Methacrylate-Modified Gold Nanoparticles Enable Non-Invasive Monitoring of Photopolymerized Hydrogel Scaffolds

Lan Li,[1] Carmen J. Gil[2], Vahid Serpooshan[2], Ryan K. Roeder[1]
[1]Department of Aerospace and Mechanical Engineering, Bioengineering Graduate Program, University of Notre Dame, USA
[2]Department of Biomedical Engineering, Emory University and Georgia Institute of Technology, USA

Statement of Purpose: Photopolymerized hydrogels such as gelMA are widely utilized for tissue engineering scaffolds and drug delivery vehicles [1], but lack a means for non-invasive, longitudinal monitoring of surgical placement, biodegradation, and drug release *in vivo*. Therefore, the objective of this study was to investigate a photocrosslinkable X-ray contrast agent, methacrylate-modified gold nanoparticles (AuMA NPs), which were photocrosslinked to gelMA hydrogels in one-step to enable a non-invasive monitoring by X-ray micro-computed tomography (micro-CT) (Fig. 1).

Methods: AuMA NPs were synthesized by surface functionalizing bare Au NPs, ~13 nm in diameter prepared by the citrate reduction method, with mercaptosuccinic acid (MSA) [2] and covalently linking 2-aminoethyl methacrylate (AEMA) using EDC/NHS chemistry (Fig. 1a). AuMA NPs were added to a gelMA solution containing 0.5% (w/v) lithium phenyl-2,4,6-trimethyl-benzoylphosphinate (LAP) photoinitiator and incubated 24 h before photocrosslinking under UV irradiation at 30 mW/cm^2 for 4 min (Fig. 1b). GelMA-Au NP hydrogels prepared with varying amounts of Au NPs were imaged by micro-CT (Scanco, µCT-80) at 70 kVp. GelMA-Au NP hydrogels were compared with hydrogels prepared from gelMA alone, physical mixing of gelMA + Au NPs, and chemical crosslinking of gelMA with Au-COOH NPs by EDC/NHS chemistry prior to photopolymerization, during *in vitro* enzymatic degradation by gravimetric analysis, micro-CT, and ICP-OES. The swelling ratio was measured by the total mass change after 24-h equilibrium in PBS relative to the initial mass.

Results: The X-ray attenuation of gelMA-Au NP hydrogels increased linearly with increasing Au NP concentration, as expected (Fig. 2a). A Au NP concentration of 10 mM was chosen to provide sufficient contrast vs. PBS or cardiac tissue, and for monitoring degradation. The degradation kinetics of gelMA-Au NP hydrogels were longitudinally measured by micro-CT for 22 days and exhibited close agreement with the mass change by gravimetric analysis and Au NP release measured by ICP-OES (Fig. 2b). The degradation kinetics of photopolymerized gelMA-Au NP (AEMA) hydrogels was slower compared to gelMA alone and gelMA with physically entrapped Au NPs (Fig. 2c) but the swelling ratio was not different from gelMA alone (Fig. 2d). Chemically crosslinked gelMA-Au NP (EDC/NHS) hydrogels exhibited more rapid degradation (Fig. 2c). This result suggested that the EDC/NHS chemical crosslinking process reduced gelMA crosslinking density, which was supported by a significantly higher swelling ratio compared to gelMA alone (Fig. 2d).

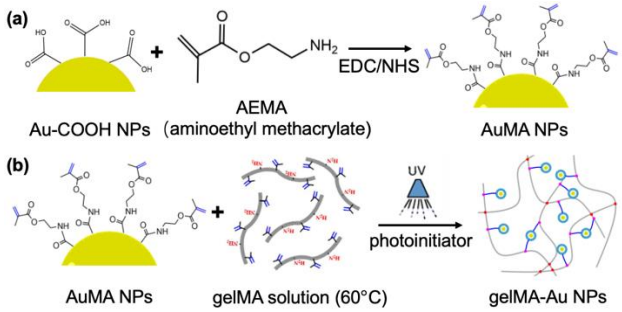

Figure 1. Scheme for synthesizing (a) AuMA NPs using EDC/NHS chemistry and (b) gelMA-Au NP hydrogels by a one-step photocrosslinking strategy.

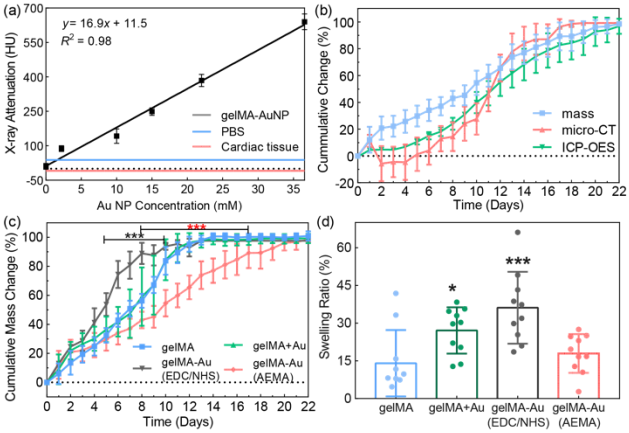

Figure 2. (a) The X-ray attenuation of gelMA-Au NP hydrogels prepared by a one-step photocrosslinking strategy. (b) Degradation kinetics of photopolymerized gelMA-Au NP hydrogels (10 mM Au NPs) measured by micro-CT, gravimetric analysis, and ICP-OES. (c) Degradation kinetics and (d) swelling ratio measured by gravimetric analysis for photopolymerized gelMA-Au NP (AEMA) hydrogels compared with hydrogels prepared from gelMA alone, physical mixing of gelMA + Au NPs, and chemical crosslinking (EDC/NHS). Error bars show one standard deviation of the mean (n = 5/group/time point in (b) and (c); n = 10/group in (d)). *p < 0.05, ***p < 0.001 vs. gelMA, ANOVA and Tukey.

Conclusions: AuMA NPs enabled the preparation of gelMA hydrogels with tunable X-ray contrast by a one-step photocrosslinking strategy, which is widely applicable to other MA-modified hydrogels. Noninvasive, longitudinal monitoring of degradation and NP release from gelMA-Au NP hydrogels was demonstrated with micro-CT *in vitro*.

References:

[1] Hu JB, *et al.*, *Appl. Phys. Rev.*, 2018;5:041106.
[2] Finamore TA, *et al.*, *Nanoscale*, 2019;11:4345.