Nondestructive, Quantitative Monitoring of Tissue Scaffolds with Spectral Photon-Counting Computed Tomography

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Statement of Purpose: Tissue-engineered medical products (TEMPs) typically employ a biodegradable material which functions as a scaffold to support tissue regeneration via the recruitment of endogenous cells and biomolecules or the delivery of exogenous cells and biomolecules. Conventional methods to monitor scaffold degradation, such as histology and mechanical testing, require invasive excision of multiple tissue samples. Nanoparticle contrast agents, such as gold nanoparticles (Au NPs), were recently demonstrated to enable nondestructive, radiographic monitoring of scaffold degradation and drug release using computed tomography (CT) [1,2]. Additionally, NPs can be incorporated into bioinks for 3D-printing scaffolds with custom designs [2]. Photon-counting computed tomography (PCCT) is a nascent imaging technology that enables quantitative molecular imaging of multiple materials simultaneously [3]. Therefore, the objective of this study was to investigate nondestructive, quantitative monitoring of tissue scaffolds with spectral PCCT.

Methods: Gelatin methacrylate (gelMA) was photocrosslinked with methacrylate-modified Au NPs (AuMA NPs, ~5 nm diameter, 10 mM concentration) to prepare hybrid gelMA-Au hydrogel scaffolds using established methods (Fig. 1a) [2]. GelMA and gelMA-Au scaffolds (n = 5/group) were incubated in Type II collagenase and imaged longitudinally over 7 days with spectral PCCT (MARS Bioimaging) at 120 kVp, 1.96 mm Al filtration, and 200 ms integration time with a 110 µm voxel size. GelMA hydrogel scaffolds were also 3D-printed in custom designs from bioinks containing Au and Gd_2O_3 NPs. Bioprinted scaffolds (n = 3/group) were imaged using a similar PCCT acquisition protocol tailored to utilize the k-edges of Gd and Au. Material decomposition was performed on spectral PCCT images using constrained maximum likelihood estimation calibrated to known material concentrations [3].

Results: Spectral PCCT imaging enabled nondestructive, quantitative monitoring of gelMA-Au scaffold degradation over 7 days, exhibiting strong correlation with gelMA alone and concurrent measurements by conventional micro-CT, gravimetric analysis, and ICP-OES (Fig. 1b). PCCT was most effective when utilizing a thresholded VOI that was larger than the scaffold volume as other methods underestimated the scaffold degradation (Fig. 1c). Bioprinted scaffolds with distinct regions comprising Au and Gd_2O_3 NPs were able to be decomposed and quantified by spectral PCCT (Fig. 2).

Conclusions: Spectral PCCT enabled nondestructive, quantitative monitoring of scaffold degradation, NP release, and multi-agent discrimination.

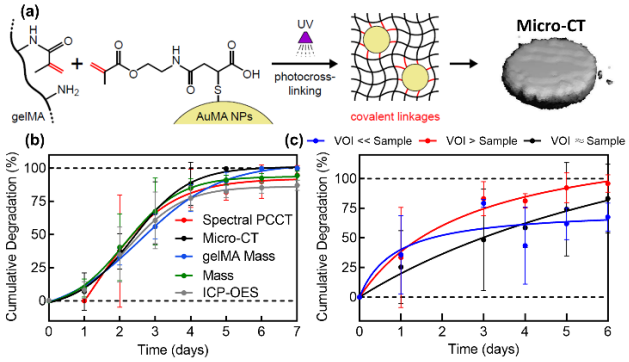


Figure 1. (a) Scheme showing the preparation of gelMA hydrogels with AuMA NPs by a one-step photo-crosslinking strategy [2] and a micro-CT image reconstruction of a gelMA-Au scaffold. (b) Degradation kinetics of gelMA-Au hydrogels measured by PCCT, micro-CT, gravimetric analysis (mass), and ICP-OES, and compared to gelMA alone as measured by gravimetric analysis (n = 5/group/time point). (c) Degradation kinetics measured by PCCT with a VOI smaller, larger (also shown in (b)), or equal to the scaffold volume.

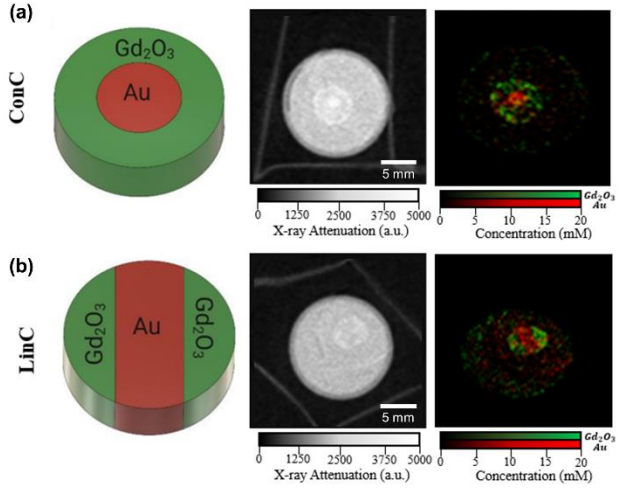


Figure 2. GelMA hydrogel scaffolds containing ~20 mM Au and Gd_2O_3 NPs 3D-printed into (a) concentric circle or (b) line-in-circle designs. Conventional grayscale CT slices of samples at the lowest energy bin (7-29 keV) are unable to discriminate different regions of the scaffold based on NP compositions. However, PCCT and quantitative material decomposition maps enable discrimination and quantification of of Gd_2O_3 (green) and Au (red) NPs, as color intensities are scaled to mM concentrations.

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References: [1] Finamore TA, *Nanoscale*, 2019;11:4345. [2] Li L, *Adv. NanoBiomed Res.*, 2022;2:2200022. [3] Curtis TE, *J. Med. Imaging*, 2019;6:013501.