## Abstract

# 3D neurons model for studying neurodegeneration

Rania Aro[1], Mathilde Wauters[3], Amandine Nachtergael[1], Laurence Ris[3], Mario Manto[2-3], Pierre Duez[1]

1 Unit of Therapeutic Chemistry and Pharmacognosy, University of Mons (UMONS), 7000 Mons, Belgium
2 Department of Neurology, CHU-Charleroi, 6000 Charleroi, Belgium
3 Department of Neuroscience, Research Institute for Biosciences, University of Mons (UMONS), 7000 Mons, Belgium

Abstract text: Reducing the number of animals that are used in research requires better in vitro systems. 2D cell cultures do not represent the real cell environment where cells spatially and chemically interact; their lack in predictivity increases the cost and failure rate of clinical trials, especially in neuroscience. Recently, 3D cell cultures have received much attention, as these are closer to tissues models; they are being used growingly to bridge the gap between *in vitro* 2D cell cultures and *in vivo* animal models. There is a consensus that 3D models are more physiologically relevant than biochemical assays and 2D cell cultures, as they more closely represent the microenvironments, cell-to-cell interactions, and biological processes that occur *in vivo.* Still the challenges with 3D models reside in end-point measurements.

We propose the use of an *in vitro* model of 3D neural spheroid cultures (*"mini-brains"*) to assess the neurotoxic effects of chemical molecules.

A model of 3D spheroids was generated from embryonic mouse cortical neurons using micro-molded agarose wells; the hydrogel microwells facilitate the assembly of neurons and glial cells spheroids that form complex 3D structures, preventing their agglomeration. We have demonstrated the ability to reproduce a 3D culture, using simple materials and routine laboratory equipment. We have improved a resazurin assay to measure cell viability and a highly sensitive fluorometric method, based on the oxidation of di-chlorodihydrofluorescein, to measure the generation of reactive oxygen species (ROS).

Despite the challenges in 3D culture and data obtaining, this 3D neural spheroid model mimics a neuronal microenvironment, allowing a fine study of neurodegenerative disorders pathologies and the effect of putative neurotoxins. These data point to a major interest in generating stem cells-based 3D models, derived from patients with well-characterized neurogenerative diseases, to investigate diseases etiology.