Assessing 2D materials safety for the nervous system in zebrafish

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The emergence of novel 2D (nano)materials (e.g. graphene related materials or transition metal dichalcogenides) and their application in multiple domains of science and technology demand careful safety (bio)assessments. We investigated the potential neurotoxicity of 2D materials, motivated on one hand by the enhanced diffusion of products containing such nanomaterials, that might favor their dispersion in the environment, on the other one by their exploitation as components of biosensors and medical devices in neurology, representing a potential direct threat for the nervous system. To study the materials interactions with a dynamic biological environment with a focus on the nervous system, we adopted the zebrafish (*Danio Rerio*) model, which allows high throughput screening of diverse 2D nanomaterials and it is relevant for *in vivo* pre-clinical evaluation of acute and chronic neurotoxicity, by monitoring sensory motor functions in combination with functional cellular assays (i.e. live calcium imaging, patch clamping) in whole organism.

By using this approach, we revealed that the tuning of single 2D materials chemical properties, such as the oxygen/carbon ratios in thermally reduced graphene oxide, induced decreased or increased swimming performances in zebrafish exposed to graphene oxide (GO) or reduced graphene oxide (rGO) nanoflakes, respectively. These opposite responses were indicative for a diverse impact of the nanomaterials on the neuronal networks governing the locomotor behavior: in the former case a specific interference of GO at synaptic level, in the latter a putative rGO ignited neuroinflammatory condition. We also investigated the effects of a bidimensional transition metal dichalcogenide, MoS_2 nanosheets. Upon ambient exposure, the nanomaterial was internalized and reached the nervous system of zebrafish, causing neuroinflammation-driven alterations, including astrogliosis, glial intracellular calcium dysregulation, and motor axons retraction, responsible for long-lasting changes of the locomotor behavior. Our investigations have proposed and validated a zebrafish based high throughput and functional work flow for assessing the biosafety for the nervous system of the heterogeneous family of 2D nanomaterials and to gain mechanistic insights on their impact on cell biology.