Nanoparticle-mediated hyperthermal therapies reveal selective efficacy in 3D neuroblastoma models

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Hyperthermia-based nanotherapies, including magnetic hyperthermia (MH) and photothermal therapy (PTT), have attracted growing attention in oncology as adjuvant strategies to improve tumor control [1]. Magnetite-based superparamagnetic iron oxide nanoparticles (SPIONs) can be activated by alternating magnetic fields (AMF) or near-infrared (NIR) light, leading to localized heating and tumor cell damage [2]. However, conventional bulk heating studies in simple media often fail to capture the complex microenvironment of tumors, and systematic comparisons of MH and PTT in 3D pediatric cancer models are scarce. Neuroblastoma (NB), the most frequent extracranial solid tumor in children, presents a heterogeneous extracellular matrix enriched in collagen I and hyaluronic acid, making it an ideal case study for engineered tissue models [3].

To address this limitation, we developed 3D neuroblastoma tissue-engineered (TE-NB) models that recapitulate the biochemical and structural complexity of pediatric tumors. Collagen I/hyaluronic acid scaffolds incorporating five NB cell lines (SK-N-BE(2), SK-N-LP, SK-N-AS, LA1-55n, and LAN-1) were loaded with polyacrylic-acid-coated SPIONs (50 µg Fe mL^{-1}) and exposed either to near-infrared laser irradiation (PTT, 808 nm, 1 W, 10 min) or to an alternating magnetic field (MH, 285 kHz, 20 mT, 60 min) **(Figure 1)**.

Heating calibration revealed that PTT was dominated by nonspecific medium absorption, producing comparable temperature rises with or without MNPs, whereas MH generated MNP-dependent heating with negligible background, confirming its specificity and reproducibility in vitro. Fluorescence microscopy, Prussian blue staining, and MRI verified efficient MNP internalization and retention across NB lines without acute toxicity, validating their use for functional hyperthermia studies.

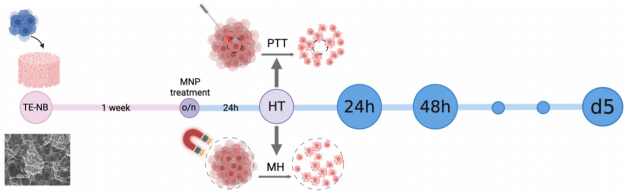

Figure 1. Experimental workflow for hyperthermia studies in TE-NB models. Tissue-engineered neuroblastoma scaffolds were cultured for 7 days, incubated overnight with magnetic nanoparticles (MNPs, 50 µg Fe mL^{-1}), and washed before hyperthermia treatment. Photothermal therapy (PTT; 808 nm NIR, 1 W, 10 min) or magnetic hyperthermia (MH; 285 kHz, 20 mT, 60 min) were applied, followed by collection at 24 h, 48 h, and 5 days to assess DNA content, viability, proliferation (Ki67), and apoptosis (caspase-3/7).

Short-term analyses showed that both modalities modulated proliferation within 24 h, but the direction and magnitude of effects varied by cell line and scaffold region. Ki67 immunohistochemistry revealed heterogeneous patterns: PTT often increased proliferation at the scaffold periphery while reducing it in inner regions, whereas MH produced opposite or mixed trends depending on NB subtype. At 48 h, the divergence between modalities became more pronounced. Hematoxylin/eosin staining demonstrated that MH disrupted scaffold architecture and reduced cell density, while PTT-treated and control samples preserved structural integrity. Quantitative assays confirmed that MH significantly decreased DNA content and Ki67 labeling, whereas PTT failed to produce measurable cytostatic or cytotoxic effects and in some cases induced a rebound in proliferation (**Figure 2**).

Caspase-3/7 activation was assessed at 24 h, 48 h, and 120 h post-treatment using pixel-based quantification of fluorescence signal. Neither modality triggered sustained caspase activation compared with untreated controls, suggesting that cytotoxicity occurred through non-apoptotic mechanisms. The absence of apoptotic signatures, together with the reversible nature of the proliferative arrest observed after MH, indicates that hyperthermia primarily acts as a modulatory rather than a strictly cytotoxic agent in these conditions.

Collectively, these results demonstrate that magnetic hyperthermia provides superior spatial uniformity of heating and stronger antiproliferative effects than photothermal therapy in 3D neuroblastoma models, yet both modalities elicit highly context-dependent responses shaped by tumor subtype and microenvironmental gradients. This study establishes 3D tissue-engineered neuroblastoma scaffolds as physiologically relevant platforms to dissect thermal therapy mechanisms and optimize treatment parameters in vitro, reducing reliance on animal experimentation. By revealing

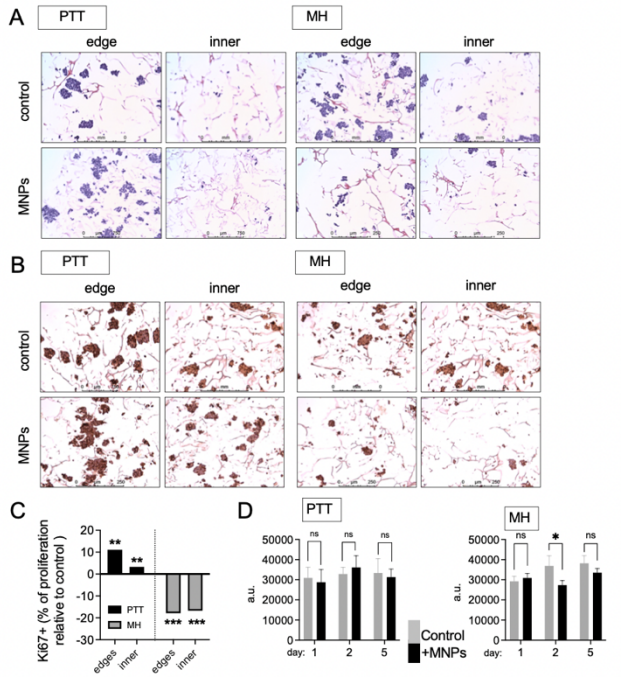

Figure 2. Effect of photothermal therapy (PTT) and magnetic hyperthermia (MH) on TE-NB models at 48 h post-treatment. (A) H&E staining showing preserved structure in control and PTT samples and marked disruption with cell loss after MH. (B) Ki67 immunostaining in SK-N-BE(2) constructs highlighting regional differences in proliferation. (C) Quantification of Ki67-positive cells: PTT increased proliferation, while MH reduced it across scaffold regions. (D) DNA quantification (PicoGreen) confirming significant DNA loss after MH ($p < 0.05$), with no major effect from PTT. Data shown as mean ± SD.

that hyperthermia's biological outcome depends not only on temperature but also on tissue architecture and cellular heterogeneity, this work reframes hyperthermia as a precision-modulating rather than purely ablative modality for pediatric neuroblastoma.

Ongoing efforts aim to enhance therapeutic durability through repeated MH exposures and combination strategies coupling MH with chemotherapeutic agents. In collaboration with the University of Porto, a cytotoxic compound identified in engineered NB models is planned to be incorporated into SPION-based nanocarriers to evaluate synergistic effects between localized hyperthermia and molecular therapy.

References

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