Enhancing X-ray sensitization using poly(2-oxazoline) coated gold nanoparticles

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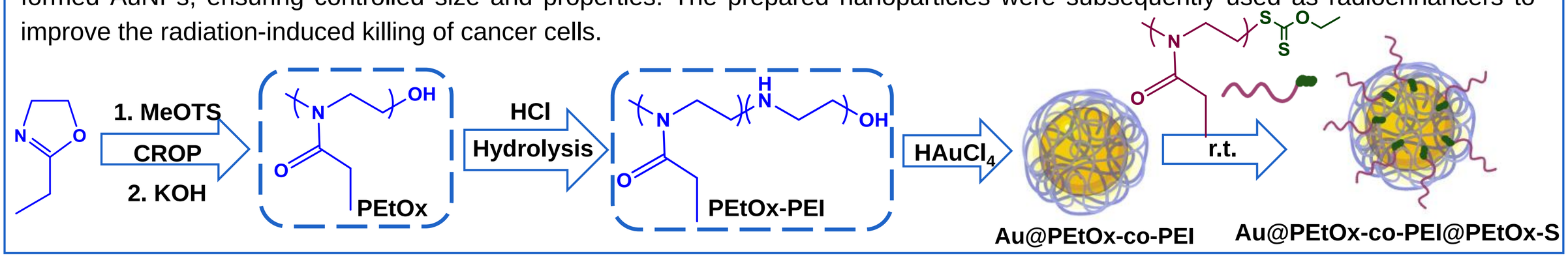
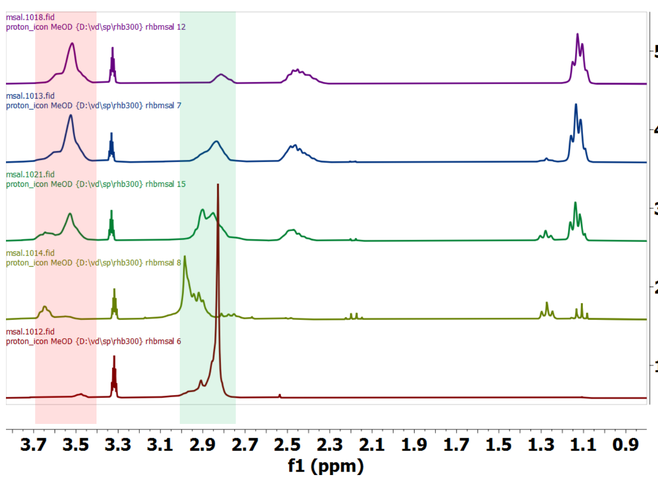
Introduction

Radiation therapy plays a vital role in cancer treatment and is applied to over half of all cancer patients. Even with significant advances in dose delivery, nearby healthy tissues still receive unwanted radiation, potentially leading to serious side effects[1]. Nanomaterials, especially high-Z nanoparticles such as gold, offer a promising way to improve both the efficacy and safety of cancer radiotherapy[2]. Here, we prepared Poly (2-oxazoline) (PAOx) coated gold NPs (AuNPs) using poly[(2-ethyl-2-oxazoline)-co-(ethylenimine)] (P(EtOx-co-EI)) as radio enhancer. The PEtOx-co-PEI copolymer was synthesized through the acid-catalyzed partial hydrolysis of poly(2-ethyl-2-oxazoline) (PEtOx)[3]. PEtOx-co-PEI copolymers retain the beneficial properties of both PAOx and PEI while exhibiting lower cytotoxicity than L-PEI. The ethylenimine (EI) groups can reduce the gold salt, while the oxazoline (EtOx) segments stabilize the formed AuNPs, ensuring controlled size and properties. The prepared nanoparticles were subsequently used as radioenhancers to improve the radiation-induced killing of cancer cells.



Characterization



No.	HCl (M)	Tem. (°C)	Time (min.)	Degrees of hydrolysis
1	6	125	60	~96
2	1	140	120	~80
3	1	140	26.5	~60
4	1	140	15	~40
5	1	140	7	~20

Fig. 1. NMR spectra and corresponding reaction conditions for partially hydrolyzed PEtOx (PEtOx-PEI) at different degrees of hydrolysis (20%, 40%, 60%, 80%, and 100%). PEtOx-PEI copolymers with varying PEI content were obtained by partial hydrolysis of PEtOx at different degrees of hydrolysis, which can be controlled by both acid concentration and reaction time.

Polymers hydrolyzed to 60% and 80% yielded NP that were smaller and exhibited greater monodispersity.

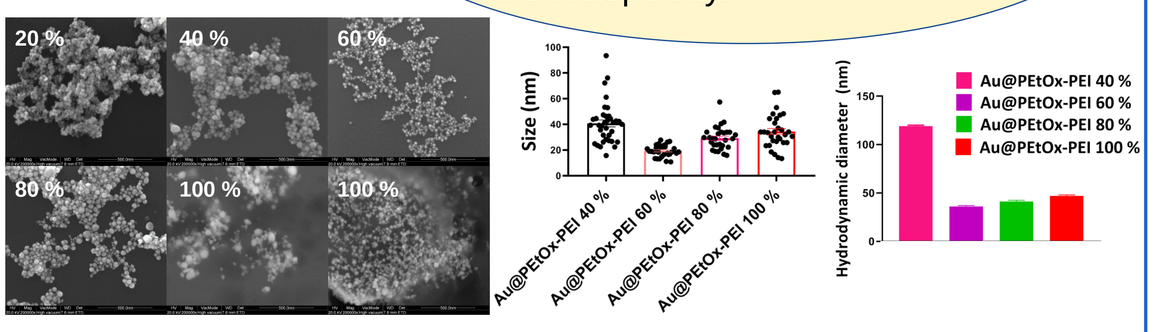
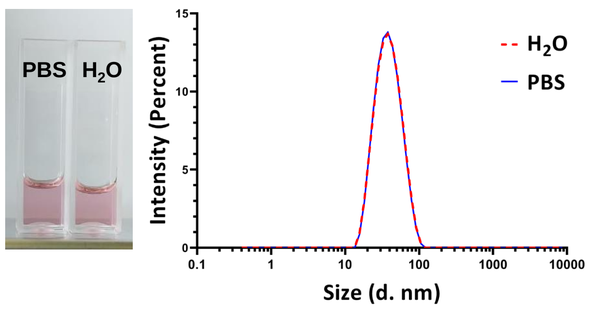


Fig. 2. Size distribution profiles measured by scanning electron microscopy (SEM) and dynamic light scattering (DLS) analysis of AuNPs synthesized using PEtOx-PEI copolymers with varying degrees of partial hydrolysis (20%, 40%, 60%, 80%, and 100%).



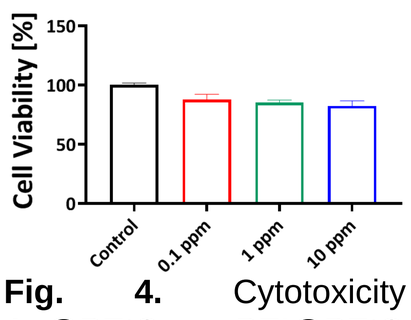
Medium	Z-Average (d. nm)	PdI	Zeta potential (mV)
H_2O	34.9	0.21	13.5
PBS	34.2	0.19	-5.6

Fig. 3. Stability of Au@PEtOx-co-PEI@PEtOx-S NPs in PBS. The PEtOx terminated with a thiol group can effectively stabilize AuNPs in PBS.

Conclusions

- We developed a new strategy to prepare stable gold NPs.
- The synthesis of AuNPs using the synthesized P(EtOx-co-EI) offers a one-step, eco-friendly, biocompatible, stable, and efficient approach without requiring additional reducing or stabilizing agents.
- Under X-ray irradiation, Au@PEtOx-co-PEI@PEtOx-S nanoparticles increase ROS production inside cells.
- X-ray irradiation combined with Au@PEtOx-co-PEI@PEtOx-S nanoparticles significantly enhances radiation efficacy, confirming their potent radioenhancing properties.

Cytotoxicity and Radiosensitizing effect of AuNPs



Au@PEtOx-co-PEI@PEtOx-S NPs induce minimal toxicity in healthy cells.

Fig. 4. Cytotoxicity of Au@PEtOx-co-PEI@PEtOx-S NPs on healthy cells (MCF-10a).

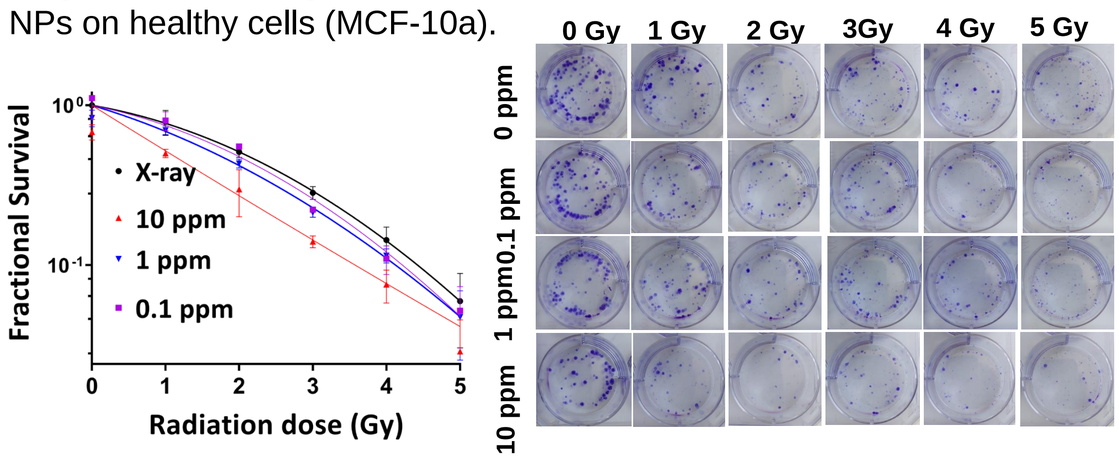


Fig. 5. Clonogenic survival and representative images of cells (MCF-7) treated with gold nanoparticles (AuNPs) and X-ray irradiation.

- Dose-dependent reduction in survival.
- Pre-treatment with AuNPs significantly enhanced the radiosensitizing effect in cells.

Intracellular ROS generation

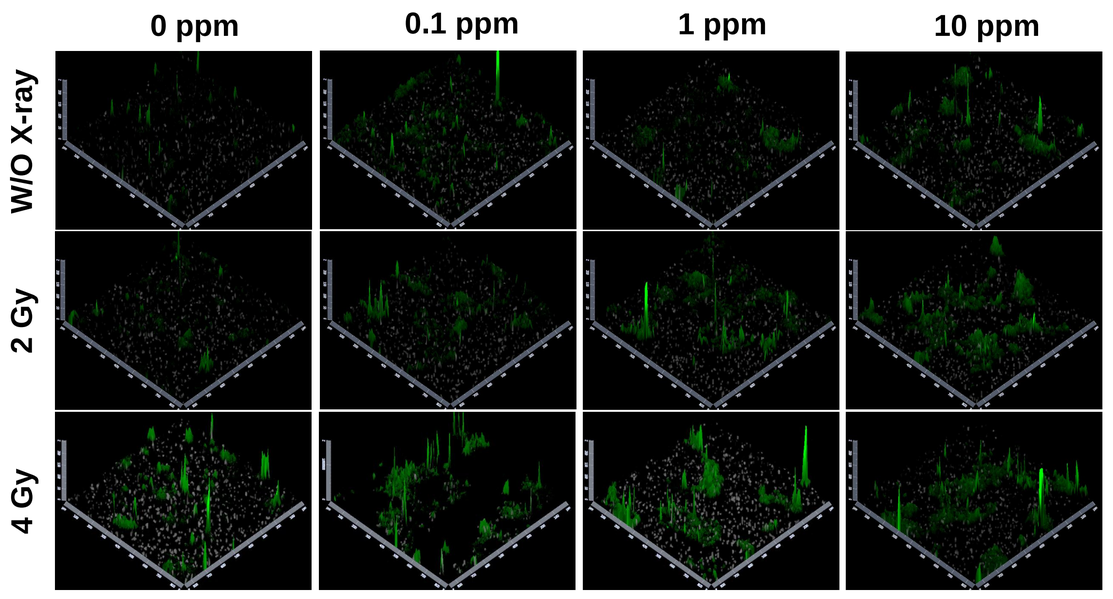


Fig. 6. Fluorescence microscopy images illustrate the levels of ROS within cells (MCF-7) under different treatment conditions.

A dose-dependent increase in fluorescence was observed with escalating NP concentrations, indicating enhanced ROS production. Furthermore, combined treatment with higher NP loading and increased X-ray irradiation resulted in synergistically elevated ROS generation.

References

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