well plates in 100µL media per well. Then free drug, released bortezomib from nanoparticles, and drug-loaded NPs were added at 50 µL/well at 1:3 dilution after 24 hrs. After incubation of the cells with the above drugs or NPs for 72 hrs, 15 µL of MTT solution was added to each well and the viability of the cells was analyzed. The absorbance of MTT solution at 570 nm correlates with the number of viable cells after 72 hrs of drug or NP exposure. Because velcade will lose some activity after exposure to light, all experiment procedures were done in the dark or in yellow light.

RESULTS:
Depending on the composition of PLGA and PLGA-PEG, the size of the NPs was around 100-200 nm (Figure 1A). Three methods had been used to load Tc inside NPs. The highest Tc loading was achieved by using Bovine Serum Albumin (BSA)-Tc conjugates (Figure 1B).

DISCUSSION:

We prepared bone-targeted nanoparticles which can be loaded with drugs inside. In vitro and in vivo binding and biodistribution studies indicated that nanoparticles did preferably bind to hydroxyapatite surface. For diseased mice, targeted NPs show significantly higher binding to bone than non-targeted NPs. Non-targeted NPs have a circulation time of around 4 hours. An efficiency study indicated that both the released drug and the nanoparticles are effective at killing the myeloma cancer cells in vitro. In vivo study of the efficacy of bone targeted NPs for treatment of myeloma cancer is underway.

REFERENCES: