FLASH Radiotherapy and an Injectable Drug Delivery Hydrogel as a Combination Treatment for Glioblastoma

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Hypothesis: Glioblastoma multiforme (GBM) remains the most aggressive form of brain tumor, with a regional location within the blood brain barrier that hinders chemotherapeutic delivery and prevents radiation dose escalation.[1][2] To overcome these obstacles, we propose the use of an injectable theragnostic hydrogel to enable local chemotherapeutic administration. To do so, we cargo-loaded SpaceOAR, an FDA approved hydrogel, with quisinostat (QS) drug and contrast-enhancing gold nanoparticles (AuNP). We also integrated FLASH radiotherapy, a novel alternative to conventional radiotherapy that administers radiation at an ultrahigh dose rate to reduce toxicity.[3] We hypothesize that this combination therapy involving a theragnostic hydrogel and FLASH-RT will elicit a synergistic anti-GBM effect.

Methods: AuNP were synthesized via modified Turkevich method and characterized via transmission electron microscopy (TEM). To determine the optimal loading of AuNP and QS within SpaceOAR, U251 glioma cells were dosed with a range of AuNP and QS concentrations, with the resulting cell viability determined by MTS assay. The elution profile of AuNP from SpaceOAR was determined using UV-vis spectroscopy. The contrast enhancing properties of AuNP were quantified via microCT. To evaluate the anti-GBM efficacy of the hydrogel system, we synthesized QS and AuNP-loaded SpaceOAR formulations **(1A)** and submerged them in cell media for 7 days, whereby aliquots of the media were sampled for dosing of U251 glioma cells. Complete elution of QS drug from SpaceOAR would correspond to a QS concentration of 320 nM in each aliquot. A series of MTS cell viability assays were then performed using free and hydrogel-eluted QS to quantify drug-mediated cell death. The MTS cell viability assays were repeated with and without proton radiation to assess the therapeutic benefits of supplementing QS-AuNP-SpaceOAR with FLASH-RT.

Results: AuNPs exhibit a spherical morphology and an average core size of 4.3 ± 1.1 nm **(1B)**. AuNPs were steadily released from SpaceOAR over 7 days **(1C)**, and their contrast attenuation was found via microCT to increase linearly as a function of concentration **(1D,1E)**. AuNP did not affect the cell viability of U251 at concentrations below 1 mg/mL and durations less than 24 hours **(1F)**. Dosing of U251 cells with free QS demonstrated both a QS dose-dependent **(1G)** and a FLASH-RT dose-dependent **(1H)** decrease in cell viability. U251 cells dosed with QS-AuNP-SpaceOAR eluent experienced significantly higher levels of cell death compared to the control **(1I)**, with greater cytotoxicity at higher doses of FLASH-RT **(1J)**. This demonstrates *in vitro* the therapeutic benefit of combining FLASH-RT and QS.

Conclusions: The QS-AuNP-SpaceOAR system is biocompatible and elicits a significant *in vitro* anti-GBM effect in combination with FLASH-RT. This research supports the synergistic efficacy of FLASH-RT against glioblastoma in combination with anti-GBM drug, supporting the need for further exploration within an *in vivo* context.

References: [1] Noorani, I. & de la Rosa, J. Breaking barriers for glioblastoma with a path to enhanced drug delivery. Nat Commun 14 , 5909, (2023). [2] Bouche, M. et al. Novel Treatment for Glioblastoma Delivered by a Radiation Responsive and Radiopaque Hydrogel. ACS Biomater Sci Eng 7 , 3209-3220 (2021). [3] Lin, B. et al. FLASH Radiotherapy: History and Future. Front Oncol 11 , 644400, (2021).

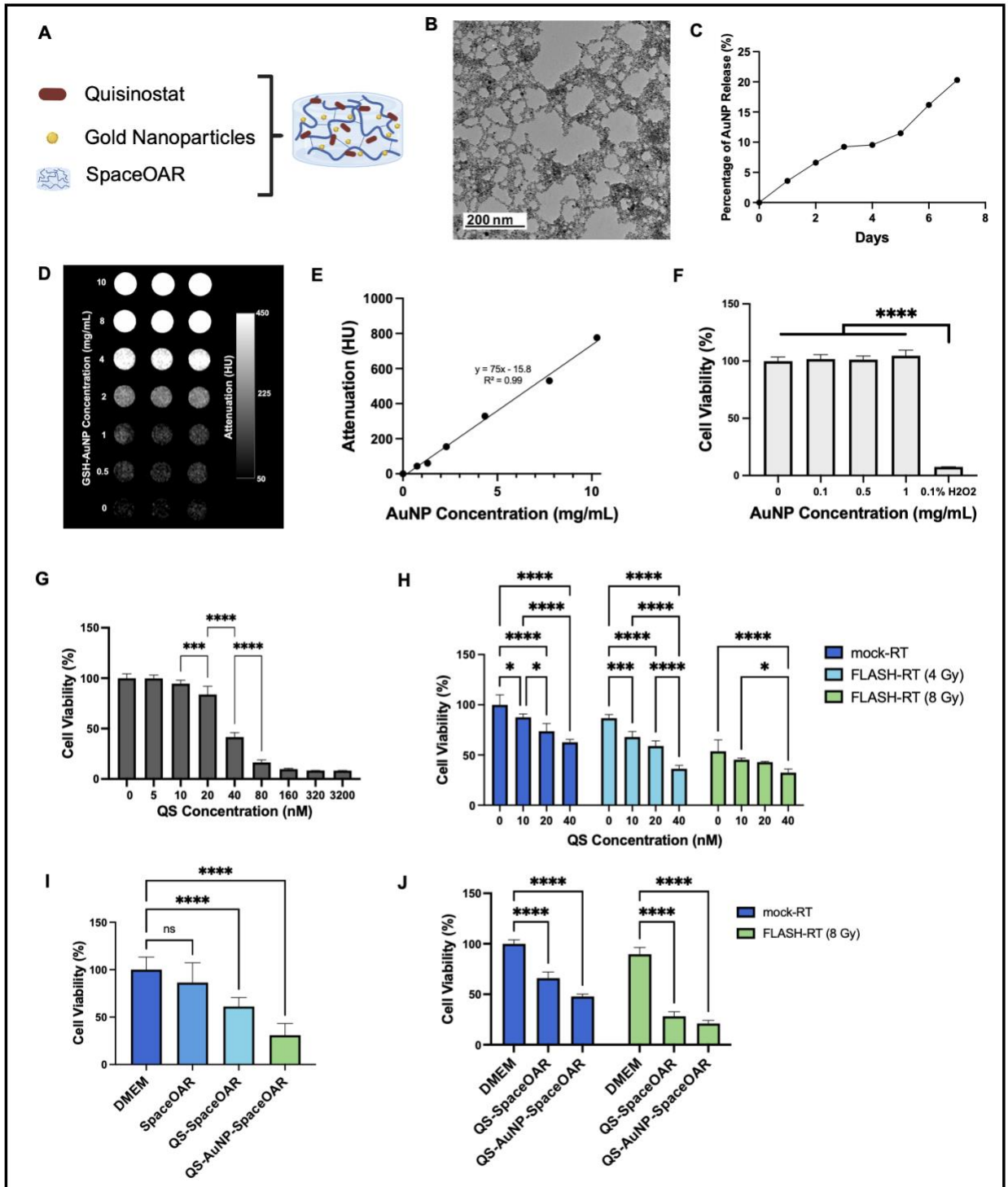

Figure 1: (A) Schematic depicting loading of quisinostat (QS) and gold nanoparticles (AuNP) within SpaceOAR. Representative AuNP (B) micrograph generated by TEM, (C) elution profile from SpaceOAR, and (D) microCT phantom expressing (E) contrast attenuation as a function of AuNP concentration. (F) Effect of AuNP on U251 cell viability after 24 hours of incubation. Effect of (G) free QS, (H) QS and FLASH-RT, (I) QS-AuNP-SpaceOAR, and (J) QS-AuNP-SpaceOAR and FLASH-RT on the viability of U251 cells (MTS Assay).