Extending the use of standardised *in vitro* ecotoxicity models to support neurotoxicity testing

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Version Française

Introduction

In this study, the OECD TG 249 was expanded in three key directions: (1) replacing RTgill-W1 gill cells with tilapia OmB brain cells to establish a model for assessing neurotoxicity; (2) transitioning from a 24-well to a 96-well plate format; and (3) advancing from a monotypic 2D culture to a 3D co-culture model by combining tilapia brain cells with rainbow trout Rtgill-W1 gill cells, which function as the initial barrier for chemical and material exposure.

Materials and methods

After establishing the in vitro models, they were exposed for 24 hours to varying concentrations of graphene, silver nanoparticles (Ag NPs), and silica nanoparticles (SiO_2 NPs), ranging from environmentally relevant levels to high concentrations (0.005–50 µg/mL). Following exposure, several endpoints were evaluated, including plasma membrane integrity, metabolic activity, lysosomal membrane stability, as well as reactive oxygen species (ROS) production.

OmB brain cells compared to RTgill-W1 gill cells

- OmB and RTgill-W1 cells were exposed to graphene, AgNPs, and SiO_2 NPs.
- **Figure 1** presents the effects on cell membrane integrity, lysosomal membrane stability, metabolic activity, and ROS production.
- The results indicate that OmB cells are more sensitive to graphene than RTgill-W1 cells. Nonetheless, a significant increase in ROS production was observed in RTgill-W1 cells—but not in OmB cells—after 24 hours of graphene exposure.

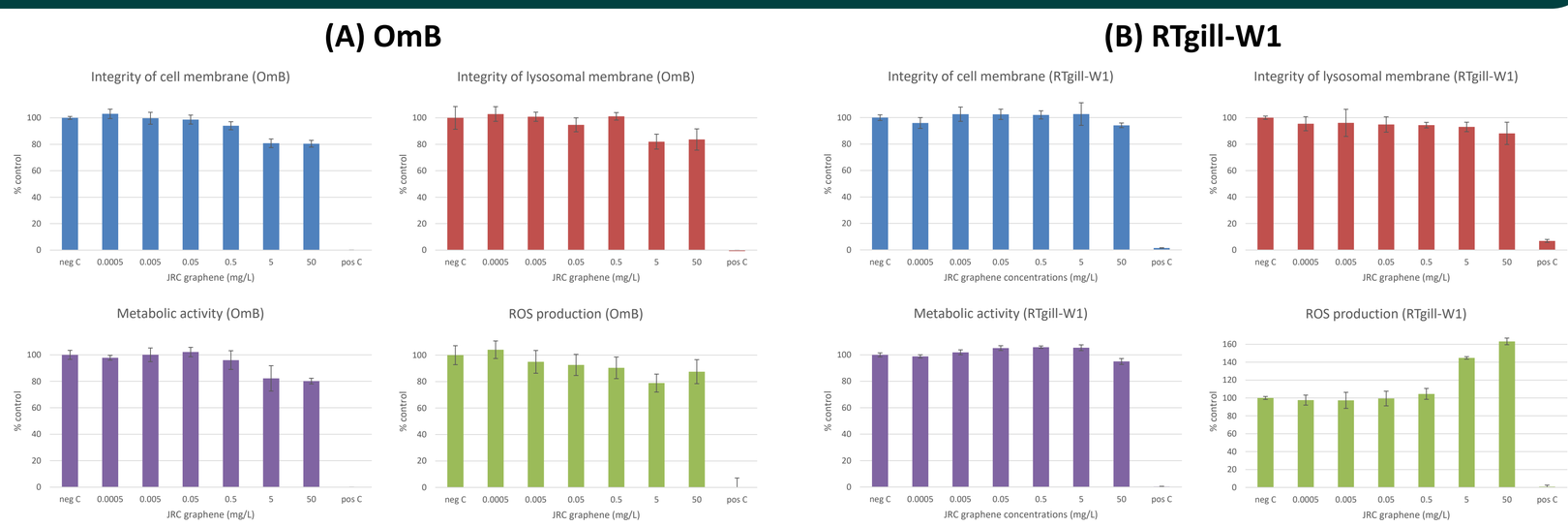

Figure 1. Cell viability and ROS production in (A) OmB line and (B) RTgill-W1 exposed for 24h to graphene.

From 24-well to 96-well plates

- RTgill-W1 cells were seeded in both 24-well plates (following OECD TG 249 guidelines) and 96-well plates in parallel experiments.
- **Figure 2** presents data on cell membrane integrity, metabolic activity, and lysosomal membrane stability following exposure to graphene, Ag NPs and SiO_2 NPs.
- Overall, the effects of graphene, Ag NPs and SiO_2 NPs were more pronounced in cells cultured in 24-well plates compared to those in 96-well plates. These effects were particularly evident at higher exposure concentrations.
- The observed differences may be attributed to the distinct particle/platelet behaviour in the two formats, with cells in 24-well plates exposed to 3 mL of medium versus 200 µL in the 96-well format.

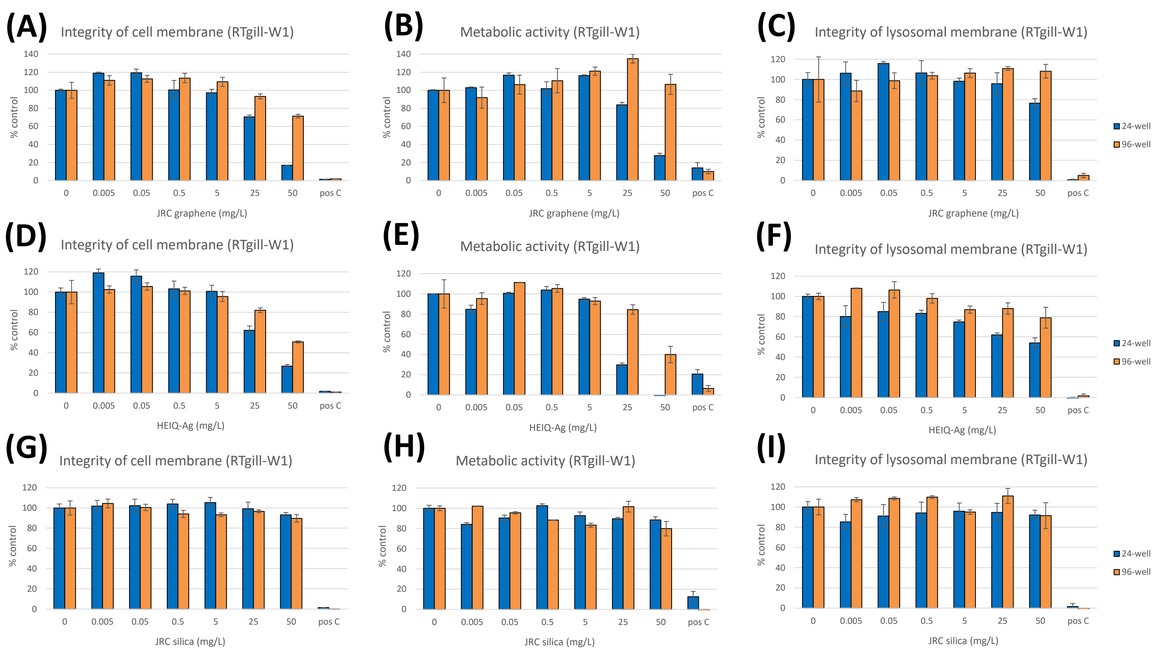

Figure 2. RTgill-W1 exposed to graphene **(A-C)** Ag NPs, **(D-F)** and SiO_2 NPs **(G-I)** for 24h in 24-well plates or 96-well plates.

Monotypic 2D to 3D co-culture models

A static fish co-culture neurotoxicity model was established using RTgill-W1 epithelium-like cells and OmB brain cells. RTgill-W1 cells began forming an epithelial barrier three days after seeding onto PET transwells with 0.4 µm pore size. By day eight, a stable barrier was established, with tight junctions clearly visible across the entire cell monolayer (**Figure 3**).

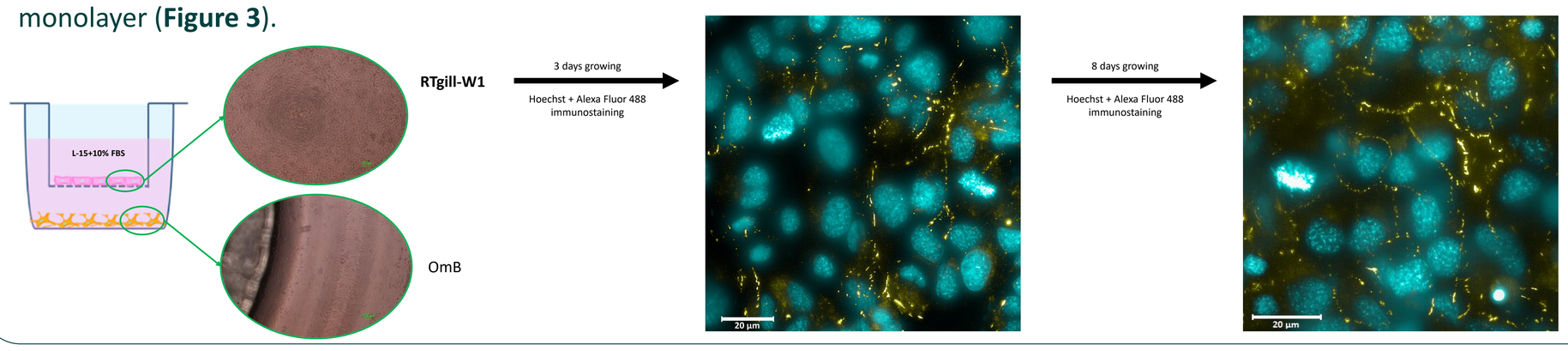

Figure 3. Tight junctions of RTgill-W1 barrier in the 3D co-culture model. Immunofluorescence of ZO-1 (yellow) and nuclei (cyan) detected in the epithelial monolayer after 3 and 8 days in culture. Scale bars = 20 µm.

Conclusions

OmB brain cells may serve as an alternative to RTgill-W1 gill cells for studying neurotoxicity. In cell viability assays, OmB cells demonstrated greater sensitivity than RTgill-W1 cells, while RTgill-W1 cells exhibited a stronger response in terms of ROS production. Transitioning from a 24-well to a 96-well plate format will require adjustments to the protocol, as the results indicated variations in response magnitude following graphene exposure. Lastly, a 3D co-culture model was successfully developed, providing a functional barrier model for future research.

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