Contrast-Enhanced Radiographic Imaging of Breast Microcalcifications *In Vivo* using Bisphosphonate-Functionalized Gold Nanoparticles

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Statement of Purpose: Microcalcifications are deposits of hydroxyapatite (HA) mineral within the breast tissue and are a common abnormality detected by mammography when screening for breast cancer due to exhibiting high X-ray attenuation [1,2]. However, the detection of microcalcifications and diagnosis of breast cancer is limited by the sensitivity and specificity of mammography [3]. Targeted contrast agents have been proposed to improve sensitivity and specificity for X-ray detection of microcalcifications. Bisphosphonate-functionalized gold nanoparticles (BP-Au NPs) were recently shown to enable contrast-enhanced radiographic detection in *in vitro* and *ex vivo* models of microcalcifications [4]. Therefore, the objective of this study was to investigate *in vivo* targeted delivery of BP-Au NPs and contrast-enhanced radiographic imaging of microcalcifications in a murine model.

Methods: Gold nanoparticles (Au NPs) were synthesized with a 13 nm mean diameter using the citrate reduction method and surface functionalized with alendronate, which provided a primary amine for binding gold opposite a bisphosphonate (BP) functional group for targeting calcium in HA [5]. Microcalcifications were created in 10-12 week old female FVB mice by injecting 5 mg/mL HA mixed with Matrigel, a hydrogel comprised of extracellular matrix proteins, into the fat pad of the left number 4 mammary gland (MG). Matrigel alone was injected into the right number 4 MG as a contralateral control. Twenty-four hours after injecting HA, mice were injected with 100 µL of a 50 mM BP-Au NP solution directly into each MG. Mice were imaged *in vivo* by computed tomography (CT) at 45 kVp and 125 µm resolution immediately prior to delivering BP-Au NPs and at 3, 6, 24 and 48 h after delivering BP-Au NPs. X-ray attenuation (HU) was measured at each time point for a volume of interest including the MG. The biodistribution of BP-Au NPs was investigated by sacrificing mice after the 48 h time point, dissecting organs, and measuring the amount of gold present in digested samples using ICP-OES.

Results: The X-ray attenuation of HA microcalcifications was significantly greater than the Matrigel control ($p < 0.001$, *t*-test) prior to delivering BP-Au NPs (0 h) and at each time point after delivering BP-Au NPs (3, 6, 24, 48 h) (Fig. 1a). After delivering BP-Au NPs, the X-ray attenuation of HA microcalcifications was increased at all time points ($p < 0.001$, *t*-test), demonstrating contrast-enhanced detection of microcalcifications (Fig 1b). After 48 h, most of the BP-Au NP dose remained within the HA microcalcification site within the MGs with only a small amount accumulating in the liver, spleen, and kidney. The X-ray attenuation exhibited by the Matrigel control initially increased at 3 h after delivering BP-Au NPs and then decreased over time, indicating that BP-Au NPs were being cleared from the site due to the absence of HA for targeted binding. Thus, the retention of BP-Au NPs in the HA site and clearance from the Matrigel control demonstrated targeted delivery of BP-Au NPs for microcalcifications *in vivo*.

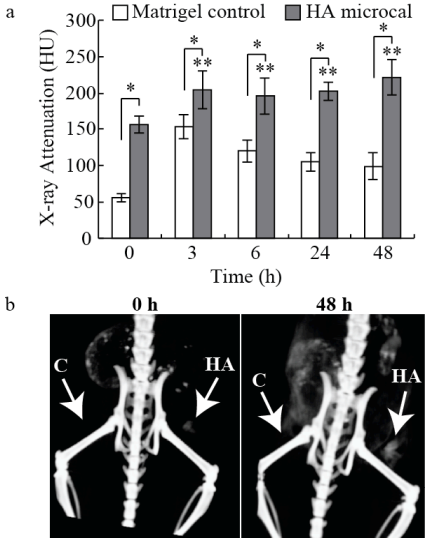

Figure 1. (a) The X-ray attenuation in Hounsfield units (HU) exhibited by HA microcalcifications and Matrigel controls immediately prior to delivering BP-Au NPs (0 h) and at 3, 6, 24, and 48 h after delivering BP-Au NPs to mouse MGs (*$p < 0.001$, HA vs. Matrigel control, *t*-test; ** $p < 0.001$ vs. HA at 0 h, *t*-test), (b) resulting in contrast-enhanced detection of HA microcalcifications (HA) compared to Matrigel controls (C).

Conclusions: This study demonstrated the first use of BP-Au NPs as a targeted X-ray contrast agent for contrast-enhanced detection of microcalcifications *in vivo*. Targeted labeling of HA microcalcifications led to an increase in the X-ray attenuation of the anatomic site and contrast-enhanced detection, which was retained for at least 48 h after delivery. Therefore, the results of this study suggest that BP-Au NPs could provide a more sensitive and specific diagnostic tool for the detection of microcalcifications during mammographic screening for breast cancer.

References:

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