

Gold Nanoparticle-Loaded Biodegradable Polyanhydride Wafers Provide Sustained Gold Availability and Enhance Radiosensitization of Glioblastoma Cells

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Abstract

Background

Local recurrence and radioresistance remain major obstacles to durable control of glioblastoma after surgical resection. Implantable biodegradable matrices could maintain radiosensitizer availability near the resection margin while limiting systemic exposure. This study evaluated gold nanoparticle (AuNP)-loaded poly[1,3-bis(p-carboxyphenoxy)propane-co-sebacic anhydride] [P(CPP:SA)] wafers as a local radiosensitizer platform for glioblastoma.

Methods

AuNP-loaded P(CPP:SA) wafers were fabricated by dry blending and compression molding and characterized by field-emission scanning electron microscopy, energy-dispersive X-ray spectroscopy, ATR-FTIR, thermogravimetric analysis, and inductively coupled plasma mass spectrometry (ICP-MS). Wafer degradation, recoverable elemental Au availability, and endpoint Au mass balance were quantified. Baseline effects were evaluated in U-87 MG glioblastoma cells and normal human astrocytes. Radiosensitization under 6-MV photon irradiation was assessed using metabolic activity, treatment-normalized clonogenic survival, sensitizer enhancement ratios, and γ -H2AX immunoblotting. Free AuNPs matched to the 24-h recoverable elemental Au concentration served as the nanoparticle comparator.

Results

AuNP incorporation preserved gross wafer morphology and the dominant polymer-associated spectral features. ICP-MS measured an Au loading of 76.88 ± 2.04 $\mu\text{g}/\text{mg}$ wafer, corresponding to 83.56% loading efficiency. The matrix degraded progressively over 28 days, while cumulative recoverable elemental Au reached $52.30 \pm 4.91\%$ by day 14. Endpoint accounting recovered $99.36 \pm 3.72\%$ of the initial Au across medium, residual specimen, and tube/sediment/wash fractions. Blank wafers produced minimal non-irradiated effects, whereas Au-containing treatments caused moderate baseline responses. Following irradiation, AuNP-loaded wafers produced the greatest reduction in clonogenic survival, with surviving fractions of 0.1514 ± 0.0071 at 4 Gy and 0.0522 ± 0.0112 at 6 Gy, significantly below radiotherapy alone, blank wafer plus radiotherapy, and Au-matched free AuNPs. The sensitizer enhancement ratio at a surviving fraction of 0.25 was 1.657 for the AuNP-loaded wafer compared with 1.357 for free AuNPs. γ -H2AX/total H2AX ratios were also highest with the AuNP-loaded wafer at 1 and 24 h after irradiation.

Conclusions

AuNP-loaded P(CPP:SA) wafers combined reproducible elemental Au incorporation, progressive matrix degradation, sustained recoverable Au availability, and enhanced megavoltage radiosensitization in U-87

MG cells. These findings establish an in vitro proof of concept for a biodegradable local Au-based radiosensitizer platform. Particle-resolved release characterization, intracellular Au quantification, clinically relevant fractionated irradiation, and validation in patient-derived and in vivo glioblastoma models are required before translational conclusions can be drawn.

1. Introduction

Glioblastoma (GBM) remains difficult to control despite maximal safe resection followed by fractionated radiotherapy (RT) with concurrent and adjuvant temozolomide [1]. Infiltrative growth prevents complete microscopic resection, and treatment failure commonly occurs within the irradiated field or near the resection cavity, where residual cells persist [2–4]. RT is therefore indispensable but constrained: dose intensification must be balanced against injury to surrounding functional brain, while cellular heterogeneity and adaptive stress responses can limit tumor control. A locally available radiosensitizer could increase the effect of a given radiation dose at the region of greatest recurrence risk without requiring indiscriminate expansion of the treated volume or dose.

Gold nanoparticles (AuNPs) are high-atomic-number materials that can modify local radiation energy deposition and amplify downstream cellular injury. Their experimental appeal includes chemical stability, size- and surface-dependent behavior, and compatibility with diverse formulations. The magnitude of AuNP radiosensitization depends on particle diameter, coating and aggregation state, delivered concentration, cellular uptake and subcellular localization, radiation energy and dose, and the biological model [5]. Efficacy under megavoltage irradiation is therefore best established experimentally. AuNP-associated reductions in clonogenic survival have been reported in U-87 MG and other GBM models exposed to clinically relevant beams [5, 6], and later studies confirm that formulation and surface properties alter the response [7, 8]. Most studies have examined freely dispersed or systemically administered Au formulations, leaving sustained postoperative-cavity exposure comparatively underexplored.

Local delivery is attractive because an implant can be placed during resection, bypass systemic dilution and blood-brain-barrier transport, and maintain material near the infiltrated margin. P(CPP:SA)-type polyanhydride wafers provide an established intracranial precedent through local carmustine delivery in malignant glioma [9, 10], while current implant research continues to use the resection cavity as a therapeutic interface [11, 12]. Polyanhydrides undergo hydrolytic bond cleavage and matrix erosion, allowing formulation behavior to be linked to local payload availability. Their use as matrices for particulate radiosensitizers, however, remains less developed than their use for conventional drugs.

A functional AuNP wafer platform requires more than successful incorporation. Loading should preserve relevant matrix features and be quantitatively reproducible; degradation and Au recovery should be referenced to the initial elemental mass; baseline material effects should remain separable from radiation-dependent effects; and the comparator should reflect the Au exposure generated during the pre-irradiation interval. Elemental analysis after digestion can close the Au mass balance, whereas

particle-resolved methods are required to distinguish intact colloidal nanoparticles from aggregates, sediment-associated material, or polymer-associated Au.

A formulation-to-function assessment must therefore connect complementary measurements rather than treating characterization and cell experiments as independent demonstrations. Microscopy and spectroscopy establish bounded aspects of incorporation, ICP-MS quantifies elemental loading and recovery, degradation defines the time-dependent matrix context, and treatment-matched clonogenic analysis determines whether the local-delivery architecture changes radiation response beyond the blank carrier and free-Au comparators.

Radiobiological endpoint selection is equally important. Clonogenic survival is the principal in vitro measure of durable loss of reproductive capacity after irradiation, whereas short-term metabolic assays provide supportive but non-equivalent information. γ -H2AX immunoblotting provides complementary population-level evidence of DNA-damage-associated signaling, while direct lesion counts, nuclear-foci analysis, and repair kinetics require dedicated assays [13, 14]. We therefore fabricated AuNP-loaded P(CPP:SA) wafers and tested whether Au incorporation preserved major matrix characteristics, produced reproducible elemental loading and sustained recoverable Au availability with endpoint mass closure, and enhanced U-87 MG radiation response relative to RT alone, blank wafer plus RT, and a free-AuNP comparator matched to the 24-h recoverable Au amount.

2. Materials and Methods

2.1. Materials and cell lines P(CPP:SA) (CPP:SA 20:80 wt%; number-average molecular mass approximately 20 kDa; dispersity approximately 2.7; P80595-CPPSA-Anh) was obtained from Polymer Source. AuNPs were supplied as a 20-nm nominal-diameter, citrate-stabilized aqueous suspension containing 0.1 mg Au/mL (OD 1; λ_{max} approximately 524 nm; J67269.AC; Thermo Scientific Chemicals). Analytical-grade dichloromethane, nitric and hydrochloric acids, crystal violet, methanol, paraformaldehyde, bovine serum albumin, and related reagents were from Sigma-Aldrich/Merck unless stated otherwise; 18.2 M Ω ·cm ultrapure water was used throughout.

U-87 MG human glioblastoma cells (ATCC HTB-14) and normal human astrocytes (NHA; ScienCell) were maintained at 37°C and 5% CO₂. U-87 MG cells were cultured in high-glucose DMEM with 10% fetal bovine serum and 1% penicillin-streptomycin; NHA were cultured in complete Astrocyte Medium. CCK-8 reagent was from Dojindo, the LDH assay kit from Thermo Fisher Scientific, and antibodies against γ -H2AX (Ser139), total H2AX, and β -actin, with HRP-conjugated secondary antibodies, from Cell Signaling Technology.

2.2. Dry AuNP feedstock and wafer fabrication The aqueous AuNP suspension was centrifuged at 6,500 $\times g$ for 30 min at 4°C. After supernatant removal, the pellet was washed once with ultrapure water, centrifuged again under identical conditions, frozen at – 80°C, and lyophilized (Labconco FreeZone). As lyophilization may affect the supplied colloidal state, subsequent ICP-MS measurements were designated as elemental Au recovery; particle state was outside the scope of this assay.

Blank and AuNP-loaded wafers were prepared by dry blending and compression molding. Lyophilized AuNP feedstock was combined with P(CPP:SA) to give a theoretical input of 92.0 µg Au/mg dry formulation (9.20 wt% Au) and mixed by geometric dilution in an agate mortar for 10 min; blank polymer was treated identically. Powder (5.0 mg) was transferred to a 5-mm flat-faced stainless-steel die and compressed at 3 metric tons for 2 min (Specac Atlas 15T). Discs were inspected for gross cracking or delamination and stored desiccated at 4°C. Release experiments used dissected specimens of 0.618 ± 0.020 mg. From the measured formulation loading, each specimen contained 47.53 ± 1.53 µg elemental Au at test initiation.

2.3. Physicochemical and mechanical characterization Surface morphology was examined by field-emission scanning electron microscopy (FE-SEM; Hitachi SU5000). Wafers were mounted on carbon tape without Au coating. Energy-dispersive X-ray spectroscopy (EDS; Oxford X-MaxN silicon drift detector) was used to detect Au M- and L-series signals and map Au within the analyzed surface region; interpretation was restricted to that region. Attenuated-total-reflection Fourier-transform infrared (ATR-FTIR) spectra of blank wafers, AuNP-loaded wafers, and dried AuNP feedstock were acquired from 4000 to 500 cm⁻¹ at 4 cm⁻¹ resolution with 32 scans (Nicolet iS50, diamond ATR), with a fresh background before each measurement.

Thermogravimetric analysis (TGA; TA Instruments Q500) was performed in triplicate on blank wafers, AuNP-loaded wafers, and dried AuNP feedstock. Samples (approximately 3–5 mg) were heated from 25 to 700°C at 10°C/min under nitrogen (60 mL/min); TGA, derivative thermogravimetry (DTG), and terminal residue were recorded. For unconfined compression, one blank and one AuNP-loaded wafer were centered between stainless-steel plates and compressed at 0.5 mm/min (Instron 5943). Force-displacement data were converted to stress-strain curves using initial specimen dimensions and used to describe representative compression behavior.

2.4. Elemental Au loading Three independently prepared AuNP-loaded wafers were weighed and digested in freshly prepared aqua regia (HCl:HNO₃, 3:1 v/v) for 2 h at 70°C, cooled, and diluted in ultrapure water containing 2% HNO₃. Elemental Au was measured by ICP-MS (Agilent 7900) against 0, 25, 50, 100, 150, 200, and 250 µg/L external standards; all samples fell within the calibration range.

Au loading (µg/mg) = measured Au mass in digest (µg) / initial dry wafer mass (mg)

Loading efficiency (%) = measured Au mass incorporated / theoretical Au input × 100

2.5. Degradation, recoverable Au release, and mass balance For degradation, individual blank and AuNP-loaded wafers were weighed (W_0) and incubated statically in 2.0 mL PBS (pH 7.4) at 37°C. Six independent wafers per formulation were destructively sampled on Days 1, 3, 7, 14, 21, and 28; Day 0 was the pre-incubation baseline. Medium pH was measured with a microelectrode (SevenCompact S220). Retrieved wafers were briefly rinsed, vacuum-dried to constant mass, and weighed (W_{∞}).

Dry mass retained (%) = $W_{\infty} / W_0 \times 100$

Because the loaded formulation contained nondegradable inorganic Au, late dry mass represented total residual specimen mass rather than residual polymer alone.

For release, standardized AuNP-loaded specimens (0.618 ± 0.020 mg; initial Au, 47.53 ± 1.53 μ g) were incubated in 1.0 mL PBS at 37°C with orbital agitation at 60 rpm ($n = 6$). At 1, 6, 12, 24, 48, and 72 h and Days 5, 7, and 14, the entire medium was collected and replaced with equal-volume prewarmed PBS. Acid-digested fractions were quantified by ICP-MS, corrected for serial replacement, and normalized to each specimen's initial Au mass.

Cumulative recoverable Au (%) = Σ Au in collected media / initial specimen Au $\times$ 100

At Day 14, cumulative medium-recovered Au, residual specimen-associated Au, and tube/sediment/wash Au were quantified separately.

Total Au recovery (%) = (medium Au + residual-specimen Au + tube/sediment/wash Au) / initial specimen Au $\times$ 100

2.6. Transwell exposure and baseline cytocompatibility Wafers were placed in 0.4- μ m polyester Transwell inserts above adherent cells to prevent direct solid-wafer contact. The free-AuNP comparator was matched to the 24-h recoverable Au amount from the standardized specimen: 8.94% of 47.53 μ g in 1.0 mL, or 4.25 μ g Au/mL. This matched the 24-h pre-irradiation exposure used in radiobiological assays.

For baseline testing, U-87 MG cells and NHA were seeded at 5×10^4 cells/well in 24-well plates containing 1.0 mL medium, allowed to adhere for 24 h, and exposed for 24, 48, or 72 h to untreated control, blank wafer, free AuNPs (4.25 μ g Au/mL), or AuNP-loaded wafer. CCK-8 was added at 10% v/v for 2 h and absorbance measured at 450 nm (Synergy H1); group-matched cell-free material blanks were subtracted and results normalized to the time-matched untreated mean. LDH was measured in supernatants, corrected with group-matched cell-free blanks, and expressed relative to matched low- and high-LDH controls. Each condition comprised four independent biological replicates measured in technical triplicate.

2.7. Irradiation and post-irradiation metabolic activity A calibrated clinical linear accelerator (Varian TrueBeam) delivered 6-MV photons at 2 Gy/min. Plates were positioned reproducibly in a tissue-equivalent build-up arrangement according to institutional dosimetry procedures. Formulation groups were pre-exposed for 24 h. Post-irradiation CCK-8 and γ -H2AX experiments used 4 Gy; clonogenic experiments used 0, 2, 4, and 6 Gy.

For post-irradiation CCK-8, U-87 MG cells were assigned to untreated control, blank wafer, free AuNPs, AuNP-loaded wafer, RT alone, blank wafer + RT, free AuNPs + RT, or AuNP-loaded wafer + RT. Metabolic activity was measured 24, 48, and 72 h after 4 Gy, corrected using group-matched irradiated or non-irradiated cell-free blanks, and normalized to the time-matched untreated mean. Each group contained five independent biological replicates measured in technical triplicate.

2.8. Clonogenic survival and sensitizer enhancement ratio Clonogenic survival was the primary durable radiobiological endpoint. U-87 MG cells received RT alone, blank wafer + RT, free AuNPs + RT, or AuNP-loaded wafer + RT. After 24-h formulation exposure, cells were irradiated at 0, 2, 4, or 6 Gy, immediately detached, counted, and replated at dose-adjusted densities. After 10–14 days, colonies were fixed in 4% paraformaldehyde for 15 min, stained with 0.5% crystal violet in 20% methanol for 30 min, and scored when they contained at least 50 cells.

Plating efficiency (PE) = colonies at 0 Gy / cells seeded at 0 Gy

Surviving fraction (SF) = colonies counted / (cells seeded × treatment-matched 0-Gy PE)

Three independent biological experiments were performed, each with technical triplicate dishes; technical replicates were averaged before inference. Survival curves were fitted to $SF = \exp[-(\alpha D + \beta D^2)]$. The sensitizer enhancement ratio was calculated at SF = 0.25, which remained within the response range supported by every fitted curve across the measured 0–6 Gy interval.

$SER_{0.25} = D_{25, \text{RT alone}} / D_{25, \text{treatment + RT}}$

2.9. γ -H2AX Western blotting U-87 MG cells assigned to untreated control, RT alone, blank wafer + RT, free AuNPs + RT, or AuNP-loaded wafer + RT were harvested 1 or 24 h after 4 Gy. Cells were lysed in RIPA buffer containing protease and phosphatase inhibitors for 30 min on ice and clarified at 12,000 ×g for 15 min at 4°C. Protein was measured by BCA assay. Equal protein (25 µg/lane) was resolved by 12% SDS-PAGE, transferred to PVDF, blocked in 5% BSA/TBST, and incubated overnight at 4°C with antibodies to γ -H2AX, total H2AX, and β -actin. HRP-based chemiluminescence was acquired with a ChemiDoc MP and quantified in ImageJ/Fiji. γ -H2AX was normalized to total H2AX and expressed relative to the 1-h RT-alone group. Three independent biological experiments were analyzed.

2.10. Statistical analysis Data are mean ± SD. GraphPad Prism (version 10) was used. The biological replicate was the unit of inference; technical replicates were averaged within each biological replicate. Baseline cytocompatibility and post-irradiation CCK-8 data were analyzed by two-way ANOVA with Tukey multiple comparisons. Clonogenic SF was analyzed by one-way ANOVA with Tukey testing separately at each dose, and γ -H2AX densitometry by one-way ANOVA with Tukey testing separately at each time point. Significance was $p < 0.05$ (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

3. Results and Discussion

3.1. AuNP incorporation preserved gross wafer morphology and dominant polymer-associated features

The P(CPP:SA) matrix was selected for its established intracranial local-delivery precedent [9, 10]. FE-SEM showed a dense blank-wafer surface without major cracks or collapse. The AuNP-loaded wafer

retained this gross architecture but displayed greater fine-scale surface texture (Fig. 1A,B), establishing morphological preservation after loading within the surface-level resolution of FE-SEM.

EDS of the loaded wafer detected the expected Au M-line near 2.1 keV and Au L-line features near 9.7 and 11.4 keV, with a weaker higher-energy signal around 13.4 keV (Fig. 1C). Mapping showed an areally distributed Au signal across the examined region (Fig. 1D), confirming local elemental Au rather than a single isolated Au-rich field.

Blank and loaded wafers retained the dominant ATR-FTIR profile of P(CPP:SA), including the anhydride carbonyl envelope at approximately $1820 - 1710\text{ cm}^{-1}$, aromatic-associated bands at approximately $1600 - 1500\text{ cm}^{-1}$, and fingerprint absorptions at approximately $1300 - 900\text{ cm}^{-1}$ (Fig. 1E). Modest intensity differences were compatible with sampling and packing variation. Features in the dried AuNP-feedstock spectrum were attributable to associated organic material rather than metallic Au. FTIR therefore confirmed retention of the major polymer-associated spectral features, with molecular-level Au-polymer interactions remaining beyond the resolution of this assay.

Together, FE-SEM, EDS, and ATR-FTIR addressed complementary levels of formulation integrity. FE-SEM showed that compression of the Au-containing blend yielded a coherent wafer surface; EDS confirmed elemental Au across the field analyzed; and ATR-FTIR showed retention of the dominant bands identifying the polyanhydride matrix. This convergence established a physically intact Au-containing P(CPP:SA) disc with a recognizable polymer-associated spectrum. Quantitative loading and mass-balance measurements extended these surface and spectral observations by defining reproducible elemental Au content at the formulation level.

3.2. Thermal behavior, elemental loading, and representative compression response

Blank and AuNP-loaded wafers showed similar multistep thermal mass-loss profiles and DTG peak regions (Fig. 2A,B). Terminal residue increased from $1.43 \pm 0.14\%$ for blank wafers to $9.19 \pm 0.19\%$ for loaded wafers; dried AuNP feedstock retained $98.24 \pm 0.13\%$. The increased stable residue provides complementary evidence of inorganic content without indicating appreciable thermogravimetric destabilization of the polymer.

ICP-MS quantified 74.90, 76.76, and 78.98 $\mu\text{g Au/mg}$ wafer in three independent batches, giving $76.88 \pm 2.04\text{ }\mu\text{g Au/mg}$, an inter-batch coefficient of variation of 2.65%, and approximately 7.69 wt% Au. Corresponding loading efficiencies were 81.42%, 83.43%, and 85.85% (mean, 83.56%). Agreement between ICP-MS loading and the TGA residue increment supports reproducible Au incorporation, although ICP-MS provides the definitive elemental quantification.

Representative stress-strain curves retained the same general sequence of seating, loading, peak, post-peak decline, and continued load bearing (Fig. 2C). Peak stress was approximately 1.55–1.60 MPa at

12.5–13.0% strain for the blank wafer and 1.34–1.40 MPa at 12.0–12.5% strain for the loaded wafer. The curves provide a descriptive comparison and a basis for future replicated mechanical characterization.

The loading data also distinguish nominal formulation composition from delivered experimental dose. A theoretical input of 92.0 $\mu\text{g Au/mg}$ would overestimate the measured wafer content by approximately 16%; using the ICP-MS value prevented that discrepancy from propagating into release normalization and the biological comparator. The low inter-batch coefficient of variation demonstrates reproducible apportionment of elemental Au by dry blending and compression at the batch scale examined. The close correspondence between the approximately 7.69 wt% ICP-MS result and the Au-associated TGA residue increment further strengthens compositional confidence because the methods rely on different measurement principles. TGA provided complementary compositional evidence, with ICP-MS supplying Au-specific quantification. The representative compression response also shows that both formulations formed coherent, testable discs and provides preliminary handling-related information for subsequent specification testing.

3.3. Progressive degradation accompanied sustained recoverable elemental Au availability

Both formulations lost dry mass progressively over 28 days (Fig. 3A). Blank and loaded wafers retained $96.65 \pm 1.22\%$ and $97.25 \pm 1.28\%$ on Day 1, $88.28 \pm 2.46\%$ and $89.99 \pm 1.21\%$ on Day 3, $49.22 \pm 2.78\%$ and $51.98 \pm 5.51\%$ on Day 14, and $12.48 \pm 2.95\%$ and $14.11 \pm 1.17\%$ on Day 28. Medium pH fell from 7.40 to 6.78 ± 0.03 and 6.78 ± 0.01 by Day 7 and to 6.16 ± 0.03 and 6.21 ± 0.02 by Day 28 for blank and loaded wafers, respectively (Fig. 3B). The comparable trajectories and acidification are consistent with hydrolytic cleavage of P(CPP:SA) anhydride linkages [15, 16]. Because late retained mass included nondegradable Au, interpretation focused on the comparable overall trajectories rather than direct polymer-mass comparison at the final time points.

Only $1.52 \pm 0.31\%$ of initial Au was recovered in medium at 1 h and $8.94 \pm 0.87\%$ at 24 h; cumulative recovery rose to $36.62 \pm 2.15\%$ on Day 7 and $52.30 \pm 4.91\%$ on Day 14 (Fig. 3C). Values were normalized to $47.53 \pm 1.53 \mu\text{g}$ initial Au in each standardized $0.618 \pm 0.020\text{-mg}$ specimen. The limited early fraction followed by continued accumulation argues against burst-dominated elemental Au loss under these test conditions.

At Day 14, residual specimen-associated Au represented $41.31 \pm 2.30\%$ of the initial amount and tube/sediment/wash fractions $5.75 \pm 0.50\%$. Together with medium-recovered Au, total recovery was $99.36 \pm 3.72\%$ (Fig. 3D). This near-complete mass closure verifies elemental Au accounting across the principal compartments. Because ICP-MS quantifies Au after digestion, the demonstrated outcome is sustained recovery of elemental Au; particle-resolved analysis can further define size, aggregation, polymer association, and colloidal stability.

The degradation and recovery experiments were intentionally interpreted together. Progressive dry-mass loss and acidification established that the P(CPP:SA) matrix was changing throughout the interval in

which medium-recoverable Au accumulated. The early 24-h fraction used for comparator matching was therefore a transient exposure value, not a surrogate for the full payload or for the cumulative Day-14 amount. Complete medium replacement at each sampling point minimized carryover between intervals and allowed cumulative recovery to be calculated from individually digested fractions, whereas normalization to the measured initial Au content controlled for specimen-mass variation. Importantly, the 41.31% remaining in the specimen at Day 14 shows that the system retained a substantial reservoir after more than half of the initial Au had become medium-recoverable.

The degradation and release studies used purpose-specific designs: whole wafers were sampled destructively in 2.0 mL static PBS for degradation, whereas standardized smaller specimens were evaluated in 1.0 mL PBS with agitation and complete serial medium replacement for Au recovery. Together, the experiments establish compatible temporal behavior—matrix erosion accompanied continuing elemental Au recovery. A future mechanistic release model can align geometry and hydrodynamics and combine denser early sampling with simultaneous measurement of polymer erosion products, Au compartmentation, and particle state.

Near-quantitative endpoint recovery strengthens the release analysis by accounting for adsorption to the vessel, settling, and material retained with eroded debris. Explicit quantification of the tube/sediment/wash fraction captured 5.75% of initial Au in addition to supernatant and terminal-specimen fractions. Accordingly, the curve reports the time-dependent availability and compartmental distribution of recoverable elemental Au under the defined in vitro conditions. Particle-resolved analysis of each compartment will determine how dispersed nanoparticles, aggregates, polymer-associated Au, and sedimented Au-bearing material contribute to that profile.

3.4. Baseline non-irradiated effects permitted radiosensitization assessment In the non-contact Transwell system, blank wafers produced little change in U-87 MG cells: metabolic activity was $97.26 \pm 6.95\%$ at 24 h and $94.92 \pm 3.58\%$ at 72 h, while LDH release was $6.61 \pm 1.05\%$ and $7.36 \pm 0.94\%$, respectively, compared with $4.22 \pm 0.61\%$ and $5.68 \pm 0.70\%$ in controls (Fig. 4A,B). Au-containing treatments caused moderate, time-dependent standalone effects. At 72 h, metabolic activity was $78.12 \pm 5.39\%$ with free AuNPs and $84.71 \pm 3.22\%$ with loaded wafers; LDH release was $23.40 \pm 1.29\%$ and $17.22 \pm 0.81\%$, respectively. At 24 h, corresponding metabolic activities were $91.48 \pm 4.68\%$ and $94.80 \pm 6.99\%$, and LDH values were $10.89 \pm 1.12\%$ and $8.38 \pm 0.60\%$.

NHA also tolerated the blank matrix: at 72 h, metabolic activity was $97.70 \pm 4.88\%$ and LDH release $5.99 \pm 0.68\%$ (Fig. 4C,D). Free AuNPs and loaded wafers yielded $89.81 \pm 4.49\%$ and $92.46 \pm 5.20\%$ metabolic activity and $15.72 \pm 1.24\%$ and $12.78 \pm 0.85\%$ LDH release, respectively. Thus, blank-wafer effects were minor and Au-associated effects were measurable, providing a suitable baseline for subsequent radiation-response analysis. The slightly smaller standalone response with the wafer is consistent with gradual Au availability and provides a focused hypothesis for dedicated mechanistic and selectivity studies.

Using both CCK-8 and LDH reduced reliance on a single operational definition of cytocompatibility. CCK-8 reflects cellular metabolic reduction capacity and can change without membrane rupture, whereas extracellular LDH primarily reports loss of membrane integrity. The agreement between preserved metabolic activity and low LDH with blank wafers supports short-term compatibility of the unloaded matrix in this non-contact configuration. The time-dependent changes with both Au conditions confirm that Au exposure influenced baseline cellular behavior and support treatment-specific 0-Gy normalization for clonogenic survival. NHA provided a preliminary normal-cell comparator, with smaller but detectable responses that inform subsequent evaluation of the therapeutic window.

3.5. The AuNP-loaded wafer produced the strongest clonogenic radiosensitization

After 4 Gy, CCK-8 metabolic activity declined from $80.17 \pm 1.58\%$ at 24 h to $61.67 \pm 2.54\%$ at 72 h with RT alone and from $78.82 \pm 2.10\%$ to $60.83 \pm 2.46\%$ with blank wafer + RT (Fig. 5A,B). Free AuNPs + RT produced values of $69.96 \pm 1.83\%$ and $46.55 \pm 3.06\%$, whereas AuNP-loaded wafer + RT produced $63.82 \pm 3.49\%$ and $36.74 \pm 2.03\%$ at the same times. The loaded formulation therefore gave the largest short-term metabolic suppression; clonogenic analysis was used to determine durable reproductive survival.

Clonogenic survival consequently provided the primary evidence of radiosensitization [13]. Treatment-specific 0-Gy PE values were 0.630 ± 0.021 for RT alone, 0.602 ± 0.011 for blank wafer + RT, 0.558 ± 0.021 for free AuNPs + RT, and 0.582 ± 0.020 for AuNP-loaded wafer + RT. Free AuNPs reduced baseline PE relative to RT alone ($p < 0.01$); normalization to group-matched PE separated this standalone clonogenic effect from radiation-dependent survival.

RT alone yielded SF values of 0.6547 ± 0.0772 , 0.3415 ± 0.0502 , and 0.1888 ± 0.0090 at 2, 4, and 6 Gy, respectively. Blank wafer + RT closely overlapped these values (0.6336 ± 0.0346 , 0.3395 ± 0.0269 , and 0.1881 ± 0.0160) and did not differ significantly at any dose. Free AuNPs + RT reduced SF to 0.4898 ± 0.0445 , 0.2381 ± 0.0105 , and 0.0840 ± 0.0033 . These values were below RT alone and blank wafer + RT at 2 and 4 Gy (both $p < 0.05$) and 6 Gy (both $p < 0.0001$).

AuNP-loaded wafer + RT produced the lowest SF: 0.4394 ± 0.0481 at 2 Gy, 0.1514 ± 0.0071 at 4 Gy, and 0.0522 ± 0.0112 at 6 Gy. Compared with RT alone and blank wafer + RT, differences were significant at 2 Gy (both $p < 0.01$), 4 Gy (both $p < 0.001$), and 6 Gy (both $p < 0.0001$). The loaded wafer also reduced SF relative to free AuNPs at 4 and 6 Gy (both $p < 0.05$), whereas the 2-Gy difference was not significant. Thus, the carrier showed no radiosensitization, whereas both Au conditions did; the loaded formulation produced an additional effect at the higher tested doses.

LQ modeling gave D_{25} values of 5.129 Gy for RT alone, 5.096 Gy for blank wafer + RT, 3.779 Gy for free AuNPs + RT, and 3.095 Gy for AuNP-loaded wafer + RT (Fig. 5E). Relative to RT alone, $SER_{0.25}$ was 1.007, 1.357, and 1.657 for blank wafer, free AuNPs, and loaded wafer, respectively. SF = 0.25 was selected to

keep the enhancement-ratio calculation within the response range supported by all fitted curves across the measured 0–6 Gy interval.

The dose-specific and model-derived results were mutually consistent. Blank wafer + RT tracked RT alone in both SF and D_{25} , whereas each Au-containing treatment shifted the response toward lower survival and a smaller dose required to reach SF = 0.25. The advantage of the loaded wafer over free AuNPs was significant at 4 and 6 Gy (both $p < 0.05$) but not at 2 Gy. $SER_{0.25}$ was chosen within the range supported by all fitted curves, avoiding extrapolation to SF = 0.10 beyond adequately sampled survival for some groups.

Prior studies support AuNP-mediated radiosensitization in GBM, including U-87 MG clonogenic models [6, 7]. Here, the Au-equivalent comparison indicates that incorporating AuNPs into the degradable wafer altered functional radiobiological performance rather than merely supplying the same nominal Au dose: under the tested exposure conditions, wafer-associated Au produced lower SF at 4 and 6 Gy and a larger $SER_{0.25}$. Potential contributors include time-dependent Au availability, extracellular presentation, cellular processing, and particle-radiation interactions. The present data demonstrate formulation-associated radiosensitization while defining intracellular Au amount and localization as focused mechanistic targets for subsequent study.

The free-AuNP comparator was designed around the exposure immediately preceding irradiation. Matching it to the 24-h medium-recoverable Au concentration, rather than to the total Au embedded in the wafer, avoided presenting cells with the entire nominal wafer payload as a freely dispersed bolus. The resulting comparison establishes a matched elemental-Au benchmark rather than physicochemical equivalence: free AuNPs and wafer-derived Au-bearing material may differ in particle state, sedimentation, protein-corona formation, diffusion through the insert, and cellular association. It therefore tests whether formulation architecture changes biological performance at matched elemental Au, while particle-state and cellular-association analyses can identify the material properties contributing to the difference.

Treatment-specific PE is central to this interpretation. Both Au conditions altered non-irradiated colony formation, most clearly for free AuNPs; use of a single untreated PE could therefore confound baseline cytotoxicity with radiosensitization. Normalization to each treatment's 0-Gy PE directly tests how irradiation changes colony-forming capacity among cells exposed to that treatment. After this correction, the blank wafer tracked RT alone across 2–6 Gy, while the progressively separated Au-containing curves support an interaction with radiation response. The dose dependence locates the measurable loaded-wafer advantage over free AuNPs at 4 and 6 Gy.

Use of a clinical 6-MV beam strengthens the functional relevance of the comparison. Because AuNP effects under megavoltage photons depend strongly on biological and formulation context, direct measurement of clonogenic survival is particularly informative. D_{25} shifted from 5.129 Gy with RT alone to 3.779 Gy with free AuNPs and 3.095 Gy with the loaded wafer, yielding $SER_{0.25}$ values of 1.357 and

1.657, respectively. These model-derived comparisons summarize the observed 0–6 Gy response and are interpreted alongside the dose-specific SF data rather than as clinical dose-conversion factors.

3.6. γ -H2AX signaling supported the radiobiological phenotype

γ -H2AX/total H2AX was measured by immunoblot 1 and 24 h after 4 Gy (Fig. 6), providing a population-level measure of radiation-associated DNA-damage signaling; nuclear foci, lesion number and complexity, and repair kinetics require dedicated analyses [14, 17]. At 1 h, untreated cells had a ratio of 0.153 ± 0.032 relative to the RT-alone reference of 1.000 ± 0.000 . Blank wafer + RT was comparable at 1.118 ± 0.044 . Free AuNPs + RT increased the ratio to 1.665 ± 0.056 , above RT alone and blank wafer + RT (both $p < 0.01$); AuNP-loaded wafer + RT gave 1.896 ± 0.071 , above RT alone and blank wafer + RT (both $p < 0.001$) and free AuNPs + RT ($p < 0.05$).

At 24 h, ratios were 0.529 ± 0.010 for RT alone, 0.503 ± 0.037 for blank wafer + RT, 0.928 ± 0.045 for free AuNPs + RT, and 1.314 ± 0.158 for AuNP-loaded wafer + RT. Free AuNPs remained above RT alone and blank wafer + RT (both $p < 0.01$); the loaded wafer remained above RT alone and blank wafer + RT (both $p < 0.0001$) and free AuNPs + RT ($p < 0.05$). This ordering parallels clonogenic suppression and is consistent with nanoparticle-associated amplification or persistence of DNA-damage signaling reported in related GBM systems [7, 18]. The result accommodates contributions from initial lesion burden, prolonged signaling, and altered processing, which can be distinguished by foci-based imaging and time-resolved repair-pathway measurements.

Together, the two time points show an early treatment-associated elevation and persistence of the same group ordering at 24 h. As population-average immunoblots, they provide complementary signaling evidence at defined post-irradiation intervals. Single-cell foci imaging across a denser time course, paired with cell-cycle and viability measurements, can distinguish damage induction, signaling persistence, and selective loss of heavily damaged cells. Accordingly, the immunoblot findings support, without uniquely specifying, the biological process associated with reduced clonogenic survival.

3.7. Integrated interpretation and limitations

The principal advance lies in connecting formulation accountability to a durable radiobiological endpoint. Surface and spectroscopic characterization confirmed an intact Au-containing polymer disc; independent-batch ICP-MS, complementary TGA, degradation measurements, serial elemental recovery, and endpoint mass closure defined the material delivered to the biological system. Baseline CCK-8 and LDH measurements contextualized treatment effects, and treatment-specific PE distinguished baseline effects from radiation sensitization. Within this integrated framework, the lower SF and larger SER_{0-25} with the loaded wafer than with the 24-h Au-matched free comparator demonstrate a formulation-dependent phenotype under the tested conditions.

This architecture is relevant to postoperative delivery because the implant functions as both a local material reservoir and an exposure-modifying interface. More than half of the initial elemental Au became recoverable over 14 days, while approximately 41% remained specimen-associated, indicating that availability extended beyond the single 24-h interval used before irradiation. The Transwell design provided a controlled non-contact test of biological effects from material crossing the insert. Resection-cavity studies can next determine how tissue fluid, proteins, phagocytic cells, interstitial transport, and repeated clinical fractions alter Au disposition. Fractionated-irradiation experiments are especially important because a sustained local platform should ultimately be evaluated across repeated exposures.

The formulation also illustrates why local radiosensitizer performance extends beyond nominal Au content. A useful implant must couple manufacturable loading with an exposure profile compatible with the radiation schedule while limiting carrier-associated injury. In this study, the same measured loading value linked the theoretical formulation input, release denominator, mass balance, and free-comparator dose, reducing ambiguity across experimental stages. The blank wafer controlled for soluble degradation products and the insert, while the free-Au group controlled for elemental Au exposure at the selected pre-irradiation time. Together, these controls isolate the central in vitro inference: the stronger clonogenic phenotype required both Au and its presentation through the degrading wafer system. Characterization of cellular delivery, persistence, and particle state will guide rational optimization of that advantage.

The present study defines an in vitro proof-of-concept scope. FE-SEM/EDS sampled surface regions, ATR-FTIR assessed dominant matrix features, ICP-MS quantified recoverable elemental Au rather than its particulate or colloidal state, and compression testing provided representative mechanical behavior. Biological evaluation used one established GBM line in non-contact two-dimensional culture; patient-derived, three-dimensional, and in vivo models and direct intracellular Au localization and processing remain priorities. NHA served as a preliminary normal-cell comparator rather than a measure of normal-brain safety or tumor selectivity. γ -H2AX immunoblotting provided population-level damage-associated signaling; direct double-strand-break counts, foci distribution, and repair kinetics require dedicated assays. These defined boundaries preserve the treatment-normalized clonogenic inference and establish a focused path for mechanistic and translational validation.

A staged validation strategy follows directly from these boundaries. Particle-resolved analysis of medium, sediment, and residual-wafer fractions should first determine whether recoverable Au remains nanoparticulate and how size or aggregation changes during erosion. Three-dimensional elemental mapping and replicated mechanical testing should establish batch and within-wafer uniformity and handling performance. Cellular studies should quantify total and subcellular Au, γ -H2AX foci, oxidative stress where appropriate, and survival after clinically relevant fractionation in additional established and patient-derived GBM models, ideally in three-dimensional tumor-normal brain co-cultures. The decisive translational experiment would then place a characterized wafer in an orthotopic post-resection model to measure local retention, tissue distribution, neurotoxicity, and tumor response with fractionated RT.

4. Conclusion

AuNP-loaded P(CPP:SA) wafers were fabricated with $76.88 \pm 2.04 \mu\text{g Au/mg wafer}$ and 83.56% loading efficiency while retaining gross surface architecture and dominant polymer-associated ATR-FTIR features. The matrix degraded progressively, and recoverable elemental Au reached $52.30 \pm 4.91\%$ by Day 14 with $99.36 \pm 3.72\%$ endpoint mass recovery. In U-87 MG cells exposed through Transwell inserts, the blank wafer had little baseline effect, whereas the AuNP-loaded wafer enhanced the response to 6-MV photons. Clonogenic survival, the primary durable endpoint, was lower than with RT alone, blank wafer + RT, and Au-matched free AuNPs at 4 and 6 Gy; $\text{SER}_{0.25}$ was 1.657 versus 1.357 for free AuNPs. Greater $\gamma\text{-H2AX/total H2AX}$ ratios at 1 and 24 h provided supporting evidence of altered DNA-damage-associated signaling. Together, these findings establish an in vitro proof of concept. The physical state of recovered Au, intracellular disposition, specific radiosensitization mechanism, and in vivo performance remain to be established through particle-resolved characterization, intracellular Au quantification, and validation in patient-derived three-dimensional GBM and orthotopic resection-cavity models.

Declarations

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Clinical trial number

Not applicable.

Author Contributions

D.Z. and T.W.: conceptualization, methodology, investigation, formal analysis, data curation, and original draft. T.Y.: methodology, formal analysis, review, and editing. T.C.: conceptualization, supervision, validation, resources, review, and editing. All authors approved the manuscript and accept accountability for the work.

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Competing Interests

The authors declare no conflict of interest.

Data Availability

Data supporting this study are available from the corresponding author on reasonable request.

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Figures

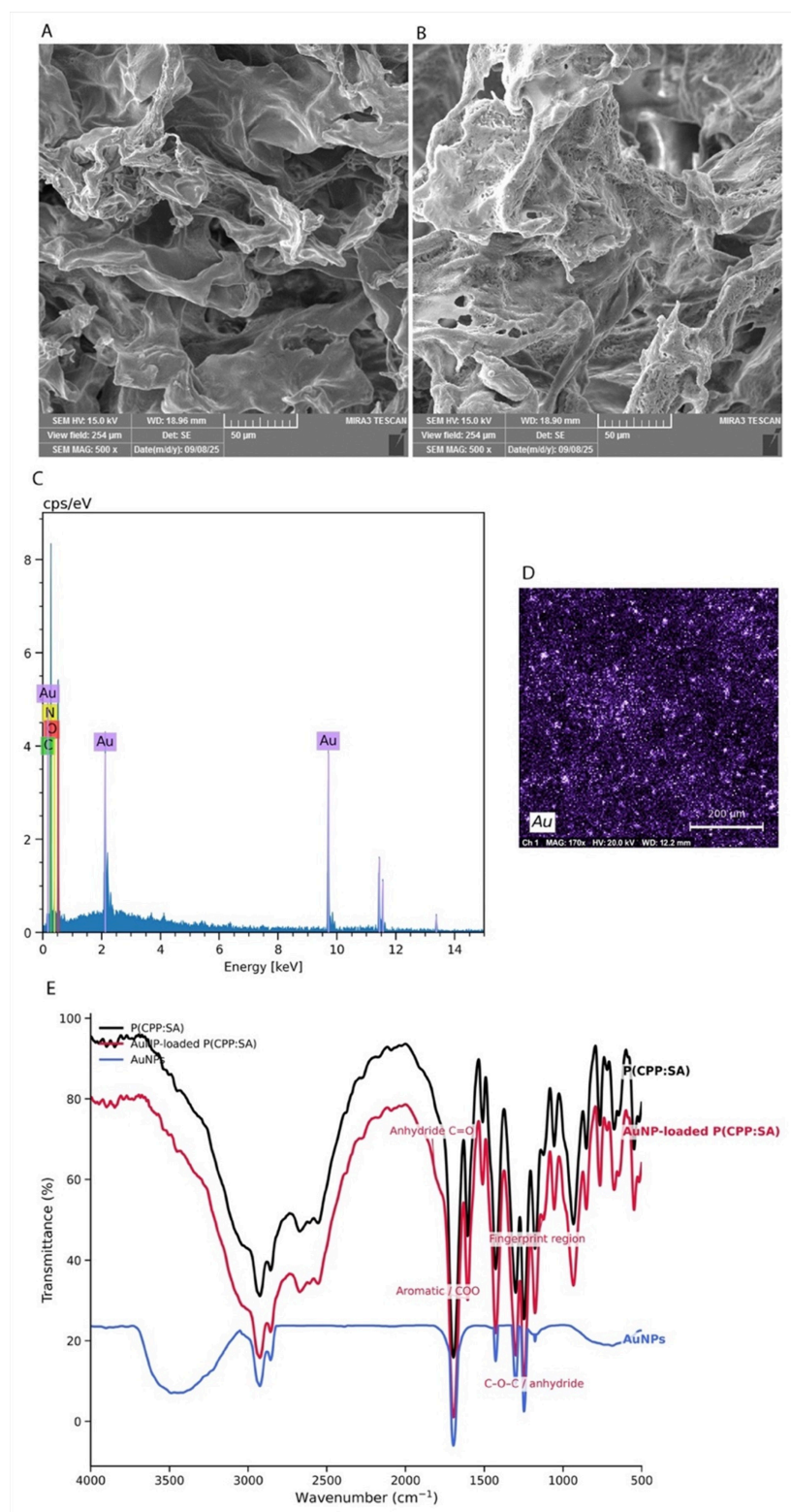


Figure 1

Morphological, elemental, and spectroscopic characterization of AuNP-loaded P(CPP:SA) wafers. (A,B) Representative FE-SEM surface micrographs of blank and AuNP-loaded wafers; scale bars are shown. (C) EDS spectrum of the loaded wafer showing Au-associated peaks. (D) Au map of the analyzed surface region (scale bar, 200 μm). (E) ATR-FTIR spectra of blank wafer, loaded wafer, and dried AuNP feedstock.

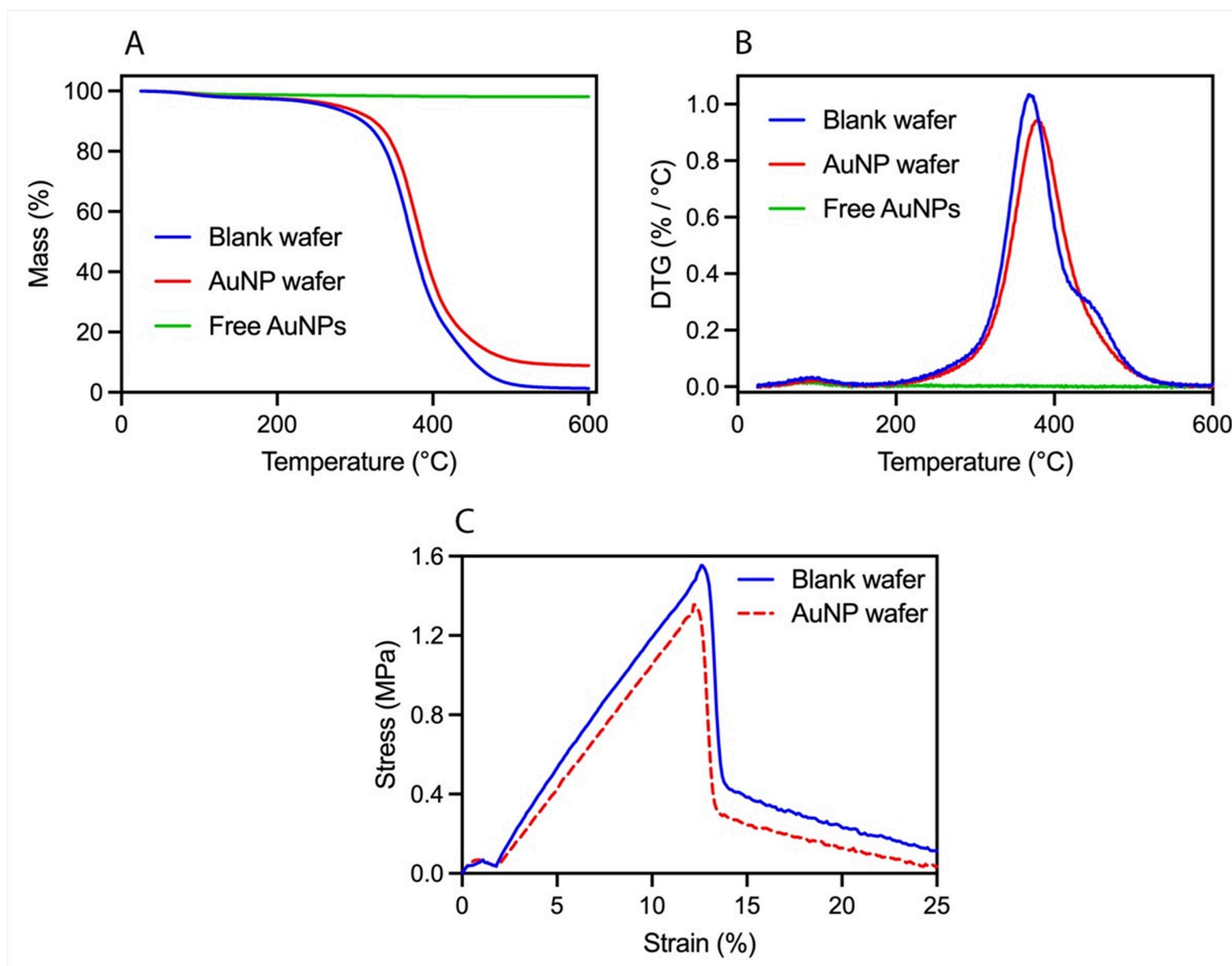


Figure 2

Thermal analysis and representative compression behavior. (A) TGA and (B) DTG profiles of blank wafers, AuNP-loaded wafers, and dried AuNP feedstock (mean $\pm$ SD; $n = 3$ independent replicates). (C) Representative unconfined-compression stress-strain curves, presented descriptively.

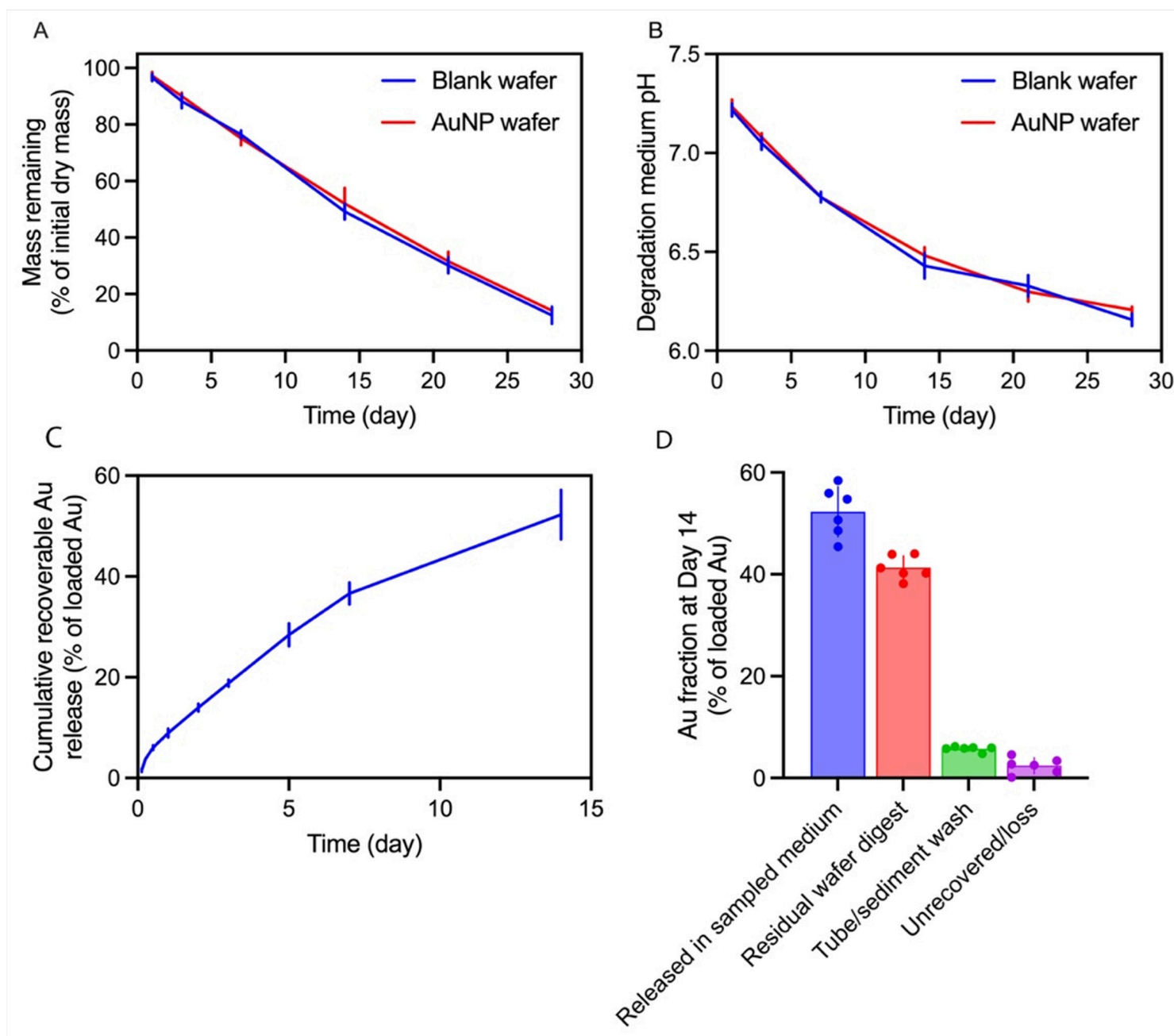


Figure 3

Wafer degradation, medium pH, recoverable Au release, and endpoint mass balance. (A) Dry mass retained and (B) medium pH during 28-day incubation of blank and AuNP-loaded wafers (mean $\pm$ SD; $n = 6$ independent specimens/formulation/time point). Day 0 was a pre-incubation baseline. (C) Cumulative recoverable elemental Au from standardized loaded-wafer specimens during 14 days and (D) Day-14 distribution among medium, residual specimen, and tube/sediment/wash fractions (mean $\pm$ SD; $n = 6$). ICP-MS quantified recoverable elemental Au after digestion; particle state requires particle-resolved characterization.

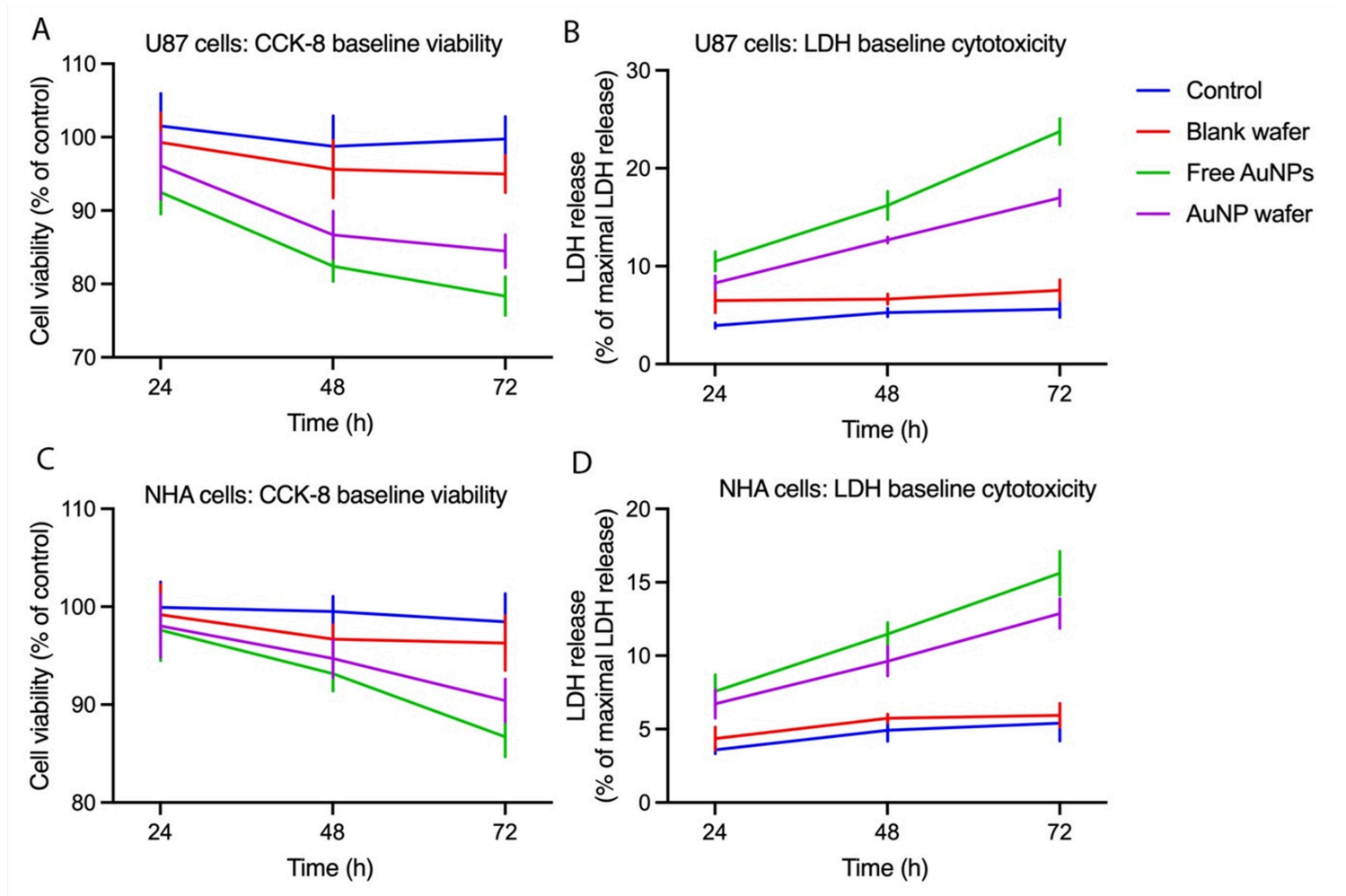


Figure 4

Baseline cytocompatibility in U-87 MG cells and normal human astrocytes. Cells were exposed for 24, 48, or 72 h to untreated control, blank wafer, free AuNPs, or AuNP-loaded wafer in Transwell inserts. (A,C) CCK-8 metabolic activity and (B,D) LDH release in U-87 MG cells and NHA. Signals were corrected with group-matched cell-free blanks; CCK-8 was normalized to the time-matched untreated mean, and LDH to matched low/high controls. Mean $\pm$ SD; n = 4 independent biological replicates, each measured in technical triplicate.

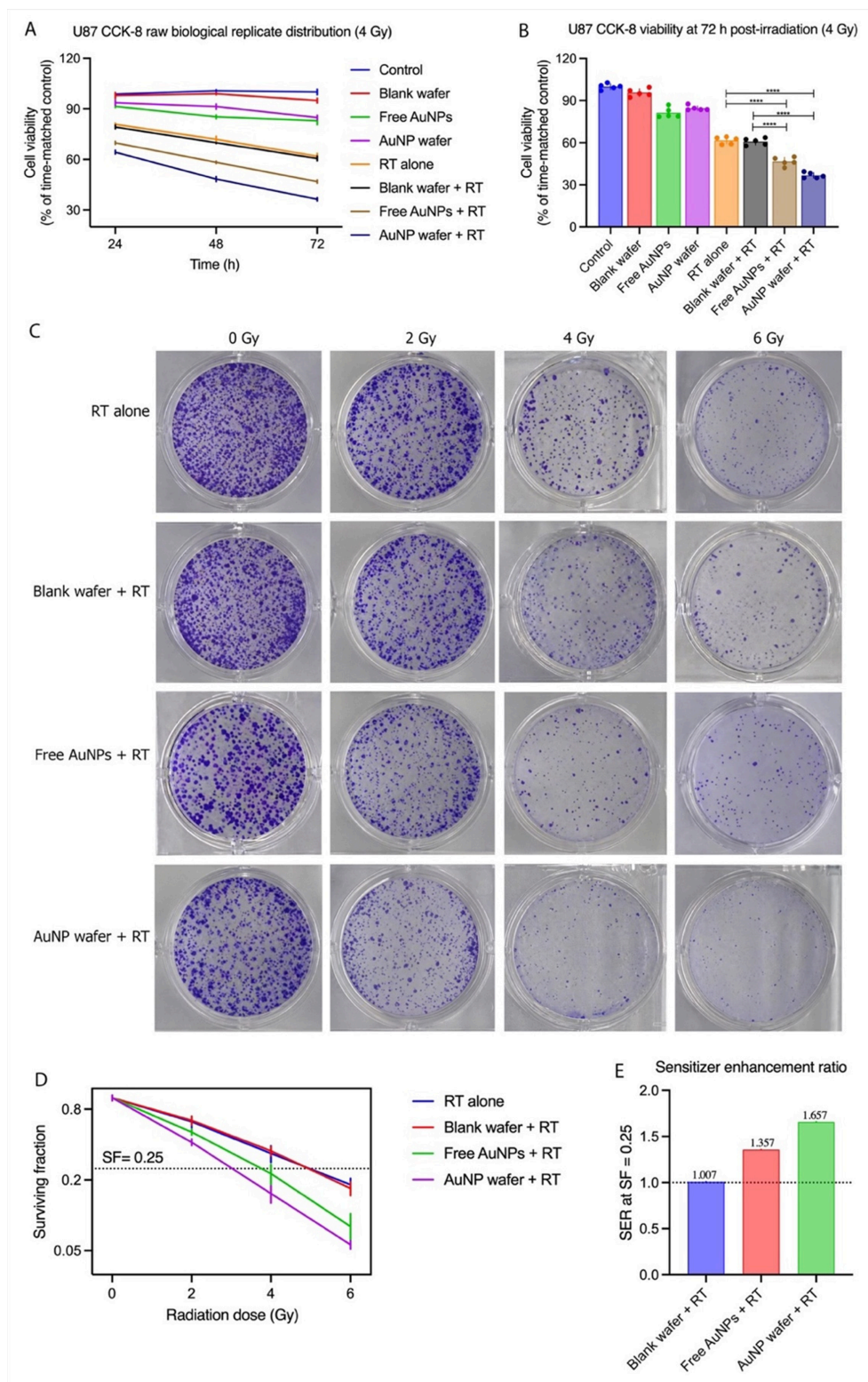


Figure 5

Short-term metabolic response and durable clonogenic radiosensitization. (A,B) U-87 MG CCK-8 activity at 24, 48, and 72 h after 4 Gy across eight treatment groups (mean $\pm$ SD; $n = 5$ biological replicates, technical triplicates). (C) Representative clonogenic plates, (D) SF curves normalized to treatment-specific 0-Gy PE, and (E) $SER_{0.25}$ after 0, 2, 4, or 6 Gy. Clonogenic data are mean $\pm$ SD from $n = 3$ independent biological experiments with technical triplicate dishes averaged before analysis. One-way

ANOVA with Tukey testing was performed separately at each dose; significance thresholds are indicated in the panels. Clonogenic survival was the primary durable endpoint

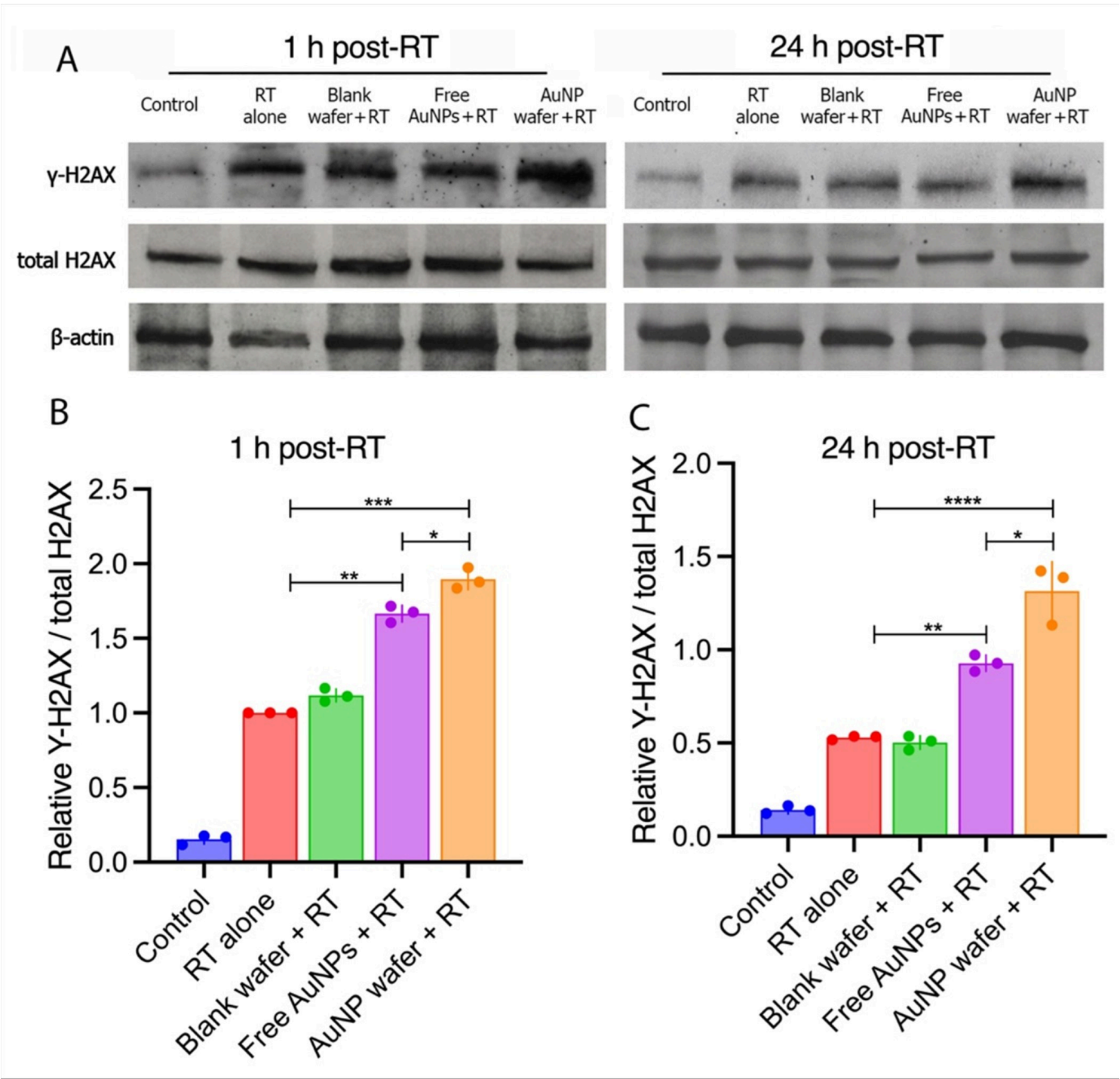


Figure 6

Early and residual γ -H2AX signaling after irradiation. U-87 MG cells were harvested 1 or 24 h after 4 Gy. (A) Representative immunoblots for γ -H2AX, total H2AX, and β -actin. (B,C) γ -H2AX/total H2AX densitometry at 1 and 24 h, expressed relative to the 1-h RT-alone group. Mean $\pm$ SD; n = 3 independent biological experiments. One-way ANOVA with Tukey testing was performed separately at each time point; significance thresholds are indicated in the panels.

Supplementary Files

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