Blood-brain Barrier Penetrant Metformin-MnO_2 Hybrid Nanoparticles for Enhancing Radiotherapy of Glioblastoma

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Introduction

- GBM is the most common and aggressive primary malignant brain tumor in adults.
- Despite aggressive treatment, GBM remains highly lethal, with only ~4.7% of patients surviving 5 years.
- Tumor hypoxia and associated abnormal microenvironment drive progression, immune evasion, and resistance to radiotherapy (RT)
- Effective radiosensitizer delivery and accumulation in the brain is limited by the presence blood-brain barrier (BBB)

Purpose

To address the radioresistance and delivery hurdles, we have developed BBB-penetrant metformin-MnO_2-loaded polymer-lipid nanoparticles (TP-MetMDNPs). With this dual pronged approach, we can leverage **MnO_2-mediated oxygen generation** and **metabolic modulation abilities of metformin** to overcome hypoxia induced RT-resistance and provide a targeted strategy for brain tumors with improved efficacy and reduced toxicity.

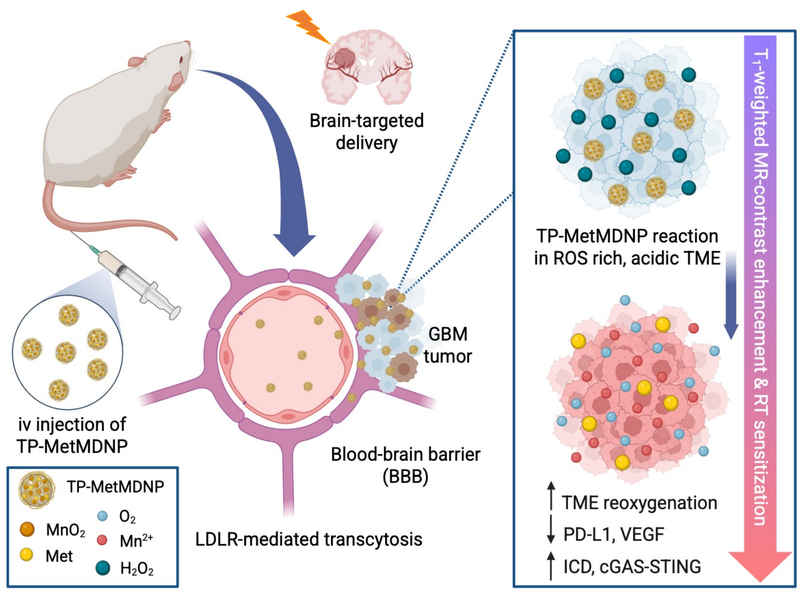


Fig 1. Schematic of proposed effects of brain-penetrative TP-MetMDNP on enhancing radiotherapy by re-oxygenation, microenvironment remodeling, and immune stimulation in an orthotopic GBM model.

Methods

- TP-MDNPs synthesis and characterization
  - Size distribution, polydispersity index, and ζ-potential
  - Metformin encapsulation efficiency and dose determination
- *In vitro* studies:
  - MTT cytotoxicity assay
  - Clonogenic survival assay
  - ds-DNA damage biomarker (γ-H2AX)
- *In vivo* and *ex vivo* studies:
  - Biodistribution

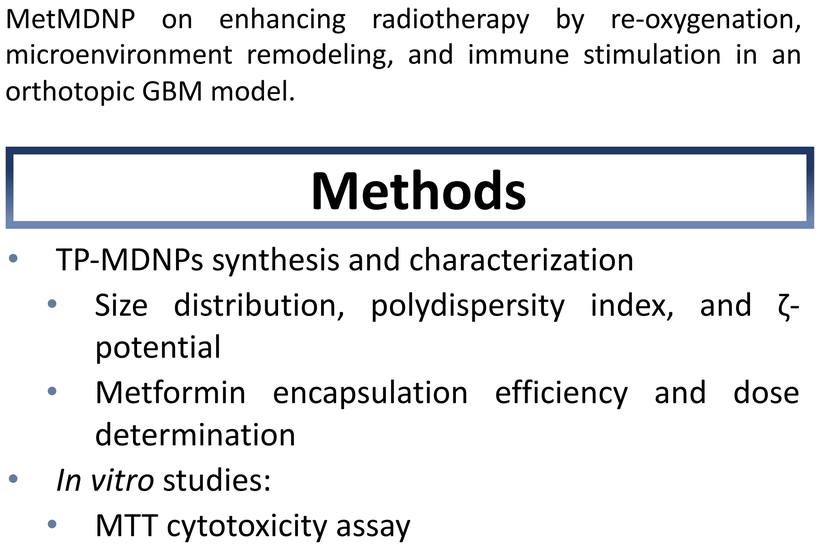
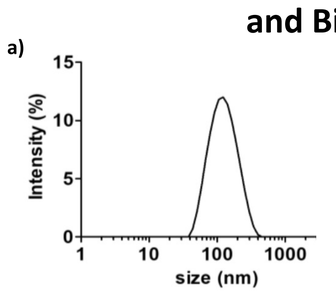


Fig 2. Proposed synthesis of TP-MetMDNP using a one pot synthesis method with BBB-penetrant terpolymer consisting of PS-80 grafted starch and poly(methacrylic acid).

Results

TP-MetMDNPs Exhibit Excellent Colloidal Properties and Biocompatibility



b)

Hydrodynamic Size (nm)	187 ± 1
PDI	0.17 ± 0.01
ζ-potential (mV)	-32.5 ± 0.9
% encapsulation of Met	96%
MnO_2 : Metformin molar ratio	1 : 11

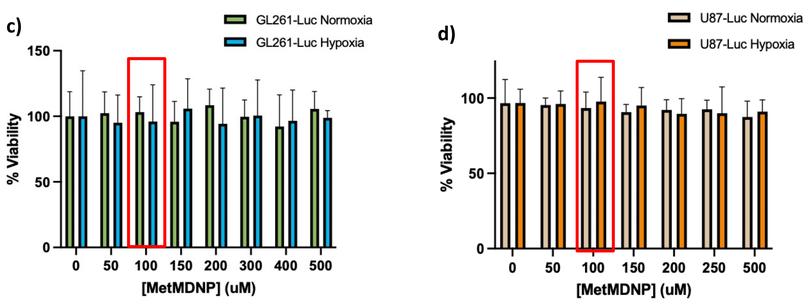
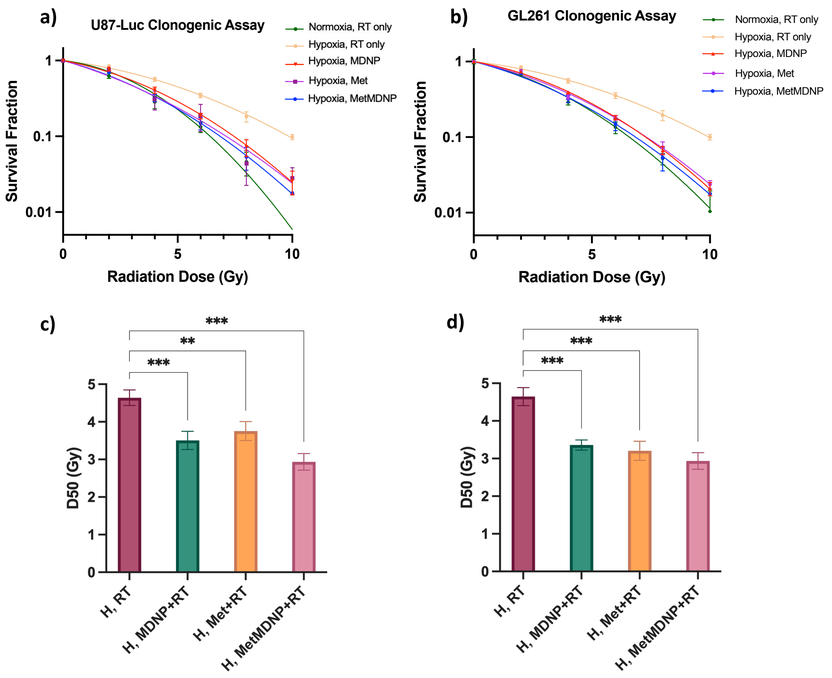


Fig 3. (a) Dynamic light scattering size distribution of TP-MetMDNPs and **(b)** summary of physiochemical properties of MetMDNP. MTT Cytotoxicity assays using 0-500 uM MetMDNP in **(c)** GL261-Luc cells **(d)** and U87-Luc cells under both normoxic (21% O_2) and hypoxic (2% O_2) conditions.

TP-MetMDNPs Sensitize Radiation Therapy *in Vitro*



e)

	Condition	% Change D50 relative to H, RT only	
U87-Luc	H, MDNP+RT	-29	*
	H, Met+RT	-30	*
	H, MetMDNP+RT	-40	
GL261-Luc	H, MDNP+RT	-28	*
	H, Met+RT	-27	*
	H, MetMDNP+RT	-38	

Fig 4. Clonogenic survival fraction vs. irradiation doses under normoxic or hypoxic condition for **(a)** human U87-Luc cells and **(b)** murine GL261-Luc cells after various treatments. Median dose of irradiation (D50) for U87-luc **(c)** and GL261 cells **(d)** by TP-MDNP, metformin, or MetMDNP treatment under hypoxia condition. **(e)** tabulated summary of % change D50 relative to hypoxic cells receiving RT only. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

TP-MetMDNPs Enhance ds-DNA Breaks

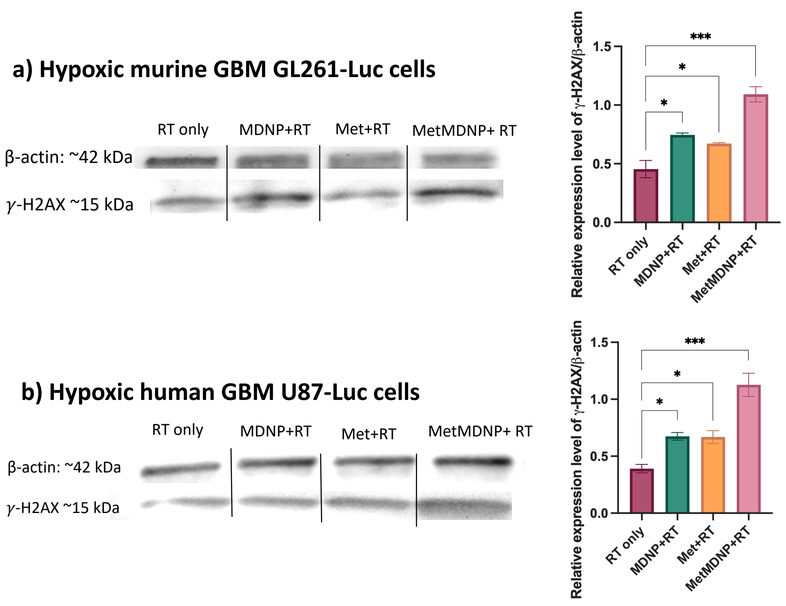


Fig 5. Western blot evaluation of ds-DNA biomarker γ-H2AX in **a)** hypoxic GL261-Luc cells and and **b)** hypoxic U87-Luc cells, treated with RT only, MDNP+RT, Met+RT, and MetMDNP+RT. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

TP-MetMDNPs Accumulate in the GBM-bearing Brain

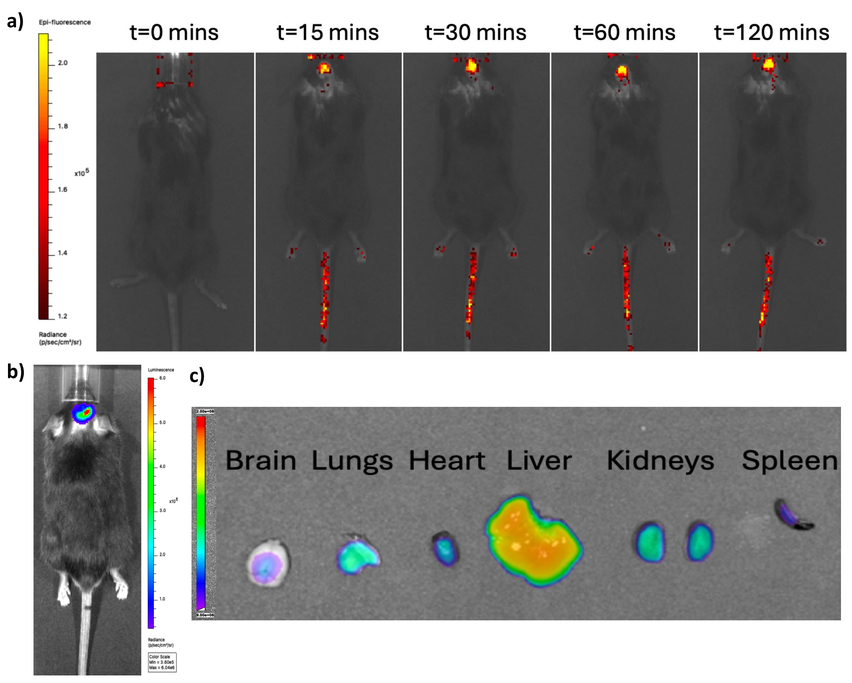


Fig 6. a) *In vivo* biodistribution study of ICG encapsulated MetMDNPs after a single IV injection monitored throughout 120 minutes, **b)** Representative bioluminescence image visualizing the luciferase expressing GBM tumour **c)** *ex vivo* biodistribution of ICG encapsulated MetMDNPs in major organs.

Conclusion

- TP-MetMDNPs were successfully synthesized with a hydrodynamic size of 187nm and PDI of 0.17
- TP-MetMDNPs demonstrated excellent biosafety *in vitro* showing minimal cytotoxicity
- TP-MetMDNPs effectively enhanced radiation therapy in both murine and human GBM cell lines reducing D50 by 38% and 40% respectively and significant increase in γ-H2AX expression
- TP-MDNPs accumulated in the brain as shown by *in vivo* and *ex vivo* fluorescence studies
- These findings support the promising potential of TP-MetMDNP as a dual pronged radiosensitizer for improving the treatment quality and outcome against GBM.

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