Chapter 2
Zebrafish Models of Nanotoxicity: A Comprehensive Account

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Contents

1 Introduction

Due to unique the physicochemical and electrical properties of materials at the nanoscale, nanomaterials are increasingly utilized these days in various fields of applications, including manufacturing, biotechnology, nanomedicine, and electronics. All these fields, particularly biomedical applications, explicitly demand nanomaterials that are bio safe. However, data concerning the possible toxic effects of the different nanomaterials are still scarce. To this end, different biological models can be used to assess nanomaterials toxicity. Well-established in vitro models are usually used to evaluate the nanomaterials toxicity. They are preferred in comparison to other biological systems, thanks to their low cost and easy maintenance.

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However, after a preliminary investigation obtained in cells, the possible nanomaterials toxicity needs to be further investigated by using an in vivo system, significantly more complex than cultured cells. Indeed, there are several in vivo toxicological models including *Drosophila Melanogaster*, zebrafish, and mouse. Each system presents several advantages and limitations for toxicology screening. In particular, the main limitation of *Drosophila melanogaster* is that it possesses only four chromosome pairs. In contrast, mice are good candidates, but the main problem is that they are expensive and time-consuming. In this framework, zebrafish represents alternative and complementary model organisms, with several peculiarities, making them established systems for toxicity screening, in comparison to other species (Kalueff et al. 2016; Wiley et al. 2017).

In this chapter, we underline the different peculiarities of zebrafish that make them excellent candidates as model systems for toxicological screening. Moreover, we describe the different parameters used as toxicological endpoints. In particular, we focus on the developmental toxicity, describing the mortality and hatching rates, cardiac and swimming activities, immunotoxicity, genotoxicity, and neurotoxicity. In addition, we give a brief overview of previous toxicity studies on different classes of nanomaterials performed using zebrafish as model. These include carbon-based nanomaterials (fullerenes, carbon nanotubes, carbon dots, nanodiamonds, carbon nano-onions, carbon nano-horns, and graphene oxide), metallic nanoparticles (gold and silver nanoparticles), and semiconductor nanoparticles (silicon-based nanoparticles).

2 Unique Characteristics of Zebrafish

Nowadays, zebrafish are employed in different studies to assess the toxicity of nanomaterials, involving both embryos and adult organisms. The increasing use of zebrafish in the nanotoxicology field is due to several powerful peculiarities they possess. Zebrafish are vertebrates and therefore share a high homology degree with mammals, including humans (Howe et al. 2013; Kalueff et al. 2014). The cardiovascular, nervous, and digestive systems of these model animals are similar to the mammal's ones. Thanks to the similarity in the cellular and developmental mechanisms with the other vertebrates, studies performed in zebrafish can give insight into human mechanisms. Other advantages of employing zebrafish in a toxicological screening over other vertebrates are their small size, their low cost, and easy maintenance. Adults are 3 cm long, reducing the housing space and costs. In addition, the minute size of embryos allows performing toxicity experiments by placing together a high number of samples in 96 multi-well plates. Embryos are able to absorb the nanomaterials dissolved in their medium directly by soaking (d'Amora et al. 2017, 2018a, b). This provides several replicates at one time and reduces the amount of nanomaterials used and therefore the cost of the toxicological screening. In addition, all these peculiarities give the possibility to create high-throughput screens for toxicity screening in which embryos and larvae can develop in testing plates

(Horzmann and Freeman 2018; Hill et al. 2005). In this way, a large number of nanomaterials can be screened contemporary and rapidly. Moreover, zebrafish have a large number of offspring in each generation. The females produce with external fertilization around 200–300 eggs per week. The organogenesis occurs quickly and the major organs are formed within 5 days post fertilization (dpf). In addition, embryos are transparent, and this allows to easily identifying the developmental staging and assessing the toxicological endpoints during a complete toxicity screening. Their transparency is very useful when immunohistochemistry (IHC) and in situ hybridization (ISH) are employed.

3 Toxicological Screening in Zebrafish

Zebrafish are powerful platforms to test the effects of different nanomaterials. The toxicity of nanomaterials is assessed by examining different toxicological endpoints during the zebrafish development (Heiden et al. 2007), based on both external phenotypic and internal organs changes (Pham et al. 2016). The mortality (or survival rate) and hatching rates are the first endpoints analyzed during a toxicological screening. Subsequently, other biological parameters, such as the cardiac (heartbeat rate) and swimming activities, could be evaluated (Fig. 2.1). In addition, the possible presence of malformations in embryos and larva exposed to different concentrations of nanomaterials is an important endpoint. Moreover, zebrafish are employed to assess immunotoxicity, genotoxicity, and neurotoxicity in a complete toxicity testing (Fig. 2.1).

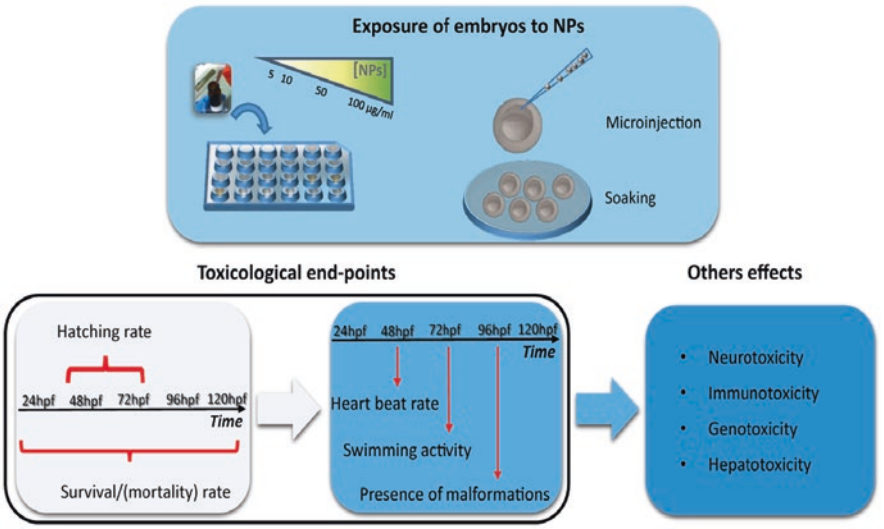

Fig. 2.1 A schematic representation of the complete toxicological screening in *C. elegans*

3.1 Developmental Toxicity

The visualization of the different toxicological endpoints in zebrafish is done at specific stages that correspond to crucial points of the development. All the observations are carried out on in vivo embryos and larvae.

3.1.1 Mortality/Survival Rate

The mortality rate of embryos and larvae is evaluated and noted at different concentrations of nanomaterials and throughout the whole exposure period. Since different studies reported the presence of mortality in the early life stage (Fraysse et al. 2006), the evaluation of this parameter starts at 4 h post fertilization (hpf). The mortality rate of zebrafish at this stage is calculated by determining the eggs blocked at the blastomeric stage and the unfertilized eggs, using a dissecting stereomicroscope. After 4 hpf, zebrafish embryos or larvae are established as dead if there is no more heartbeat rate or no longer moving or the appearance of the tissue changed from the normal transparency to the opacity (Ali et al. 2011). The mortality rate is subsequently recorded every 24 h (at 24, 48, 72, 96 and 120 hpf). Treatment with acute doses of nanomaterials enhances the increase in the mortality rate. For instance, the increase of the exposure concentrations of silica nanoparticles induced an increase in mortality and cell death (Duan et al. 2013). Moreover, different studies indicated a relationship between the incidences of the mortality and the different shapes of nanomaterials. Hence, the toxicity induced by zinc oxide nanoparticles (ZnO NPs) with a different shape, including nanospheres, nano-sticks, and cuboidal submicron particles, was investigated (Hua et al. 2014). Nano-sticks were found to be the most toxic compared to the other nanoparticle shapes, leading to an increase in the mortality and hatching rate.

3.1.2 Hatching Rate

Successful hatching is a very important parameter to conceive the toxicity. Hatching occurs from 48 to 72 hpf (Kimmel et al. 1995). A delay in the hatching rate is clear evidence of toxicity. Normally, the number of hatched pro-larvae is counted within 80 hpf, every 2 h. It is possible to establish pro-larvae as hatched when the whole body (from the head to the tail) comes out from the chorion. The hatching rate is counted in a multi-well plate as the percentage of hatched larvae from the total animal per plate. After the number of hatched zebrafish in each replicate is pooled to calculate the mean hatching time (HT50). In 2014, Samaee et al. employed titanium oxide nanoparticles to conceive the hatching occurrence and the toxicity by estimating the correlation among hatching success and hours post-treatment. The authors reported premature hatching or delay in the hatching in zebrafish embryos/larvae treated with titanium oxide nanoparticles (Samaee et al. 2015) with a concentration-dependent toxicity.

3.1.3 Possible Presence of Malformations

The presence of malformations in zebrafish embryos and larvae exposed to nanomaterials is another endpoint frequently assessed during toxicological testing. Since development has been well characterized (Kimmel 1989; Kimmel et al. 1995) and embryos and larvae are transparent, it is possible to easily observe the main zebrafish developmental defects and abnormalities using little magnification. The discrimination between normal and anomalous development is generally made using the organogenesis description of Kimmel et al. (Kimmel et al. 1995). Zebrafish are placed in a multi-well under a common stereomicroscope at different developmental stages and the malformations are noted. These involve incomplete organ development, or defects in different body parts, including the heart, notochord, and brain. In this framework, silica nanoparticles were found to induce typically zebrafish malformations, including yolk sac edema, tail malformations, pericardial edema, and head malformation. The pericardial edema was the most incident defect caused by silicon-based nanoparticles (Duan et al. 2013).

3.1.4 Neurotoxicity

Zebrafish have emerged as powerful model to assess the neurotoxicity (Giordani and d'Amora 2018). It is well known that the developmental processes of the central nervous system of zebrafish and other vertebrates are highly conserved (Belousov 2011). This homology comprises also the blood brain barrier (BBB) development (Eliceiri et al. 2011). Moreover, many brain subdivisions of mammals during the development have a counterpart in zebrafish (Wullimann 2009). In the neurotoxicity assessment, thanks to the transparency of zebrafish, it is possible to visualize specific neurons or axonal tracts in vivo, by means of different biomarkers and dyes or in fixed samples using in situ hybridization and immunohistochemistry techniques. Different endpoints can be investigated in order to investigate the possible neurotoxicity induce by nanomaterials, including neurobehavioral profiling and neural morphogenesis (Kalueff et al. 2014; Truong et al. 2014). Different studies evaluated the neuronal apoptosis by using specific staining and in situ hybridization. Kim et al. showed that treatment of zebrafish with small gold nanoparticles leads to neuronal damage. Moreover, using in situ hybridization, the expression of several transcription factors involved in the eye development, including *sox10*, *pax6a,* and *pax6b*, were analyzed and found to be re-pressed (Kim et al. 2013).

Nowadays, another crucial parameter to understand and evaluate the neurotoxicity is represented by the zebrafish behavioral response to the nanomaterials exposure (Locomotion and Behavioral Toxicity in Larval Zebrafish: Background, Methods, and Data). It is possible to assess the swimming activities in terms of spontaneous movements or number of movements by means of video recording tools. Normally this measurement is performed on larvae at 72 hpf or after 72 hpf. Before this developmental stage, the zebrafish rolled up in the chorion and this membrane disturbs their movements. Treatment of zebrafish with different carbon-based nanomaterials

revealed that the frequency of movements of larvae at 96 hpf had no significant reduction in the case of carbon nano-onions and carbon nano-horns, while a significant reduction was found in the case of graphene oxide exposure (d'Amora et al. 2017). In contrast, the number of spontaneous movements was not affected in zebrafish embryos treated with single-walled carbon nanotubes (Ong et al. 2014). Duan et al. evaluated the total distance of swimming of zebrafish treated with silicon-based nanoparticles (Duan et al. 2013). The results reported a decrease on the swimming distance concentrations dependent. In addition, zebrafish exposed to graphene quantum dots (GQDs) presented a decrease in the total swimming distance and speed. In both studies, the total distance of swimming was measured by means of a visible light test (Wang et al. 2015).

3.1.5 Cardiotoxicity

The heart is the first organ to develop in zebrafish and the heartbeat starts around 22 hpf. The cardiovascular system is formed and completely functional within 48 hpf (Thisse and Zon 2002). Even if there are physiological differences between the heart of zebrafish and mammalian, zebrafish can be considered a prominent candidate to assess the cardiotoxicity. In fact, the mechanisms of heart ontogenesis are well conserved between vertebrates (Staudt and Stainier 2012) and the electrical properties are highly homolog to the human's one (Arnaout et al. 2007; Sedmera et al. 2003). Moreover, the optical transparency of embryos allows in vivo real-time imaging. A study on zebrafish cardiotoxicity assays reported that embryos can live for days in the presence of abnormalities affecting the circulatory system, validating zebrafish as good tools in this field (Chen et al. 1996). Moreover, it was found that tyrosine kinases are conserved expressed during the early developmental stage (Challa and Chatti 2013). All these peculiarities make zebrafish suitable and powerful systems to assess the cardiotoxic effects (Cheng et al. 2011). In particular, the embryos have been employed to assess the effects of different nanomaterials on cardiovascular development, while adults have been used to investigate acute and chronic effects on cardiac function (Sarmah and Marrs 2016). Thanks to the optical transparency of the zebrafish, the cardiac function, including heartbeat rate, the presence of malformations can be easily evaluated by using different in vivo biomarkers.

During a cardiotoxicity screening, the effects of nanomaterials on zebrafish are assessed at 48hph by using a common stereomicroscope. Zebrafish were used to investigate the possible cardiotoxicity induced by several nanomaterials. The possible cardiotoxicity of silica nanoparticles (Si NPs) during the development was deeply evaluated via intravenous microinjection. Silica nanoparticles caused bradycardia and pericardial edema. Moreover, treated embryos presented oxidative stress and neutrophil-mediated cardiac inflammation. Histology techniques on the heart of embryos and larvae treated with silica nanoparticles allowed observing the presence of inflammatory cells in the atria (Duan et al. 2016).

3.2 Immunotoxicity

The immune system is known to be highly sensitive to nanomaterials exposure in particular, in terms of inflammation induction and activation of macrophages and neutrophils (Johnston et al. 2018). Different studies reported that nanomaterials modulate cytokines production by generating free radicals. Moreover, exposure to nanomaterials can induce allergic sensitization and asthma (Di Gioacchino et al. 2011). Zebrafish treated with small gold nanoparticles (1.5 nm core) functionalized with three different ligands presented a perturbation in the inflammation and immune response mechanisms (Truong et al. 2013). On the other side, silver nanoparticles (Ag NPs) induced immunotoxicity in adult zebrafish (Krishnaraj et al. 2016).

3.3 Genotoxicity

An important component in nanotoxicity is the evaluation of genotoxicity. Nanomaterials can induce genotoxicity, leading to DNA damage and gene mutations. Genotoxicity is a crucial risk determinant for long-term toxicity, including tumorigenesis. In the past, mice have been widely used to assess the genotoxicity of nanomaterials, by using micronucleus assays and gene profiling techniques (Manjanatha et al. 2014). Recently, zebrafish come out as powerful genotoxic tools. The genotoxicity induced by several nanomaterials was studied and assessed in zebrafish by using different techniques. Dedeh et al. and Geffroy et al. evaluated the effects of gold nanoparticles (AuNPs) using RAPD coupled with PCR genotoxicity test (Geffroy et al. 2012; Dedeh et al. 2015). After gold nanoparticles exposure, an alteration of genome composition was found (Geffroy et al. 2012). Subsequently, other techniques were employed to assess the genotoxicity of compounds in zebrafish (Villacis et al. 2017) (Rocco et al. 2015). Adult animals were treated with different doses of iron oxide nanoparticles (IONPs). The results demonstrated significant concentration-dependent genotoxic effects of IONPs. In particular, a high number of transcripts of liver samples were found to have a different expression in comparison to the controls, by using microarray analysis (Villacis et al. 2017). In addition, the potential genotoxicity of titanium dioxide nanoparticles (TiO_2 NPs) was analyzed in zebrafish by means of diffusion assay, RAPD-PCR technique, and comet assay (Rocco et al. 2015). The maximum concentrations of nanoparticles tested caused the highest genotoxicity.

3.4 Hepatotoxicity

The organogenesis of the liver begins at 72 hpf and is completely functional within 120 hpf (Chu and Sadler 2009). Several reports indicated that compounds are metabolized by zebrafish during the development with mechanisms similar to those of humans (Quinlivan and Farber 2017; Vliegenthart et al. 2014). Different nanomaterials can cause liver injury. It is possible to evaluate the hepatotoxicity in zebrafish by means of different tests, including enzymes assays and histology techniques. The hepatotoxicity can be easily evaluated visually on the liver tissue. Another technique consists in use biomarkers for liver injury. The levels of these biomarkers are measured in the circulation of treated animals (Vliegenthart et al. 2014). The visualization of the liver damage can be performed by using transgenic lines with a liver-dye expression. The size and the number of hepatocytes can be calculated by analyzing the intensity of the fluorescence before and after the treatment (Zhang et al. 2014).

4 Toxicity Studies in Zebrafish

4.1 Carbon-Based Nanomaterials

Carbon nanomaterials (CNMs) have gained increased interest in different fields, thanks to their unique electronic, optical, and physical characteristics (d'Amora and Giordani 2018). They include fullerenes (C_{60}) (Kroto et al. 1985), nanodiamonds (NDs) (Greiner et al. 1988), carbon nanotubes (CNTs) (Iijima 1991), carbon nano-onions (CNOs) (Ugarte 1992), carbon nano-horns (CNHs) (Iijima et al. 1999), carbon dots (CDs) (Xu et al. 2004), and graphene (Novoselov et al. 2004). Their biocompatibility plays a crucial role in their different applications, including nanomedicine and bioimaging. In the last few years, a careful evaluation of the possible toxic effects of different CNMs in zebrafish during the development has been carried out, reporting their in vivo biosafety (d'Amora et al. 2017; Nicholas et al. 2018).

4.1.1 Fullerenes

Fullerenes are employed in several biomedical and biological applications, including imaging and drug delivery, thanks to their intrinsic photoluminescence, nano-meter size, and hollow cavity (Levi et al. 2006). Using zebrafish as model system, the toxicity of different fullerenes was tested. The effects of the dendro [C_{60}]fullerene DF-1, with antioxidant properties, were assessed, by monitoring the survival rate and the possible presence of malformations (Daroczi et al. 2006). DF-1 exerted no detectable toxicity on zebrafish at the tested concentration. Usenko et al. exposed zebrafish to C_{60}, its hydroxylated derivative $C_{60}(OH)_{24}$, and C_{70} (Usenko et al. 2007, 2008). All

these fullerenes caused a high percentage of developmental abnormalities and mortality, with $C_{60}(OH)_{24}$ less toxic than C_{60}. In another study, dendrofullerenes (monoadducts of C_{60}) and e,e,e-trismalonic acid-like fullerenes (C3-like fullerenes), anionic water-soluble fullerenes were found to be more toxic than oxo-amino fullerenes, anionic fullerenes with similar structures (Beuerle et al. 2007). In addition, it was observed that the toxicity of anionic fullerenes varied from very low to moderate depending on the structures. The biological consequences of different fullerenes on zebrafish were also studied in terms of effects on the proteomic profiles. For instance, the comparison of proteomic profiles between the phosphatidylcholine-based phospholipid nanoparticles containing fullerene C_{60} and the control reported low toxicity of the nanoparticles on zebrafish (Kuznetsova et al. 2014).

4.1.2 Carbon Nanotubes

Since their discovery (Iijima 1991; Iijima and Ichihashi 1993), CNTs have raised increasing interest from different fields for their unique chemical, optical, electrical, and thermal (Bachilo et al. 2002; Ruoff and Lorents 1995) properties. Thanks to these properties, they are employed in different applications. CNTs comprised sp2 carbon atoms organized in single or multiple coaxial tubes of graphitic sheets resulting in single-walled carbon nanotubes (SWCNTs) and multiple-walled carbon nanotubes (MWCNTs), respectively. In 2009, Cheng et al. studied the biological consequences and in vivo biodistribution of fluorescent-labelled MWCNT (FITC-BSA-MWCNTs) in zebrafish at the different developmental stages (Cheng et al. 2009). No lethal effects and no developmental defects were observed after FITC-BSA-MWCNTs injection. Moreover, the data suggested that purification and functionalization of carbon nanotubes improved their biosafety. Subsequently, the same group evaluated the effects of BSA-MWCNTs sonicated in nitric acid for 24 h (MWCNTs-24 h) and 48 h (MWCNTs-48 h). The sonication time affected the length of the MWCNTs. MWCNTs-24 h presented a length of 0.8 ± 0.5 μm, while MWCNTs-48 h had a length of 0.2 ± 0.1 μm. Zebrafish embryos were microinjected with MWCNTs-24 h and MWCNTs-48 h to check their effects. MWCNTs-24 h did not affect the embryos, while the MWCNTs-48 h caused significant toxic effects (Cheng and Cheng 2012). The authors suggested that shorter BSA-MWCNTs were more toxic in zebrafish embryos after injection. Perhaps another factor could be the production of carbonaceous fragments during the nitric acid treatment (Del Canto et al. 2011; Salzmann et al. 2007).

4.1.3 Carbon Dots

Carbon dots, also known as carbon quantum dots (C-dots) or graphene quantum dots (GQDs), are interesting materials to be used in imaging application as they have high photo-stability and exhibit an intrinsic fluorescence (Zheng et al. 2015). The toxicity of C-dots has been investigated in zebrafish in terms of liability after

soaking or microinjection of C-dots in embryos (Kang et al. 2015). Zebrafish grow normally, with a low percentage of developmental abnormalities. C-dots demonstrated good biocompatibility. Recently, Khajuria et al. reported similar results for carbon dots doped with nitrogen (N-CDs). Embryos soaked in N-CDs solutions with different concentrations presented viabilities of more than 75%, with no malformations. These data confirmed the biosafety of C-dots after soaking (Khajuria et al. 2017). On the other hand, GQDs exhibited high biocompatibility, without affecting zebrafish at a concentration lower than 2 mg mL^{-1} (Roy et al. 2015).

4.1.4 Nanodiamonds

Nanodiamonds (NDs) have been employed in several biomedical applications, including drug delivery and imaging, thanks to good optical and biological properties (Mochalin et al. 2011). Lin et al. evaluated the possible adverse effects as well as the persistent effects on larval behavior of nanodiamonds. After microinjection in the yolk, only high concentrations (5 mg/ml) of NDs affected the zebrafish growth, inducing body axis curvature (Lin et al. 2016). Recently, we assessed the possible toxicological effects induced by small carbon dot decorated nanodiamonds (CD-DNDs) on zebrafish (Nicholas et al. 2018). CD-DNDs caused no significant effect on the survival, hatching, and heartbeat rates, and the zebrafish organogenesis. Our results clearly demonstrated the biosafety of CD-DNDs.

4.1.5 Carbon Nano-Onions

Multi-shell fullerenes, known as carbon nano-onions (CNOs), are promising CNMs for imaging (Bartelmess et al. 2015; Lettieri et al. 2017a) and diagnostic applications (Lettieri et al. 2017b; Giordani et al. 2014). Small CNOs (average diameters of 5 nm) show high cellular uptake and weak inflammatory potential (Yang et al. 2013).

Our group has been investigating the toxicity of benzoic acid functionalized CNOs (benz-CNOs) and fluorescent boron dipyrromethene (BODIPY) tagged CNOs (BODIPY-CNOs) in zebrafish during the development. We evaluated the survival and hatching rates, cardiac activity, frequency of movements, and possible morphological abnormalities of zebrafish embryo and larvae treated with 5, 10, 50, and 100 μg mL^{-1} of benz-CNOs and BODIPY CNOs for 120 hpf. We observed no considerable changes in all the toxicological endpoints analyzed in treated embryos and larvae. In particular, the survival and hatching rates of treated zebrafish were found to be similar to the untreated control. Moreover, no reduction in the total frequency of movements and no cardiac effects were observed and the total percentages of abnormalities during the organogenesis was less than 4%. Our result clearly revealed that benz-CNOs and BODIPY-CNOs presented non-toxicity and good biocompatibility in zebrafish (d' Amora et al. 2016).

Furthermore, we reported that oxi-CNOs possessed higher biocompatibility than other classes of CNMs such as oxi-carbon nano-horns (CNHs) (d'Amora et al. 2017).

4.1.6 Carbon Nano-Horns

Carbon nano-horns (CNHs) are conical carbon nanostructures, suitable for biomedical applications, such as drug delivery (Xu et al. 2008).

Our group assessed for the first time the in vivo biological consequences of carbon nano-horns in zebrafish during the development. We exposed the embryos to different concentrations of oxidized CNHs (oxi-CNHs) of 5–8 nm in horn diameter and 30–50 nm in length. Oxi-CNHs induced no significant differences in survival/hatching rates and heartbeat rate of treated embryos and larvae. Moreover, no reduction in the cardiac and swimming activities was observed in the larvae treated with the different concentrations of CNHs. Our results demonstrated that oxi carbon-horns presented no toxicity.

4.1.7 Graphene Oxide

Graphene oxide (GO) presents different properties such as high surface area, layer number, and lateral dimensions that make them able to transport drugs, genes, and proteins in certain cell types or specific body regions. Thanks to these properties, GO is employed in cancer treatment, biological imaging, and drug delivery. Several groups have assessed the in vivo toxicity of graphene oxide. In 2014, Liu et al. (Liu et al. 2014) treated zebrafish eggs with different concentration of GO (1, 5, 10, 50, 100 mg/l) and analyzed different biological parameters. GO (average size 512 nm) resulted to be toxic, inducing a disturbance in the hatching and larvae length. Subsequently, Chen and his group reported that, after exposure to zebrafish, part of the GO adhered to the chorion of the embryo, occluding the pore and consequently causing hypoxia and hatching delay (Hu et al. 2016). Moreover, the amount of GO up taken by the embryos induced damage in the mitochondria, a reduction of the heartbeat rate and different developmental abnormalities affecting the eye, the heart, and the tail.

Our group has investigated the toxicity of commercially available GO (lateral size 15 μm) in zebrafish (d'Amora et al. 2017). GO caused adverse effects on zebrafish development at high concentrations (50 and 100 μg ml^{-1}). The treated embryos/larvae presented a developmental delay. The heartbeat rates and the frequency of movements of treated larvae were reduced. Moreover, different developmental abnormalities in zebrafish, including pericardial and yolk sac edema, fold flexure and tail flexure have been found. The percentage of malformations reached 13.5% and 11% in the case of pericardial and yolk sac edema, respectively.

4.2 Metallic Nanoparticles

Metallic nanoparticles have a wide range of applications in different fields, including nanomedicine. Among metallic nanoparticles, the gold and silver NPs are mainly employed; therefore, an accurate assessment of their toxicity is needed.

4.2.1 Gold Nanoparticles

Gold nanoparticles (Au NPs) are mainly employed in nanomedicine applications, as diagnostic agents (Huang et al. 2006) or drugs carriers (Dykman and Khlebtsov 2011). Since gold nanoparticles can induce cytotoxicity (Gerber et al. 2013), their possible effects are further investigated in zebrafish.

Adult zebrafish treated with gold nanoparticles of two sizes presented genome alterations and different dysfunctions (Geffroy et al. 2012) in several tissues. It has been reported the surface functionalization of gold nanoparticles can influence their toxicity. In particular, gold nanoparticles functionalized with positively charged N,N,N-triethylammoniumethanol (TMAT) caused a high mortality rate in zebrafish, without a significant presence of developmental abnormalities. On the other hand, gold nanoparticles functionalized with 2-mercaptoethanatesulfonate (MES) have completely different behavior. In fact, zebrafish treated with these nanoparticles have no significant percentage of mortality while presented a high incidence of developmental defects. Other studies confirmed the dependence of the gold nanoparticles toxicity from the functionalization (Truong et al. 2012). Small gold nanoparticles caused a disruption in eye development with consequent neuronal damage and changes in the behavioral profile (Kim et al. 2013). One factor mediating the toxicity of gold nanoparticles is represented by the different shapes. Gold nanoparticles of different shapes were exposed to adult zebrafish (Sangabathuni et al. 2017). Rod-shaped Au NPs presented higher uptake and faster clearance compared to spherical gold nanoparticles and stars NPs.

4.2.2 Silver Nanoparticles

Silver nanoparticles (Ag NPs) are extensively applied in different biomedical applications as antimicrobial agents (Kőrösi et al. 2016), drug delivery systems (Jin and Ye 2007), therapeutic agents (Czupryna and Tsourkas 2006), and biosensors (Jin and Ye 2007). Because of the widespread use and the increased exposure to Ag NPs (Benn et al. 2010), it is important to access the toxic effects related to their exposure. In 2008, Asharani et al. reported for the first time toxic effects and biodistribution of Ag NPs on zebrafish during the development (Asharani et al. 2008). Embryo treated with different concentrations of Ag NPs presented high mortality, a hatching delay, and different malformations, including pericardial edema and tail flexure. In addition, Ag NPs localized preferentially in the yolk, heart, and brain. The toxicity of silver nanoparticles was found to be dependent on their size (Bar-Ilan et al. 2009). In the last decade, many studies further investigated the toxicity of Ag NPs of different sizes and with different surface coatings. In order to evaluate the toxicity dependence from the different coating surfaces, Lee et al. synthesized Ag NPs functionalized with three biocompatible peptides (CALNNK, CALNNS, CALNNE). They investigated the toxic effects of Ag-CALNNK NPs$^{+\zeta}$, Ag-CALNNS NPs$^{-2\zeta}$, and Ag-CALNNE NPs$^{-4\zeta}$ and demonstrated charge-dependent toxicity. Ag-CALNNK NPs$^{+\zeta}$ and Ag-CALNNE NPs$^{-4\zeta}$

were the most and less biocompatible nanoparticles, respectively (Lee et al. 2013). Recently, the different behavior of flat and spherical Ag NPs was investigated in zebrafish. Silver nanoplates were found to be more toxic than Ag nanospheres (Abramenko et al. 2018). The effects of silver nanoparticles on zebrafish larvae were also investigated in terms of bio-interactions with subcellular structures (d'Amora et al. 2015), by evaluating the possible effects of small-sized NPs on the cytoskeletal architecture.

4.3 Semiconductors Nanoparticles

Over the past few years, semiconductor nanomaterials such as silicon and germanium became attractive materials for bio photonics and personalized medicine, i.e., imaging and therapeutic agents, applications (Hashim et al. 2014) (Maji et al. 2014; Li et al. 2014). Nevertheless, principal trouble that can restrict their use in these applications are the toxicity due to heavy metal (Ambrosone et al. 2012).

4.3.1 Silicon-Based Nanoparticles

During the last decade, the possible toxicity of silicon-based nanomaterial has been deeply investigated in zebrafish. Duan et al. reported that silicon-based nanoparticles caused high mortality and a hatching delay concentration-dependent (Duan et al. 2013). Moreover, Si NPs induced different types of developmental defects, such as head malformation and yolk sac edema, and a decrease in the total swimming distance. Another study reported that Si NPs did not internalize in the embryos and were mostly accumulated in the chorion surface (Fent et al. 2010).

The effects of silicon-based nanoparticles produced by laser ablation have been studied in zebrafish during the development (d'Amora et al. 2018a, b). The results showed that these NPs did not affect any biological parameters in the zebrafish embryos and larvae, demonstrating their biosafety.

5 Conclusions

As the use of nanomaterials in daily life, and in particular in nanomedicine applications, constantly increases, their possible adverse effects need to be carefully evaluated. In this chapter, we report that zebrafish have become excellent in vivo systems for toxicological screening at the whole animal level. These models are cheaper, quicker, and more efficient than other vertebrates, including mice. We have highlighted how their use gives the opportunity to investigate specific physiological impacts at the different stage of the zebrafish growth. Notwithstanding the toxicity studies of different nanomaterials based on vertebrate models have been reported in

the recent past, the use of zebrafish in nanotoxicity is relatively new. In the near future, zebrafish may become an alternative to other mammalian organisms in evaluation of the toxicity of different nanomaterials.

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