Functionalized Gold Nanoparticle X-ray Contrast Agents for Bone Tissue

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Introduction: Repetitive loading of bone tissue can cause damage in the form of microcracks and diffuse damage.[1-3] *In vivo* fatigue microdamage has been shown to correlate with a degradation of mechanical properties including a loss of stiffness and a decrease in fracture toughness, which leads to an increased risk of fracture.[1,3] Current methods for imaging and quantifying microdamage are inherently invasive, destructive and two-dimensional. Therefore, gold nanoparticles (Au NPs) are being investigated as a potential damage-specific X-ray contrast agent due to their biocompatibility, ease of surface functionalization and high X-ray attenuation. Preliminary experiments using glutamic acid functionalized Au NPs showed specificity to damaged regions in bone tissue.[4] The objective of this work was to investigate the effects of different functional groups on specificity and imaging of the contrast agents.

Methods: Au NPs were prepared from $HAuCl_4 \cdot 3H_2O$ (Aldrich) and sodium citrate to a mean particle size of ~ 20 nm using a citrate reduction reaction.[5] Au NPs were then surface functionalized using glutamic acid, 2-aminoethylphosphonic acid or alendronate (Fig. 1). Briefly, 25 mL solution of 0.5 mM Au NP solution was mixed with 1.5 mL 2% poly(vinyl alcohol) (Aldrich, M_w = 50,000-85,000) and 60 mg ion exchange resin (Sigma, Amberlite MB-150) was added to the mixture to remove citrate ions. After stirring overnight, the solution was filtered and 1 mL 0.01 M solution of functional molecule was added. Binding experiments were preformed by adding hydroxyapatite to functionalized Au NP solutions. The suspension was incubated for 4 h to allow for binding and centrifuged. The supernatant solution was collected and the gold concentration was measured using ICP-OES (Perkin-Elmer). Binding constants were derived using a half-reciprocal linearization of Langmuir isotherms. Bovine cortical bone specimens were scratched with a scalpel to induce controlled surface damage and then soaked in a solution of functionalized Au NPs. Specimens were characterized using backscattered scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDXA) and x-ray tomography using a synchrotron light source.

Results: Binding constants of the functionalized Au NPs were 3.82, 0.72 and 0.25 mg/L for alendronate, glutamic acid and phosphonic acid respectively, corresponding to a maximum of 7.33, 1.22 and 0.48 mg Au NPs bound per gram of hydroxyapatite. Thus, alendronate functionalized Au NPs exhibited the highest specificity, as expected, and consequently provided the most visible staining of damaged tissue in backscattered SEM (Fig. 2). Surface damage on similarly prepared specimens labeled with alendronate and glutamic acid functionalized Au NPs was able to be detected using x-ray tomography with a synchrotron light source (Fig. 3).

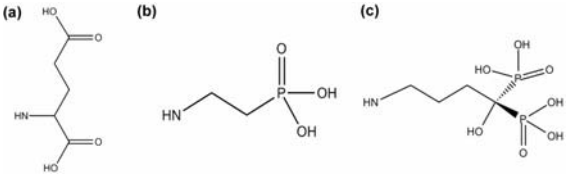

Figure 1. Au NPs were functionalized with (a) glutamic acid (b) 2-aminoethylphosphonic acid or (c) alendronate. Note that each molecule has amino groups for binding on gold surface which are opposite (a) carboxylic acid, (b) phosphonate or (c) bisphosphonate groups for calcium binding in damaged tissue.

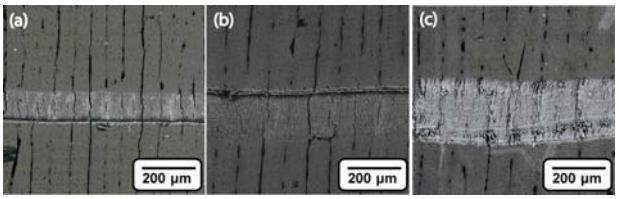

Figure 2. Backscattered SEM micrographs of bovine cortical bone surface damage (scratch) labeled with (a) glutamic acid, (b) phosphonic acid and (c) alendronate functionalized Au NPs, showing enhanced contrast between damaged and non-damaged tissue correlating to the binding affinity of each functional group.

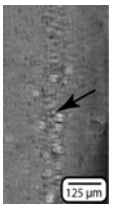

Figure 3. X-ray tomography image of alendronate functionalized Au NP stained bovine cortical bone sample using synchrotron light source, showing enhanced contrast in scratched region (labeled with an arrow) corresponding to the presence of Au NPs.

Conclusions: The binding affinity and imaging contrast of functionalized Au NPs was greatest using alendronate followed by glutamic acid and phosphonic acid. Additional binding experiments are being preformed in fetal bovine serum to simulate an *in vivo* environment.

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