## P26

# Developmental neurotoxic effect of nanoparticles: towards real-time assessment using zebrafish embryos as vertebrate model

Luana Magalhães[1], Mário Fernandes[1], Sofia Machado[1], Anabela Gonçalves[1], Espiña B[2], Marisa P. Sárria[2] and Andreia C Gomes[1,*]

*[1] Centre of Molecular and Environmental Biology (CBMA), Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal, [2]International Iberian Nanotechnology Laboratory (INL), 4715-330, Braga, Portugal*
** agomes@bio.uminho.pt*

Exposure to neurotoxic agents during embryonic development can cause irreparable short- and long-term damage to vertebrates, including for humans, as it may affect the central nervous system (CNS) and the blood brain barrier (BBB)[1]. The reported CNS susceptibility during embryogenesis suggests that there may be a critical window of exposure during brain development. Nowadays, therapies using nanoparticles are an important alternative for most of the diseases, since they allow, for example, more effective and localized delivery of drugs and higher success in treatments [2]. However, the exposure to them at an embryonic stage can cause neurotoxic damages.
In this study, the putative toxicity of two different nanoparticle formulations was evaluated, namely gold nanoparticles, which were produced by Green synthesis, and liposomal nanoparticles. Both have known and interesting bioactivities, such as anti-inflammatory, antioxidant, antifungal and antibacterial[3][4].
Thus, the capacity of the neuroprotective effect of the nanoparticles in contact with toxins was tested, the evaluation was done through the ZET assay, with the endpoits shown in the figure and following parameters, mortality, epiboly, malformation, heart beat, spotaneus movements, hedd-trunk angle, hatching and free-swimming.
2D cell models are easy to reproduce, however they fail to mimic real conditions. Animal models are better than 2D models, however many ethical problems are involved. Zebrafish embryos compose a low-cost and highly informative non- animal alternative to evaluate early developmental responses associated with neurotoxicity[5][6]. As model has multiple advantages for its use in toxicity tests including *ex-utero* fertilization, transparency of the embryos which allow observations at real time, rapid development and high fecundity[5][6]. Zebrafish embryos possess neural development characteristics similar to the vertebrate ones, which makes them ideal to assess embryo neurotoxicity *in vivo*.
Obtained results show that zebrafish is a profitable and reliable model organism for neurotoxicity tests. Nevertheless, there is a need to continue these neurotoxic tests in more complex models, so that these nanoparticles can become an alternative therapy.

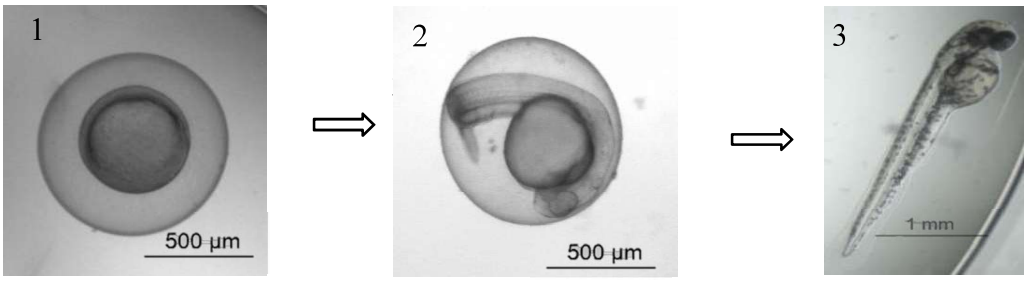


Figure 1: Different endpoints of zebrafish development at 1-8 $h_{pf}$, 2- 32 $h_{pf}$ and 3- 56 $h_{pf}$ *$h_{pf}$- hours post fertilization.

**Acknowledgements:** This work was supported by the strategic programme UID/BIA/04050/2019, funded by national funds through the FCT IP, and project FUN2CYT: Harnessing the potential for biomedical applications of pleiotropic cytokines LIF and oncostatin M (PTDC/BTM-MAT/30568/2017, POCI-01-0145-FEDER-030568) supported by POCI through FEDER and FCT IP.

**References**

[1] D. Paquet *et al.*, "A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation Find the latest version: Technical advance A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation," *J. Clin. Invest.*, vol. 119, no. 5, pp. 1382–1395, 2009.

[2] N. González-Ballesteros, S. Prado-López, J. B. Rodríguez-González, M. Lastra, and M. C. Rodríguez-Argüelles, "Green synthesis of gold nanoparticles using brown algae Cystoseira baccata: Its activity in colon cancer cells," *Colloids Surfaces B Biointerfaces*, vol. 153, pp. 190–198, 2017.

[3] C. Bruno de Sousa *et al.*, "Antileishmanial activity of meroditerpenoids from the macroalgae Cystoseira baccata,"
*Exp. Parasitol.*, vol. 174, pp. 1–9, 2017.

[4] G. Marslin *et al.*, "Curcumin Encapsulated into Methoxy Poly(Ethylene Glycol) Poly(ε-Caprolactone) Nanoparticles Increases Cellular Uptake and Neuroprotective Effect in Glioma Cells," *Planta Med.*, vol. 83, no. 5, pp. 434–444, 2017.

[5] J. M. Panzica-Kelly, C. X. Zhang, and K. A. Augustine-Rauch, "Optimization and performance assessment of the chorion-off[Dechorinated] Zebrafish Developmental toxicity assay," *Toxicol. Sci.*, vol. 146, no. 1, pp. 127–134, 2015.

[6] L. Chen, C. Huang, C. Hu, K. Yu, L. Yang, and B. Zhou, "Acute exposure to DE-71: Effects on locomotor behavior and developmental neurotoxicity in zebrafish larvae," *Environ. Toxicol. Chem.*, vol. 31, no. 10, pp. 2338–2344, 2012.