

## U87 glioblastoma spheroids as a 3D model for magnetic hyperthermia: defining thermal response through nanoparticle loading and experimental setup

Marco A. Morales Ovalle<sup>1,2</sup>; Enio Lima Jr.<sup>1,2</sup>; Marcelo Vasquez Mansilla<sup>1,2</sup>; Elin Winkler<sup>1,2,3</sup>; Roberto Zysler<sup>1,2,3</sup>; Mariana Raineri<sup>1,2,4</sup>

<sup>1</sup> Instituto de Nanociencia y Nanotecnología, Nodo Bariloche, CNEA-CONICET, Centro Atómico Bariloche, Av. Bustillo 9500, 8400, San Carlos de Bariloche, Argentina

<sup>2</sup> Departamento de Magnetismo y Materiales Magnéticos, Gerencia de Física (CNEA), Centro Atómico Bariloche, Av. Bustillo 9500, 8400, San Carlos de Bariloche, Argentina

<sup>3</sup> Instituto Balseiro, CNEA-Universidad Nacional de Cuyo, Centro Atómico Bariloche, Av. Bustillo 9500, 8400, San Carlos de Bariloche, Argentina

<sup>4</sup> Facultad de Medicina, Universidad Nacional de Río Negro, Sede Andina, Mitre 630, San Carlos de Bariloche, Argentina

maruraineri@gmail.com

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Magnetic hyperthermia is a promising therapeutic strategy for glioblastoma; however, its experimental implementation in vitro requires careful control of both biological and physical parameters. In this work, we developed a three-dimensional spheroid model based on the U87 glioblastoma cell line to investigate the factors governing heating performance under an alternating magnetic field.

The magnetic nanoparticles (MNP) used in this study consisted of zinc ferrite nanoparticles, with a mean size of  $21.5 \pm 4.05$  nm as determined by TEM. Their crystalline structure was confirmed by XRD, while surface chemistry was evaluated by FTIR. Elemental composition was assessed by EDS. Magnetic characterization by VSM showed superparamagnetic behavior, with a saturation magnetization of 61.16 emu/g.

U87 spheroids were generated and exposed to magnetic nanoparticles under different experimental configurations. The nanoparticles were applied at  $4.5 \mu\text{g}$  MNP/spheroid, resulting in a retained nanoparticle load of  $3.8 \mu\text{g}$  MNP/spheroid under the selected experimental conditions. By increasing the number of spheroids per sample, a threshold condition was identified, enabling the system to reach the hyperthermia temperature window. Using samples containing different numbers of spheroids or nanoparticle loading, two distinct thermal regimes were obtained: a hyperthermia condition ( $\sim 43$  °C) associated with a significant decrease in viability, and a non-hyperthermia condition with no detectable loss of viability.

These differences suggest that the effective nanoparticle load and heat dissipation conditions play a critical role in determining the thermal response. Additional experiments varying the thermal setup confirmed that system insulation significantly influences temperature increase under magnetic field exposure.