Nanotoxicity Assessment in Freshwater Planarians: A Multilevel Approach

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Freshwater planarians, particularly *Schmidtea mediterranea*, are increasingly recognized for their regenerative capabilities and sensitivity to environmental stressors, positioning them as promising bioindicators in toxicological research [1]. Their ease of cultivation and responsiveness to chemical exposure make them suitable for evaluating the biological effects of emerging pollutants such as nanoparticles (NPs) and nanomaterials (NMs), which are widely used for industrial and biomedical applications, for example as a tool for the setting up of alternative therapeutic approaches in the treatment of cancer [2]. Emerging contaminants do not only comprehend NPs and NMs. Aquatic environments are also prone to being the receptacle of micro- and nanoplastics (MPLs-NPLs) [3-4]. The urge to find safer (and potentially biodegradable) substitutes such as Polylactic Acid (PLA) has led to the necessity to test the harmlessness of these new polymers as well, and since planarians are mostly colonizing aquatic environments, they appear to be more than suitable for this kind of investigation.

This study, conducted within the framework of both the European iCare project, and within the “Flagships for Sustainability” program promoted by IIT, investigates the suitability of *S. mediterranea* (Figure 1) as an *in vivo* model for assessing the toxicity of various nanomaterials at different levels of biological organization, from molecular to behavioral one. The evaluation includes survival rate, genotoxicity, tissue alterations, neurobehavioral changes and proteomic profiling.

Nanoparticles tested include silica dioxide (SiO_2), graphene, and silver (AgPure®). Polydopamine nanoparticles (PDNPs), polydopamine nanotubes (PDA NTs), and zinc oxide (ZnO) nanowires (Figure 2) were also tested. Exposure was carried out over seven days, with concentrations ranging from environmentally relevant to high doses. Co-exposure with tert-butyl hydroperoxide (TBH) was also performed to assess the antioxidant potential of PDNPs. For what concern microplastics, a double route of exposure was investigated. The MPLs were resuspended in planarian water, and the animals were exposed for 7 days to increasing concentrations. Moreover, the internalization by oral administration was also tested. Three experimental points were chosen, 1%-15%-40% of PLA in 50 mg of liver (which was calculated as the mean weight of liver eaten by 10 animals, which was the *n* for each experimental point).

With referral to NPs and NMs, the results of this study obtained confirm the suitability of *Schmidtea mediterranea* as a sensitive and versatile *in vivo* model for the assessment of nanomaterial and microplastic toxicity. Overall, the tested nanomaterials exhibited limited acute toxicity, except for silver nanoparticles at the highest concentrations, which negatively impacted survival (Figure 3). Graphene and polydopamine-based nanostructures showed divergent effects on cell proliferation. In addition, preliminary evidence of an antioxidant role of PDNPs during oxidative stress co-exposure highlights the potential dual activity of certain nanomaterials, combining toxicity evaluation with therapeutic relevance (Figure 4).

With respect to biodegradable polymers, PLA microplastics did not affect survival or regenerative capacity, regardless of exposure route. Their ingestion was confirmed (data not shown), indicating that planarians represent a suitable model for evaluating ingestion-related effects of emerging plastic substitutes. However, issues related to dispersion in aquatic media were observed, emphasizing the importance of considering environmental behavior in parallel to biological toxicity.

While proteomic, genotoxic, neurobehavioral, and histological analyses are still in progress, these findings reinforce the value of freshwater planarians as a bridge model for nanotoxicology. They provide insights across multiple levels of biological organization, from survival and proliferation to molecular pathways, thus supporting their role as a complementary alternative model in the assessment of both established and emerging contaminants.

References

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Figures

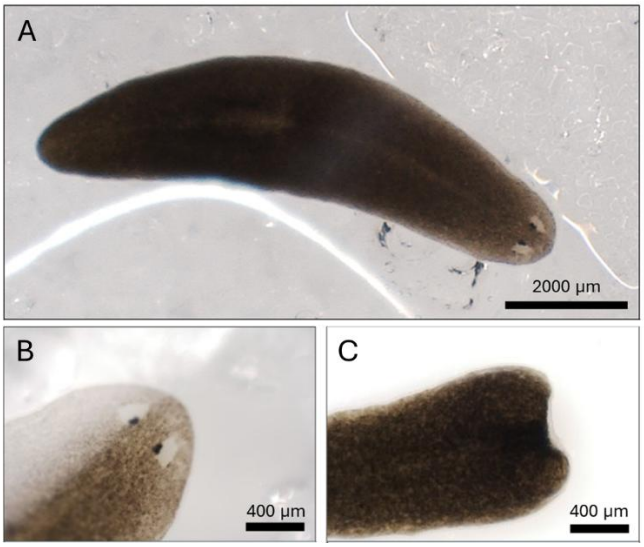

Figure 1: general overview (A) and close-up spots (B, C) of a *S. mediterranea* specimen.

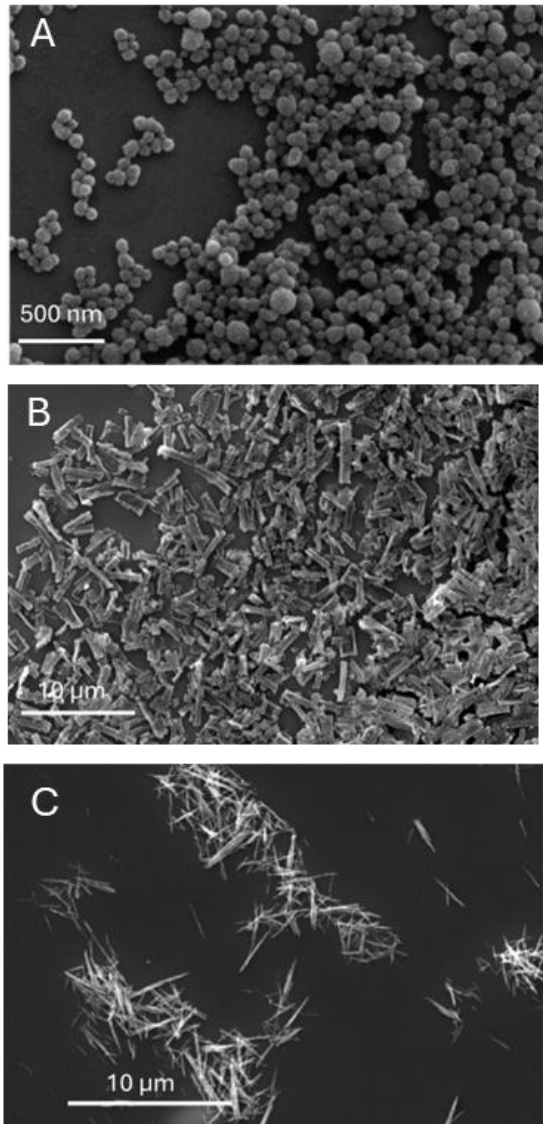

Figure 2: Representative SEM images of nanostructures tested: (A) PDNPs, (B) PDA NTs, (C) ZnO nanowires

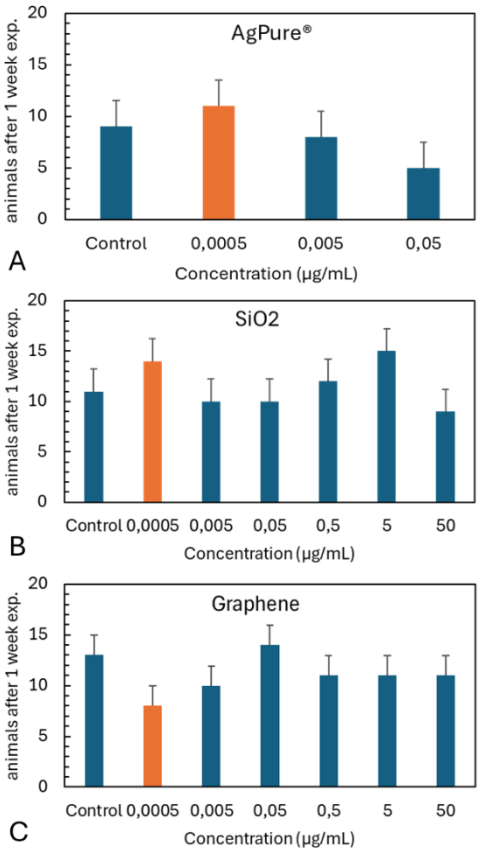

Figure 3. Toxicology studies after 7 days of exposure for (A) AgPure, (B) SiO_2, (C) Graphene.

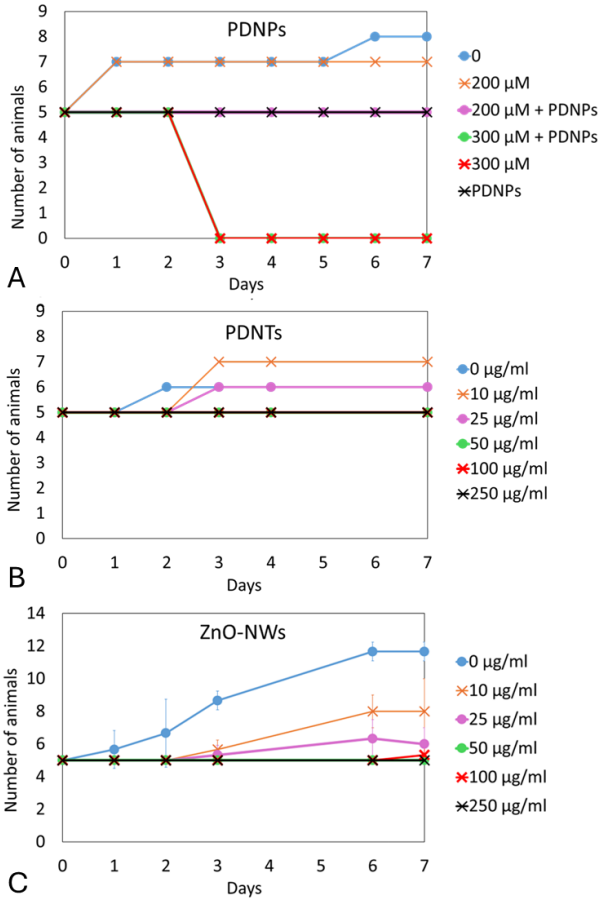

Figure 4. Survival curves of animals monitored over a 7-day period under PDNPs also in co-exposure with TBH (A), PDNTs (B) and ZnO-NWs (C) treatments.