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Polymer-Supported Gold Nanoparticle Radiosensitizers with Enhanced Cellular Uptake Efficiency and Increased Cell Death in Human Prostate Cancer Cells

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Polymer supported gold nanoparticles (AuNPs) show increased stability to biological and physiological conditions compared to free AuNPs. Here, we demonstrate an increase in cellular uptake efficiency of Au by DU145 human prostate cancer cell line for 4.5 nm AuNPs loaded onto 100 nm (13%) and 200 nm (18%) polystyrene particles compared to free AuNPs (4%). Composite formulations were well tolerated by the cell with minimal direct toxicity. Under X-ray irradiation significant radiation dose enhancement of 1.23 @ 4 Gy was observed, outperforming previously reported radiosensitization experiments using free AuNPs, demonstrating the potential application of polymer supported AuNPs as radiosensitizers.

Radiotherapy continues to be a key form of cancer therapy. However, effectiveness is restricted by the need to limit off target dose deposition to surrounding healthy tissues, and by the inherent differences between normal and tumor cells in their response to ionizing radiation. A solution to these limitations lies in the use of radiosensitizing molecules or materials, which increase the effective dose to cells, offering a means to treat radiation resistant tumours.¹ In particular, metal enhanced radiation therapy is attractive as exploits the increased absorption of X-rays by high-Z materials to produce locally damaging secondary electrons such as Auger electrons.² Low energy electrons can damage surrounding biological molecules by (a) reacting directly or by (b) reacting with water molecules to yield reactive oxygen species (ROS) such as hydroxyl radicals, which lead to indirect damage.^{3,4} In the last two decades gold (Au, Z = 79) nanoparticles (AuNPs) have received significant attention as radiosensitizers due to their high photoelectric cross-section, chemical inertness, biocompatibility, and low toxicity.³⁻⁶ A wide variety of AuNP systems have been tested for their radiosensitization properties, examining physical properties such as the influence of nanoparticle size, shape and the composition of the surface ligands.^{7,8} The use of AuNP loaded drug molecules has also been investigated for radiotherapy combined with chemotherapy treatment or imaging.^{9,10} Previous studies have shown that the high surface area associated with small, sub 5 nm, AuNPs results in a high ROS yield.¹¹ The choice of AuNP stabilizing ligands also influences the magnitude of radiosensitization and in general small charged ligands enhance the radiosensitizing properties of AuNPs while bulky neutral ligands reduce the effect.

Retaining the stability of electrostatically stabilized AuNPs is an important consideration as biological environments may impose hostile conditions to nanoparticles. This can be overcome by preparing AuNP composite materials. For the latter, a wide range of support materials have been described in the literature, including polymer beads¹² and silica particles.¹³ Furthermore, composite assemblies of AuNPs avoid the more rapid clearance of small particles, which allow

for increased intra-cellular concentrations and greater efficacy due to their biological stability.¹⁴ We recently reported the preparation of composite materials comprised of AuNPs immobilized on a polystyrene (PS) scaffold, which exhibit increased stability to ionic solutions and biological medium at physiological temperatures.¹⁵ Composite particles with over a 1000 AuNP (4.5 nm) per 200 nm, possessing a AuNP surface area of 18.5 m²/g, were prepared by immobilizing citrate stabilized AuNPs on amine functionalized PS carriers. Furthermore, the composites were found to be efficiently internalized by the radiation resistant MDA-MB-231 breast cancer cell line.¹⁵

The development of composite nanoparticle based radiosensitizers is not widely reported. These systems typically focus on nanomaterials supported molecular sensitizers such as the successful use of poly lactic-co-glycolic acid (PLGA) polymer particles to deliver molecular radiosensitizing agents to prostate cells.¹⁶ However, to our knowledge, the report of graphene oxide supporting FePt radiosensitizing nanoparticles is one of the few examples of composite supported metal nanoparticles, with no examples of supported AuNPs radiosensitizers appearing.¹⁷ In this study we now report on the cytotoxicity, internalization and radiosensitizing potential of 4.5 nm citrate stabilized AuNPs supported on 100 nm and 200 nm PS beads, comparing their performance to that of discrete 4.5 nm AuNPs, see Fig. 1. DU145 androgen independent prostate cancer cells were selected to investigate the ability of the composite nanoparticle formulations to overcome their inherent radioresistance driven efficient DNA damage repair and free radical scavenging capabilities.¹⁸

Figure 1 Schematic representation of radiosensitization using AuNP@PS composite particles.

Negatively charged citrate stabilized AuNPs were prepared using a modified procedure reported by Jana *et al.*(see SI Methods).¹⁹ The formation of spherical AuNPs with a mean core diameter of $4.5 \text{ nm} \pm 0.7 \text{ nm}$ was confirmed by transmission electron microscopy (TEM) and supported by the characteristic surface plasmon band at 513 nm (Fig. S1). The AuNP concentration was estimated from absorption spectroscopy to be $(2.44 \pm 0.16) \times 10^{14}$ AuNPs per mL (Table S1). AuNP composites were prepared according to the method previously reported to prepare 200 nm composites, which exploits the affinity of amine modified PS for gold.¹⁵ Briefly, 100 nm and 200 nm amine-modified PS beads were agitated in the presence of 4.5 nm citrate stabilized AuNPs in aqueous solution at RT for 1 h. This was followed by repeated centrifugation (5000 rpm, 10 min) to remove the excess AuNPs in the supernatant before re-dispersing the composites in sterile H₂O. A large excess of AuNPs (1000 equivalents for 100 nm PS and 2750 equivalents for 200 nm PS) was used to ensure maximum loading on the PS surface. TEM images recorded for the **AuNP@PS-100** show well dispersed AuNPs on the surface of the PS beads. Importantly, the images show no evidence of free AuNPs, see Fig. 2a. Similar images were also recorded for the 200 nm composites, see Fig. S2. The UV-visible absorption spectrum of the 100 nm composite showed a surface plasmon (SPR) peak at 528 nm, which represents a 15 nm shift in the position from the SPR of the free AuNP (513 nm),

see Fig. 2b. This shift is indicative of AuNP-AuNP interactions at the PS surface.²⁰ Dynamic light scattering (DLS) measurements show the hydrodynamic diameter of the composites as similar to that of the parent PS bead, Table S2. Importantly, the DLS measurements for the 100 nm and 200 nm composites reveal the presence of discrete composites in solution, with no evidence of aggregation or the presence of individual AuNPs (Fig. 2c and Fig S2). ζ -potential measurements of the composites show the surface charge of the PS bead changing from positive ($+23 \pm 2$ mV for **PS-100 nm** and $+27 \pm 3$ mV for **PS-200 nm**) to negative values for the composites (-11 ± 2 mV for **AuNP@PS-100 nm** and -13 ± 2 mV for **AuNP@PS-100 nm**) due to the immobilization of negatively charged AuNPs, (Fig. 2d, Table S2). Composite AuNP loading was calculated by atomic absorption spectroscopy (AAS) quantification of Au atoms following acid digestion of (a) composite particles and (b) the free gold remaining in the supernatant following PS loading. These alternative approaches were found to be in good agreement, with AuNP loadings of 440 ± 39 and 1780 ± 45 respectively for the 100 nm and 200 nm PS supports, see Table S3.

Figure. 2 (a) TEM image of AuNP@PS-100 composite (Main scale bar 200 nm, inset scale bar 100 nm.). (b) DLS, (c) ζ -potential and (d) UV-visible spectra of 4.5 nm AuNPs and AuNP@PS-100 composite. All measurements were recorded in ddH₂O at pH 7.

The cell viability to AuNPs and AuNP composites was assessed against characteristically radioresistant DU145 androgen independent cells. These cells have previously been shown to tolerate the uptake of AuNPs²¹ and polymer particles.²² Cytotoxicity assays were performed using the fluorescent resazurin based Alamar Blue assay, deriving survival fractions of NP treated samples relative to untreated controls.²³ Cells were incubated with 10 $\mu\text{g/mL}$ of 4.5 nm AuNPs, **AuNPs@PS-100** and **AuNP@PS-200** for 24 h, as our previous study showed good tolerance of the cells to this amount.¹⁵ When the contribution of the polymer mass is accounted for, these incubation amounts are found to represent effective Au concentrations of 10 $\mu\text{g/mL}$, 4.3 $\mu\text{g/mL}$ and 2.9 $\mu\text{g/mL}$ for the respective systems. All AuNP and composite treated cells

exhibited a maximum 10% reduction in viability and as such the formulations can be deemed as non-toxic at the assayed concentrations (Fig. 3a). To ensure composite AuNP/PS nanoparticle exposure has no long-term anti-proliferative effect that may influence subsequent radiosensitization experiments, colony forming assays were performed. Cells were incubated with 10 µg/mL of composite nanoparticles for 24 h, after which cells were maintained in fresh (particle free) medium allowing colony formation. After 12 days, colony formation was assessed, and no significant toxicity was observed for any formulation (Fig. 3a).

A potential advantage of AuNP composite particles is the enhanced intracellular Au loading following uptake of the PS carrier particles. To assess this, the internalization of AuNPs and the AuNP composites was investigated using ICP-AAS. Briefly, 1.5×10^5 DU145 cells were treated with 10 µg/mL of each material for 24 h (representing effective Au concentrations of 10 µg/mL, 4.3 µg/mL and 2.9 µg/mL for the 4.5 nm AuNPs, **AuNPs@PS-100** and **AuNP@PS-200** systems respectively). After this time the cells were washed and dissolved in aqua regia to determine the exact amount of elemental gold per cell (Fig. 3b). Cells incubated with 4.5 nm AuNPs were found to accumulate a mean Au concentration of 2.62 ± 1.26 pg/cell. Significant amounts of internalized Au were also found for the cells treated with the **AuNP@PS-100** (3.70 ± 0.98 pg/cell) and **AuNP@PS-200** (3.56 ± 1.43 pg/cell). However, when the effective amount of Au incubated is considered, the uptake efficiency of the composite systems is found to be significantly enhanced, with values of 3.9 ± 1.9 % (AuNPs), 12.9 ± 3.4 % (**AuNPs@PS-100**) and 18.4 ± 7.4 % (**AuNPs@PS-200**) determined, see Fig. 3c. These results clearly show that the composite systems result in improved transport of AuNPs with ~ 3.1 and ~ 4.7 times greater efficiency observed for the **AuNPs@PS-100** and **AuNPs@PS-200** composites respectively.

We have previously demonstrated the improved biostability of these AuNP composite formulations, which was shown to be more stable against aggregation than discrete citrate stabilized AuNPs.¹⁵ This increased stability to ionic conditions may provide an extended

window of opportunity for nanoparticle endocytosis and may contribute to the increased cellular uptake. It is notable that there is significant deviation in uptake of AuNPs in all samples, with the greatest % deviation of 48% found for the ‘free’ AuNPs compared to a 26% and 29% deviation for the **AuNP@PS-100** and **AuNP@PS-200** composites respectively. This is may arise due to the inherent instability of free AuNPs to the washing procedure post-incubation, which incorporates a PBS wash. If some of the 4.5 nm AuNPs are aggregating during this washing step, it could potentially distort the cell internalization data through the formation of non-internalized aggregates.

The influence of size and surface charge on nanoparticle uptake has been well documented in the literature.²⁴ In this case, the surface charge for the two composite systems is similar (ζ -potential shift = -11 ± 2 mV (100 nm) vs. -13 ± 2 mV (200 nm)). Therefore, it is expected that size primarily influences composite nanoparticle uptake. Small nanoparticles afford greater surface area to volume and improved radiosensitization effects. Critically, while individual small 4.5 nm AuNP are likely to enter the cells through passive uptake, the size of the composite particles is likely to facilitate active Clathrin or Caveolae mediated uptake, which is a probable factor in the enhanced transport.²⁵ An uptake of *ca.* 8930 **AuNP@PS-100** and 2180 **AuNP@PS-200** composite particles was calculated based on intra-cellular Au concentration. When these values are considered against the estimated number of composites introduced to the cells the 200 nm particles are more efficiently internalized at 18.4 ± 7.4 %, see Table S4.

Figure. 3 (a) Cell viability of DU145 cells assayed 24 h (resazurin assay) and 12 days (clonogenic assay) post treatment with free 4.5 nm AuNPs and AuNP@PS composites. Treatment duration was 24 h for both assays using a nanoparticle concentration of 10 $\mu\text{g/ml}$. (b) ICP-AAS measurements of total intracellular Au (pg/cell) for 4.5 nm AuNPs, AuNP@PS-100 nm, and AuNP@PS-200 nm composites. (c) Uptake efficiency normalised against total Au mass per treatment (AuNP 10 $\mu\text{g/ml}$; AuNP@PS-100 nm 4.2 $\mu\text{g/ml}$; AuNP@PS-200 nm 2.9 $\mu\text{g/ml}$). One-way ANOVA with Bonferroni correction. * $p<0.05$, *** $p<0.001$.

As single agents, a model radiosensitizer should avoid inducing direct cell death. However, in combination with ionizing radiation (IR), these biologically inert materials should augment the cell killing effect of IR alone, generating a dose enhancement factor (DEF). The radiosensitizing capacity of citrate-AuNPs and AuNP@PS was determined using the previously described clonogenic assay. Briefly, this involved irradiation of cells between 1.5 Gy and 6 Gy, using a 160 kV X-ray source with a dose rate of 0.77 Gy/min. Radiation survival curves in the presence and absence of the AuNP systems are presented in Fig. 4 (representative images of colony formation, see Fig. S3). Across both the control and AuNP families there is a decrease in the survival fraction in a dose dependent manner, arising from a combination of both direct and indirect radiation mediated damage. The 4.5 nm citrate-AuNPs (Fig. 4a) conferred a small decrease in radiation sensitivity, by approximately 10%, of radiation protection, an effect dominated by radiation doses below 3.5 Gy. This effect could be potentially attributed media stimulated agglomeration of inherently unstable citrate-AuNPs, resulting in comprised internalization, and subsequent extracellular nanocluster photon absorption reducing the efficacy of radiation alone. This result agrees with published works where 1.9 nm AuNPs failed to confer substantial radiosensitization in DU145 cells. Typical dose enhancement factors ranged between 0.81 and 1.06 reported (Table 1).²⁶⁻²⁸ A significant ($p=0.0039$) increase in the sensitizer enhancement ratio to 1.17 was observed for the **AuNP@PS-100** composites (Fig. 4b), with a similar sensitizing effect observed using the **AuNP@PS-200** composite particles ($p=0.0048$) (Fig. 4c). Interestingly, radiation dose dependency was observed with the sensitizing effect further enhanced by dose per fraction. This is clearly demonstrated by calculating the dose enhancing factor at 4 Gy which indicates increased radiation sensitivity by 23% for both composite nanoparticle formulations. This suggests that in the increasingly common scenario of clinical hypofractionated radiotherapy, these composite nanoparticles could provide additional benefit.

Figure 4 (a-c) Graphs showing the clonogenic survival fractions as a function of radiation dose for all samples tested. Survival data was fitted to a linear quadratic survival curve, with dose enhancement factors at 4 Gy calculated. (a) Radiation dose modifying effect of 4.5 nm citrate-AuNP; (b) AuNP@PS-100; (c) AuNP@PS-200 composite nanoparticles.

This data represents superior radiosensitization performance compared to similar studies quoted in the literature (Table 1). Both the **AuNP@PS-100** and the **AuNP@PS-200** composites had similar cell viability and total Au internalization, therefore it is perhaps not overly surprising that the radiosensitizing effects were very similar. Notably, the absolute Au

concentration used in these experiments is significantly lower than those reported previously in the literature, with increased relative enhancement factors.

Table 1 Summary of the dose enhancement factors calculated for each family tested and comparison with literature values for DU145 cells.

Sample	Au Concentration μg/mL	Sensitizer Enhancement Ratio	Reference
4.5 nm AuNP	10	0.90	this work
4.5 nm AuNP@100 nm PS	4.3	1.17	this work
4.5 nm AuNP@200 nm PS	2.9	1.14	this work
1.9 nm AuNPs	10	0.91	²⁶
1.9 nm AuNPs	100	0.81	²⁶
1.9 nm AuNPs	500	1.06	²⁷
1.9 nm AuNPs	500	0.92	²⁸

Overall, these composite materials exhibit excellent cell viability and uptake, along with increased efficiency of uptake in respect to Au treatment concentration. Importantly, there were no observed long-term cytotoxic effects arising from exposure to these materials. Both the **AuNP@PS-100** and the **AuNP@PS-200** composites outperformed previously reported radiosensitization experiments using the same cell line and similar sized AuNPs, with superior sensitizer enhancement ratios measured. This was achieved using a reduced Au concentration, without any surface modification of the AuNPs.

AuNPs been applied to many biological applications from drug delivery to cellular imaging and cancer therapy. Light-activated tailored AuNPs offer access to combine radiosensitization with targeted photothermal effects,²⁹ which can be combined with the SERS activity of the composites to report on the cellular environment.²⁰ Future work may take advantage of priming of blood vessels by radiation treatment to enhance the uptake of nanoparticles in combination

with the modification of either the composite support or the AuNP surface to increase the “targeted” selectivity of these particles towards cancerous regions.^{30,31}

ASSOCIATED CONTENT

Supporting Information

The supporting information is available free of charge from the ACS Publications website at

DOI: xxxxxx

Additional experimental section detailing AuNP formation, composite preparation, Alamar blue toxicity study and cellular uptake of nanomaterials. 4.5 nm AuNP and 4.5 nm@PS-200 characterization by UV-vis, DLS, ζ -potential and TEM.

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‡L.B and S.A.B contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interests.

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REFERENCES

- (1) Wang, H.; Mu, X.; He, H.; Zhang, X. D. Cancer Radiosensitizers. *Trends Pharmacol. Sci.* **2018**, *39*, 24–48.
- (2) Hainfeld, J. F.; Dilmanian, F. A.; Slatkin, D. N.; Smilowitz, H. M. Radiotherapy Enhancement with Gold Nanoparticles. *J. Pharm. Pharmacol.* **2008**, *60*, 977–985.
- (3) Sicard-Roselli, C.; Brun, E.; Gilles, M.; Baldacchino, G.; Kelsey, C.; McQuaid, H.; Polin, C.; Wardlow, N.; Currell, F. A New Mechanism for Hydroxyl Radical Production in Irradiated Nanoparticle Solutions. *Small* **2014**, *10*, 3338–3346.
- (4) Rosa, S.; Connolly, C.; Schettino, G.; Butterworth, K. T.; Prise, K. M. Biological Mechanisms of Gold Nanoparticle Radiosensitization. *Cancer Nanotechnol.* **2017**, *8*, 2.
- (5) Hainfeld, J. F.; Slatkin, D. N.; Smilowitz, H. M. The Use of Gold Nanoparticles to Enhance Radiotherapy in Mice. *Phys. Med. Biol.* **2004**, *49*, N309–N315.
- (6) Coulter, J. a; Hyland, W. B.; Nicol, J.; Currell, F. J. Radiosensitising Nanoparticles as Novel Cancer Therapeutics--Pipe Dream or Realistic Prospect? *Clin. Oncol. (R. Coll. Radiol).* **2013**, *25*, 593–603.
- (7) Jain, S.; Hirst, D. G.; O’Sullivan, J. M. Gold Nanoparticles as Novel Agents for Cancer Therapy. *Br. J. Radiol.* **2012**, *85*, 101–113.
- (8) Ma, N.; Liu, P.; He, N.; Gu, N.; Wu, F. G.; Chen, Z. Action of Gold Nanospikes-Based Nanoradiosensitizers: Cellular Internalization, Radiotherapy, and Autophagy. *ACS Appl. Mater. Interfaces* **2017**, *9*, 31526–31542.
- (9) Setua, S.; Ouberaï, M.; Piccirillo, S. G.; Watts, C.; Welland, M. Cisplatin-Tethered Gold Nanospheres for Multimodal Chemo-Radiotherapy of Glioblastoma. *Nanoscale* **2014**, *6*, 10865–10873.

- (10) Alric, C.; Serduc, R.; Mandon, C.; Taleb, J.; Le Duc, G.; Le Meur-Herland, A.; Billotey, C.; Perriat, P.; Roux, S.; Tillement, O. Designed for Combining Dual Modality Imaging. *Gold Bull.* **2008**, *41*, 90–97.
- (11) Cheng, N. N.; Starkewolf, R. An. D.; Sharmah, A.; Lee, C.; Lie, J.; Guo, T. Chemical Enhancement by Nanomaterials under X-Ray Irradiation. *J. Am. Chem. Soc.* **2012**, *134*, 1950–1953.
- (12) Liu, Y.; Li, M.; Chen, G. A New Type of Raspberry-like Polymer Composite Sub-Microspheres with Tunable Gold Nanoparticles Coverage and Their Enhanced Catalytic Properties. *J. Mater. Chem. A* **2013**, *1*, 930–937.
- (13) Westcott, S. L.; Oldenburg, S. J.; Lee, T. R.; Halas, N. J. Formation and Adsorption of Clusters of Gold Nanoparticles onto Functionalized Silica Nanoparticle Surfaces. *Langmuir* **1998**, *14*, 5396–5401.
- (14) Feng, Q.; Shen, Y.; Fu, Y.; Muroski, M. E.; Zhang, P.; Wang, Q.; Xu, C. Self-Assembly of Gold Nanoparticles Shows Microenvironment-Mediated Dynamic Switching and Enhanced Brain Tumor Targeting. *Theranostics* **2017**, *7*, 1875–1888.
- (15) Belhout, S. A.; Kim, J. Y.; Hinds, D. T.; Owen, N. J.; Coulter, J. A.; Quinn, S. J. Multifunctional and Robust Composite Materials Comprising Gold Nanoparticles at a Spherical Polystyrene Particle Surface. *Chem. Commun.* **2016**, *52*, 14388–14391.
- (16) Menon, J. U.; Tumati, V.; Hsieh, J. T.; Nguyen, K. T.; Saha, D. Polymeric Nanoparticles for Targeted Radiosensitization of Prostate Cancer Cells. *J. Biomed. Mater. Res. - Part A* **2015**, *103*, 1632–1639.
- (17) Yang, C.; Peng, S.; Sun, Y.; Miao, H.; Lyu, M.; Ma, S.; Luo, Y.; Xiong, R.; Xie, C.; Quan, H. Development of a Hypoxic Nanocomposite Containing High-Z Element as 5-Fluorouracil Carrier Activated Self-Amplified Chemoradiotherapy Co-Enhancement. *R. Soc. Open Sci.* **2019**, *6*.
- (18) Jayakumar, S.; Kunwar, A.; Sandur, S. K.; Pandey, B. N.; Chaubey, R. C. Differential Response of DU145 and PC3 Prostate Cancer Cells to Ionizing Radiation: Role of Reactive Oxygen Species, GSH and Nrf2 in Radiosensitivity. *Biochim. Biophys. Acta* **2014**, *1840*, 485–494.
- (19) Jana, N.; Gearheart, L.; Murphy, C. Seeding Growth for Size Control of 5-40 Nm Diameter Gold Nanoparticles. *Langmuir* **2001**, *17*, 6782–6786.
- (20) Belhout, S. A.; Baptista, F. R.; Devereux, S. J.; Parker, A. W.; Ward, A. D.; Quinn, S. J. Preparation of Polymer Gold Nanoparticle Composites with Tunable Plasmon Coupling and Their Application as SERS Substrates. *Nanoscale* **2019**, *11*, 19884–19894.
- (21) Coulter, J. A.; Jain, S.; Butterworth, K. T.; Taggart, L. E.; Dickson, G. R.; McMahon, S. J.; Hyland, W. B.; Muir, M. F.; Trainor, C.; Hounsell, A. R.; Sullivan, J. M. O.; Schettino, G.; Currell, F. J.; Hirst, D. G.; Prise, K. M. Cell Type-Dependent Uptake, Localisation and Cytotoxicity of 1.9 Nm Gold Nanoparticles. *International J. Nanomedicine* **2012**, *7*, 2673–2685.

- (22) Chandratre, S. S.; Dash, A. K. Multifunctional Nanoparticles for Prostate Cancer Therapy. *AAPS PharmSciTech* **2014**, *16*, 98–107.
- (23) Rampersad, S. N. Multiple Applications of Alamar Blue as an Indicator of Metabolic Function and Cellular Health in Cell Viability Bioassays. *Sensors (Switzerland)* **2012**, *12*, 12347–12360.
- (24) He, C.; Hu, Y.; Yin, L.; Tang, C.; Yin, C. Effects of Particle Size and Surface Charge on Cellular Uptake and Biodistribution of Polymeric Nanoparticles. *Biomaterials* **2010**, *31*, 3657–3666.
- (25) Panzarini, E.; Mariano, S.; Carata, E.; Mura, F.; Rossi, M.; Dini, L. Intracellular Transport of Silver and Gold Nanoparticles and Biological Responses: An Update. *Int. J. Mol. Sci.* **2018**, *19*, 1305–1325.
- (26) Butterworth, K. T.; Coulter, J. A.; Jain, S.; Forker, J.; McMahon, S. J.; Schettino, G.; Prise, K. M.; Currell, F. J.; Hirst, D. G. Evaluation of Cytotoxicity and Radiation Enhancement Using 1.9 Nm Gold Particles: Potential Application for Cancer Therapy. *Nanotechnology* **2010**, *21*, 1–9.
- (27) Taggart, L. E.; McMahon, S. J.; Currell, F. J.; Prise, K. M.; Butterworth, K. T. The Role of Mitochondrial Function in Gold Nanoparticle Mediated Radiosensitisation. *Cancer Nanotechnol.* **2014**, *5*, 1–12.
- (28) Jain, S.; Coulter, J. A.; Hounsell, A. R.; Butterworth, K. T.; McMahon, S. J.; Hyland, W. B.; Muir, M. F.; Dickson, G. R.; Prise, K. M.; Currell, F. J.; O’Sullivan, J. M.; Hirst, D. G. Cell-Specific Radiosensitization by Gold Nanoparticles at Megavoltage Radiation Energies. *Int. J. Radiat. Oncol. Biol. Phys.* **2011**, *79*, 531–539.
- (29) Ma, N.; Jiang, Y. W.; Zhang, X.; Wu, H.; Myers, J. N.; Liu, P.; Jin, H.; Gu, N.; He, N.; Wu, F. G.; Chen, Z. Enhanced Radiosensitization of Gold Nanospikes via Hyperthermia in Combined Cancer Radiation and Photothermal Therapy. *ACS Appl. Mater. Interfaces* **2016**, *8*, 28480–28494.
- (30) Bennie, L. A.; McCarthy, H. O.; Coulter, J. A. Enhanced Nanoparticle Delivery Exploiting Tumour-Responsive Formulations. *Cancer Nanotechnol.* **2018**, *9*, 1–20.
- (31) Pearce, A. K.; O’Reilly, R. K. Insights into Active Targeting of Nanoparticles in Drug Delivery: Advances in Clinical Studies and Design Considerations for Cancer Nanomedicine. *Bioconjug. Chem.* **2019**, *30*, 2300–2311.

Table of Contents Graph

Increased cellular uptake efficiency of Au by DU145 human prostate cancer cell is observed for polymer composites compared to free AuNPs. The composites show minimal direct toxicity and a significant radiation dose enhancement of 1.23 @ 4 Gy X-ray irradiation.