Spanish Conference
on Biomedical Applications
of Nanomaterials

Dependence of intracellular magnetic nanoparticles amount in the apoptotic death pathway triggered by magnetic hyperthermia treatment

Lilianne Beola[1*], Laura Asín[1], Catarina Roma-Rodrigues[2], Yilian Fernández-Afonso[1], Raluca M. Fratila[1], David Serantes[3], Sergiu Ruta[4], Roy W. Chantrell[4], Alexandra R. Fernandes[2], Pedro V. Baptista[2], Jesús M. de la Fuente[1], Valeria Grazú[1], Lucía Gutiérrez [1,5]

[1]Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, 50009 Zaragoza, Spain; Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN),50009 Zaragoza, Spain. [2]UCIBIO, Departamento de Cičncias da Vida, Faculdade de Cičncias e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal. [3]Applied Physics Department and Instituto de Investigacions Tecnoloxicas, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain. [4]Department of Physics, University of York, YO105DD York, United Kingdom. [5]Department of Analytical Chemistry, University of Zaragoza.

e-mail: lbeola@unizar.es

Magnetic hyperthermia is a promising approach for the localized cancer treatment based on the exposure of magnetic nanoparticles to an alternating magnetic field. In this work, 3D cell culture models were prepared to observe the effect a different number of internalized particles had on the mechanisms of cell death triggered upon the magnetic hyperthermia treatment (MHT). In this way, 11 nm superparamagnetic iron oxide nanoparticles were synthesized and coated with amphiphilic polymer and rhodamine fluorophore, and then, functionalized with glucose to improve cellular internalization. Macrophages were selected as cell line model by their high capacity to uptake nanoparticles. Intracellular nanoparticle concentrations were measured both by elemental analysis and magnetic characterization techniques. Cell death studies were performed by flow cytometry and RT-PCR. In order to provide an understanding of the local thermal effects occurring within the cells during the AMF exposure, a theoretical simulation was performed. Our results demonstrated that different apoptotic routes were triggered depending on the number of internalized particles (Figure 1). At low intracellular magnetic nanoparticle amounts, the intrinsic route was the main mechanism to induce apoptosis, as observed by the high *Bax/Bcl-2* mRNA ratio and low caspase-8 activity. In contrast, at higher concentrations of internalized magnetic nanoparticles, it is noted the extrinsic route activation through the increased activity of *caspase 8*. Nevertheless, both mechanisms may coexist at intermediate iron concentrations. In addition, theorical calculation suggested a correlation between the number of particles and size of the vesicles where the particles are trapped after their internalization might result in different localized thermal effects. In contrast, the global cell temperature may not increase significantly. Knowledge on the different mechanisms of cell death triggered after the magnetic hyperthermia treatment is crucial to establish the optimal treatment conditions and predict the future efficacy in the therapy.

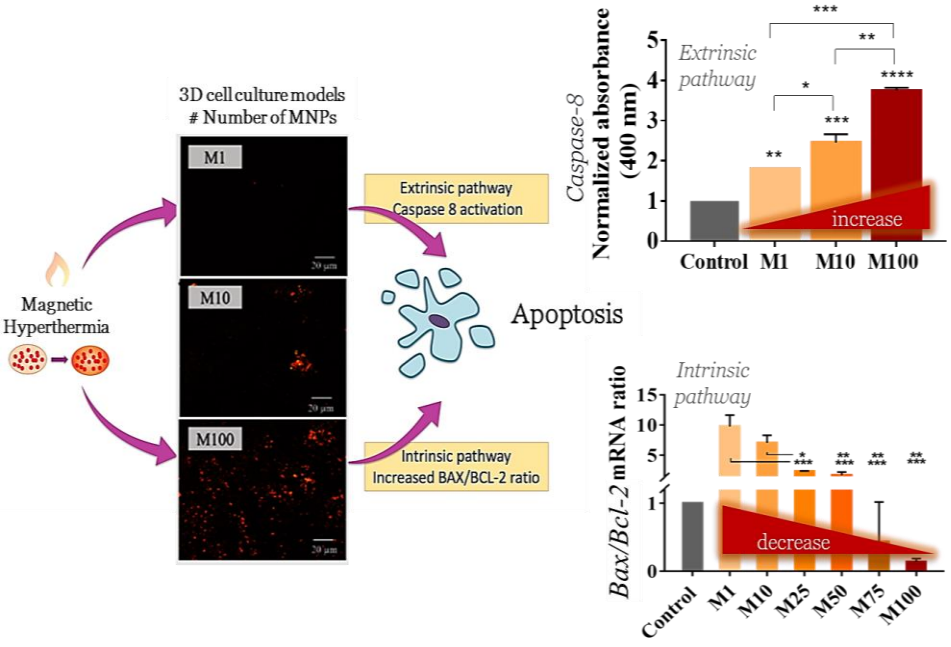

Figure 1. Effect of the MNPs quantity internalized on the different regulated cell death pathways triggered by MHT. *Our approach was based on the preparation of 3D cell culture models containing different amounts of particles per cell, named M1, M10, M25, M50, M75, and M100 depending on the iron amount used to treat the cells before they were entrapped within the collagen matrix. To Statistical significance between was determined using a one-way ANOVA followed by Tukey's multiple comparisons test (****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p \leq 0.05$; $p > 0.05$ no significance).*