Functionalized Gold Nanoparticles as a Damage Specific Contrast Agent for Bone

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Introduction: Accumulation of microdamage in bone tissue can lead to an increased risk of fracture, including stress fractures in active individuals and fragility fractures in the elderly. Current methods for detecting and imaging microdamage are inherently invasive. Therefore, micro-computed tomography (micro-CT) was proposed and demonstrated using precipitated PbS[1] or $BaSO_4$[2,3] as a non-specific contrast agent. The objective of this study was to improve specificity and deliverability by preparing functionalized gold nanoparticles (Au NPs) as a new contrast agent. Au NPs with a mean diameter of 20 and 40 nm were synthesized and functionalized with L-glutamic acid. Au NPs are well known to have affinity with carboxy, amine, and thiol groups.

Methods: Au NPs were synthesized from $HAuCl_4 \cdot 3H_2O$ (Aldrich) and sodium citrate following the method developed by Turkevich and co-workers.[4,5] The obtained sol was wine red and the concentration was adjusted to approximately 0.5 mM. Au NPs were functionalized with L-glutamic acid (Fig. 1). 236 mL of the Au solution was mixed with 14 mL 2% poly(vinyl alcohol) (PVA, Aldrich, M_w = 50,000-85,000) and then 5.8 g ion exchange resin (Sigma, Amberlite MB-150) was added to the mixture to remove citrate ions. After stirring overnight, 3 mL 0.72 mM L-glutamic acid was added and stirred for 3 days to functionalize the Au NP surface. The amount corresponded to one monolayer of glutamic acid on the Au NP surface assuming a surface coverage of 40 $Å^2$ per amine group. Au NPs were also synthesized by $NaBH_4$ reduction[6] and functionalized with one monolayer of glutamic acid using methods similar to those described above. Au NPs synthesized by either method were characterized by UV-Vis spectroscopy (Cary 50 Bio UV-Vis Spectrophotometer) and Dynamic Light Scattering (DLS, Malvern Zetasizer Nano ZS).

Six bovine cortical bone specimens (12×5×5 mm) were soaked in 0.5 mM calcein for 1.5 h under vacuum to stain damage from specimen preparation. Specimens were scratched with a scalpel to create new damage and soaked in solutions containing functionalized Au NPs for 24 h.

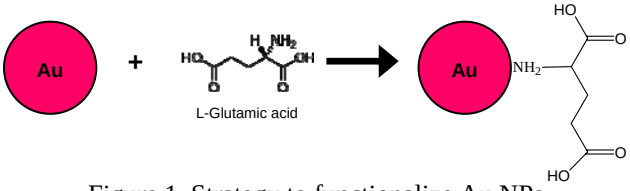

Figure 1. Strategy to functionalize Au NPs

Results/Discussion: Au NPs synthesized by citrate and $NaBH_4$ reduction were measured to have a mean size of approximately 20 and 40 nm, respectively as determined by DLS (Fig. 2). The characterictic plasmon bands of Au NPs were observed in UV-Vis spectra (Fig. 3). The peak positions were 516 nm and 534 nm for 20 and 40 nm Au NPs, respectively. The plasmon band position was unchanged after functionalization indicating that the Au NPs were dispersed. Scratches created on the surface of bone specimens were visibly stained red by the functionalized Au NPs (Fig. 4). Future work will investigate the feasibility of imaging the Au NPs by micro-CT.

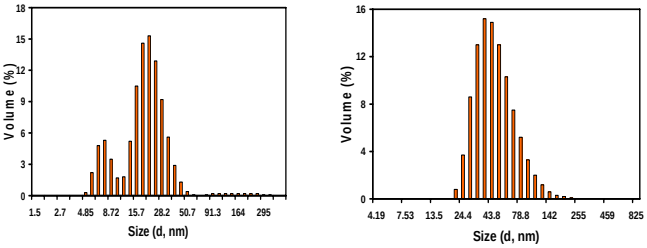

Figure 2. Particle size distribution measured by DLS for Au NPs synthesized by citrate (left) and $NaBH_4$ (right) reduction.

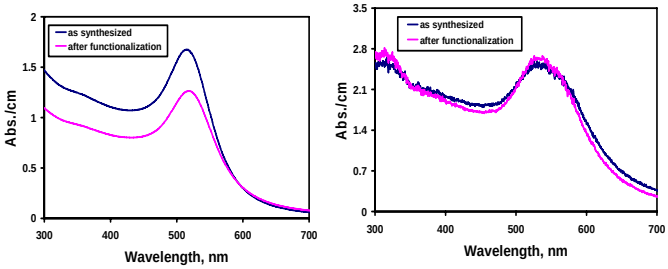

Figure 3. UV-Vis spectra of Au NPs synthesized by citrate (left) and $NaBH_4$ (right) reduction before (blue) and after (pink) functionalization with L-glutamic acid.

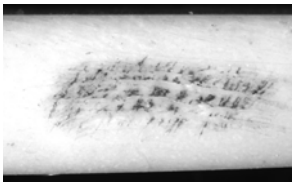

Figure 4. Optical micrograph showing scratches on the surface of a bone specimen stained by Au NPs (dark). The width of the specimen is 5 mm.

Conclusions: Au NPs were synthesized, functionalized with L-glutamic acid, and demonstrated to bind to damage in bone.

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