Evaluation of Nanotoxicity Using Zebrafish: Preclinical Model

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Abstract

Throughout the globe, nanotechnology has emerged as a segment which produces a multitrillion-dollar business opportunity that covers a wide range of industries such as medicine, electronics, and chemistry. Due to the rapid development of application-oriented nanoparticles, from targeted drug delivery to diagnostics, in vivo toxicological examinations for assessing the potential hazardous effects of nanoparticles on natural and human safety are in urgent need. Therefore, it is essential to assess their toxicity and possible hazards to humans and ecosystem. Zebrafish is considered as the "gold standard" among animal models for assessment of several metal and metal oxide nanoparticle toxicity due to its cost-effectiveness, high fecundity, optical transparency, short life cycle, well-characterized developmental stages, etc. The chapter emphasizes on how zebrafish (*Danio rerio*) can be utilized to assess nanotoxicity at different levels, including genotoxicity, developmental toxicity, immunotoxicity, cardiovascular toxicity, teratogenicity, neurotoxicity, reproductive toxicity, hepatotoxicity, as well as change in behavior and disruption of gill, skin, and endocrine system. The harmful impacts of chosen metal and metal oxide nanoparticles are also reviewed. The advantages, drawbacks, and future aspects of utilization of zebrafish model in nanotoxicity studies are also argued. Overall, zebrafish is projected to fulfill as a high-throughput screening platform for drug delivery assessment and nanotoxicity, which may help in designing safe and more effective nanomedicines.

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Keywords

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7.1 Introduction

7.1.1 Overview of Nanoparticles

Nanotechnology is the engineering of functional systems at the atomic, molecular, and supramolecular scale. In recent times, nanoparticle (NP)-based research achieved overwhelming attention of scientific community due to its widespread area of applications. In nanotechnology, a particle is termed as a small entity which acts as an entire unit due to its unique properties and transportation capabilities. A particle having reduced dimension (1–100 nm) is described as nanoparticles (NPs) by agencies like "International Organization for Standardization," "American Society of Testing and Materials," and "National Institute of Occupational Safety and Health" (Horikoshi and Serpone 2013). Over the years, exponential growth in preparation, characterization, and innovative application of NPs has been observed (De Crozals et al. 2016). Extensive research on NPs resulted in engineered nanosized particles like various metal and metal oxide NPs, nanopolymers, fullerenes, carbon nanotubes (CNT), and crystalline materials, which possess numerous properties and are labeled as multifunctional NPs (Seaton et al. 2010; McNamara and Tofail 2013).

7.1.2 Applications of Nanoparticles

NPs are used, or are being evaluated for usefulness, in many fields due to its widespread area of applications. NPs possess diverse properties and are useful in industrial manufacturing as chemically inert additives, anticaking agents, pigments, and fillers and more prominently to generate functional surfaces/membranes which exert UV protection, antimicrobial property, catalytic function, filtration, etc. (Stark et al. 2015). Newer areas like nanomedicine have evolved as a cumulative outcome of well-known subjects like medicine, physics, and chemistry which are the driving force behind various biomedical applications. Characteristic electrochemical, piezoelectric, optical, and photoluminescence properties of NP are the basis of making biosensors for drugs, proteins, pathogens, nucleic acids, metabolites, cancer cells, etc. (Stark et al. 2015; Das et al. 2013). Most of the time NPs are designed to act as a delivery system where containment of surface characteristic and dimension is a prerequisite for drug-release pattern to exert site-specific action of the drug at an optimal rate and dose (McNamara and Tofail 2013; Das et al. 2013; McNamara and Tofail 2015). Site-specific distributions of NPs are possible due to their unique physicochemical properties when compared with fine particles (FPs). NPs are available in various forms and compositions like metallic based and carbon-based

nanomaterials, polymeric particulate materials, and quantum dots (Wang and Tang 2018; Wu and Tang 2018). Among all, metal NPs and metal oxide NPs contribute majority of them in terms of manufacturing output and application (Djurisic et al. 2015). Specific metal NPs like silver (AgNPs), gold (AuNPs), nickel (NiNPs), copper oxide (CuNPs), and metal oxide NPs (titanium dioxide [TiO_2], zinc oxide [ZnO], iron oxides [Fe_2O_3, Fe_3O_4]) are produced in large quantity and supplied in various fields of healthcare, medicine, transportation, construction, energy, defense, etc. along with engineered nanoparticles (ENPs) as major components or as additives for performance enhancement (Kessler 2011; Rudramurthy and Swamy 2018). Researchers are exploring the possible anticancer activity of biologically synthesized AgNPs, AuNPs, and platinum NPs (PtNPs) (Bendale et al. 2017; Ning et al. 2017; Yamada et al. 2015; Zhang et al. 2016), whereas manufacturers of sunscreen products are using TiO_2 and ZnO NPs in the formulation due to their capability to block ultraviolet radiation. Research on drug delivery uses iron oxide NPs (IONPs), including Fe_3O_4 and γ-Fe_2O_3, and magnetic resonance imaging uses superparamagnetic IONPs widely (Ding and Guo 2013; Namvar et al. 2014). However, assessment of adverse impact on the environment and human health has explored a new area of research.

7.1.3 Measurement of Nanotoxicity

Nontoxicity is a prerequisite for NPs used in biomedical field. However, environmental exposure of toxic NPs used in manufacturing and other applications is a major concern (De Crozals et al. 2016; Friedman et al. 2013). Metal and metal oxide NPs possess good dispersibility and stability in the presence of organic matter present in water and thus can pollute aquatic environment by means of direct discharge and waste discharge and during routine use. Metal and metal oxide NPs entering into the aquatic environment can reach and accumulate in the human body through food chain while drinking water and eating vegetables, fish, and livestock and can be a threat to human health (Xing et al. 2016; Nowack and Bucheli 2007; Wang and Wang 2014). Researchers have been working on developing newer assessment or evaluation methodologies to check exposure levels and toxicity of specific nanomaterials. Largely, toxicological assessment of NPs is carried out using in vitro and in vivo models starting with in vitro cell culture assays to basic model organisms, such as algae, protozoa, zooplankton, and advanced higher vertebrate models, such as rodents, rabbits, and nonhuman primates (Li and Chen 2011; Schrand et al. 2010). Cellular level toxicity and genotoxicity can be assessed using simple organism and cell lines, whereas complex physiological interactions can be assessed only on higher vertebrates. However, rodents and rabbits have a drawback as an animal model due to their ethical concerns, cost, slower and inaccessible embryo development, and amount of testing material required (as per animal size), whereas primate model shows similar issues with greater extent (Gad 2006). Therefore, zebrafish can be used as a compelling alternative model for the evaluation of

in vivo nanotoxicity due to its efficiency, cost-effectiveness, and smaller size (Chakraborty et al. 2016).

7.2 Zebrafish: Preclinical Model

7.2.1 Outline

Zebrafish (*Danio rerio*) has been a well-established vertebrate model since 1960s (Kalueff et al. 2014) and is being used extensively in preclinical and toxicity studies due to the number of favorable traits available (Strähle et al. 2012; Chakraborty and Agoramoorthy 2010). In recent times, zebrafish (*Danio rerio*) had drawn much attention as an in vivo model as it carries unique features like lower cost, high fecundity, embryonic transparency, rapid and well-characterized growth, shorter reproduction time, and gene manipulation accessibility. Ecotoxicology research recognizes zebrafish to assess embryo toxicity and it is used as one of the standard methods for evaluating toxicity due to single chemical entity as per the guidelines of national standards organizations (Fako and Furgeson 2009). A fully grown adult fish shown in Fig. 7.1 can be used for studying a large number of testing materials due to their advantage of having lower size. They possess high fertility rate where a single female can produce about 300 eggs, which proves the completeness as a model (Westerfield 1995; Ribas and Piferrer 2014). The genome of zebrafish and humans shows ~70% resemblance (Howe et al. 2013; Kettleborough et al. 2013). Majority of investigations using *Danio rerio* concentrate on teratogenic and developmental effects of materials on the larvae and on the fry. *Danio rerio* is commonly used to assess the potential toxic effects of NPs due to its capability of rapid reproduction, ease of breeding, availability of embryos round the year, and transparency of the larva body.

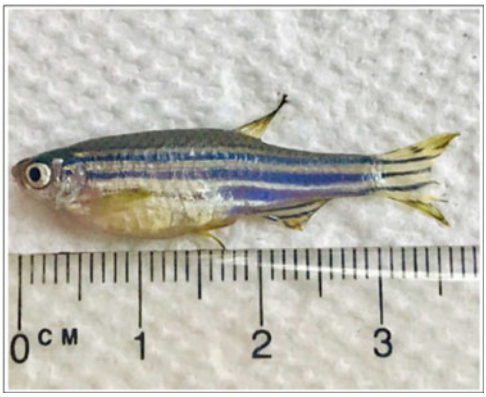

Fig. 7.1 Medium-size adult zebrafish (*Danio rerio*) (De León et al. 2019)

7.2.2 Advantages of Zebrafish in Nanotoxicity Research

In recent times, utilization of the zebrafish model has become popular in the screening of toxicants (Chun et al. 2017; Da et al. 2018; Sangabathuni et al. 2017; Vicario-Pares et al. 2018). Various attributes make zebrafish a substitute model for toxicological investigations of nanomaterials as follows.

Firstly, as a multicellular entity, zebrafish can provide more comprehensive data regarding kinetic, passage, and transformation of nanomaterials against in vitro cell culture analysis, despite the fact that in vitro analysis is mostly used to assess toxicological impacts of nanomaterials and is recognized as a successful model for toxicity studies even at the cellular level (Gad 2015).

Secondly, due to the smaller size, ease of cultivation, shorter life cycle, and higher fecundity compared to rodents, zebrafish became the most accessible model for the vast majority of research facilities around the world. They achieve mature reproductive system in laboratories within a short span of time (3–6 months) postfertilization under optimum temperature, food supply, and rearing densities (Spence et al. 2008). A fully grown female fish can yield about 100–300 embryos per day, and therefore may be useful in high-throughput analysis which improves the statistical power of experiments (Castranova et al. 2011; Spence and Smith 2005).

Third, the rapid embryogenesis and developmental processes in zebrafish compared with other animals make it a superior model for evaluating developmental toxicity (Haffter et al. 1996; Kimmel et al. 1995; Westerfield 2007; Lin et al. 2013). Toxicological effects like lethality, reproductive toxicity, and teratogenicity can be observed easily due to their transparency during embryo stages (Choi et al. 2016; Ma et al. 2018; Mesquita et al. 2017; Pecoraro et al. 2017).

Fourth, information gathered post-gene sequencing elaborates that zebrafish have 26,206 protein-coding genes and around 85% of these are similar to their human counterparts, making zebrafish a popular model for investigating genotoxicity and developmental toxicity (Collins et al. 2012; Howe et al. 2013; Renier et al. 2007; Garcia et al. 2016; Rizzo et al. 2013; Sarmah and Marrs 2016; Zhu et al. 2014).

7.2.3 Developmental Stages of Zebrafish

Eggs of zebrafish are robust in nature and grow externally, so it is possible to engineer them easily for high-throughput applications. In addition, optical transparency of zebrafish permits impeccable visual examination, including fluorescent and different markers (Stainier and Fishman 1994; Dooley and Zon 2000). The basic development of zebrafish requires only 24 h postfertilization (hpf), whereas embryogenesis completes by 72 hpf, and it takes 96 hpf to develop organs and around 3 months to arrive at adulthood (Stainier and Fishman 1994). There are a number of screening methods to study the developmental stages of zebrafish to measure toxicological responses to metal and metal oxide NPs, in terms of developmental toxicity, neurotoxicity, hepatotoxicity, genotoxicity, immunotoxicity, cardiovascular toxicity, reproductive toxicity, etc. (Fig. 7.2).

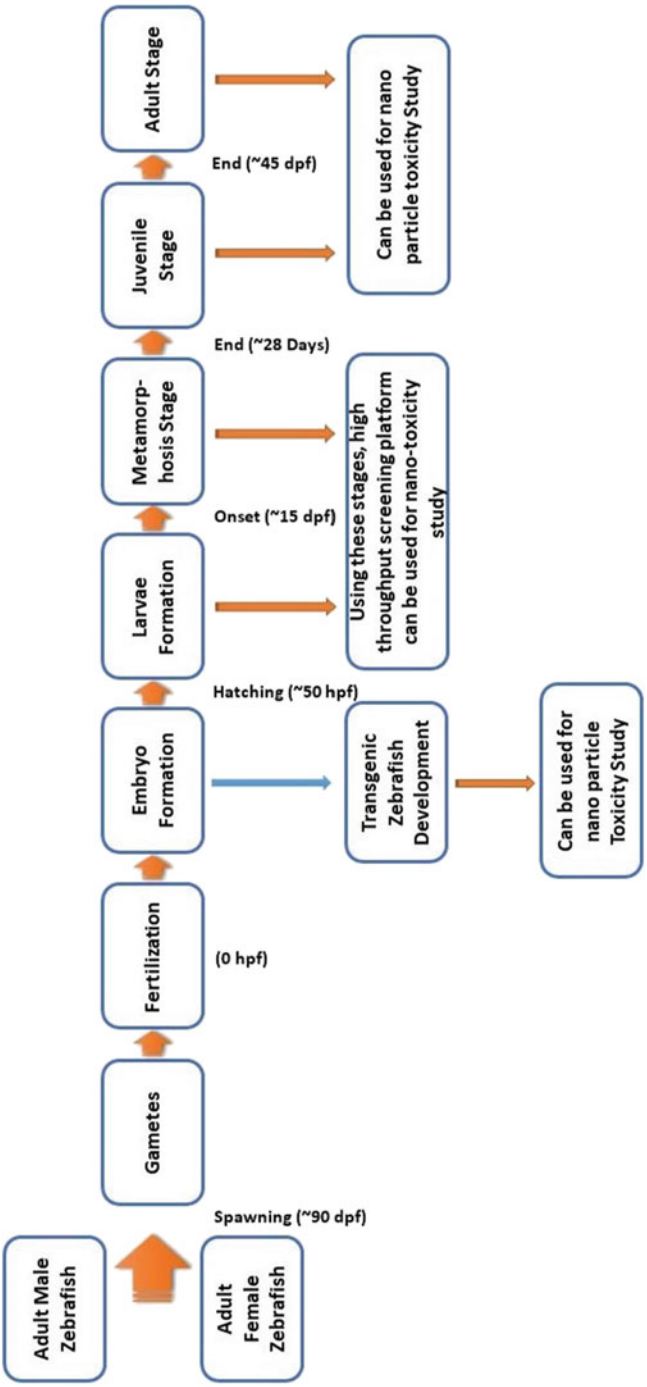

Fig. 7.2 Schematic diagram of different stages of zebrafish development and their relevance to nanotoxicity study (Chakraborty et al. 2016)

7.3 Various Methods to Assess Nanotoxicity

7.3.1 Developmental Toxicity

Teratogenicity, mortality, and hatching rate are the developmental toxicity parameters of nanomaterials in zebrafish. NP assessment on zebrafish used for the evaluation of developmental toxicity of embryos has been found to be mature than the toxicity evaluation of target organs or other systems. It is also appropriate for image-based detection and is capable of recording a range of teratogenic indicators like cell movement throughout intestinal phase, blood circulation, brain formation, and heartbeat due to its in vitro fertilization and lucidity during embryo stages. Embryonic development events are capable of being utilized as endpoints for toxicological assessment (Fig. 7.3). Additionally, the embryo teratogenic test cycle in zebrafish is short and appropriate for gene mutant screening and analysis in large scale.

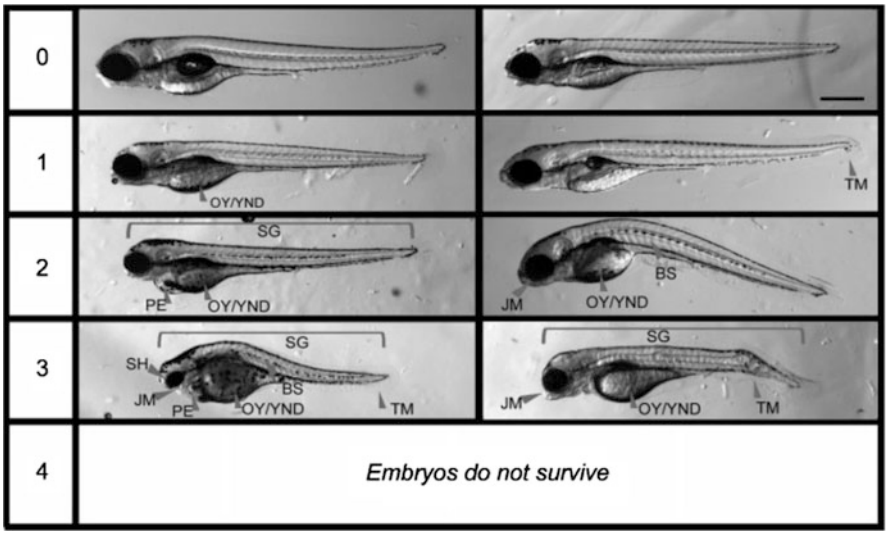

Fig. 7.3 The scoring spectrum utilized for screening nanoparticle-induced toxicity is portrayed by representative micrographs of 120 hpf zebrafish embryos that were exposed to different toxicants. This screening method was used as a semiquantitative analysis for scoring at 4, 24, 48, 96, and 120 hpf time points. Embryos were scored for severity of morphological defects, survival, and toxic adverse effects. Scores range from 0 to 4, with 0 indicating no visible deleterious effects and 4 signifying death. The intervening numbers correspond to various degrees and quantities of morphological anomalies (i.e., 1 = one to two minor toxic effects; 2 = one moderate or three to four minor toxic effects; and 3 = one (or more) severe or more than four minor toxic effects). Scores were used to yield a mean cumulative toxicity score for each treatment group at each time point to evaluate toxicity. Most of the sublethal endpoints included in the studies are depicted in the figure: bent spine (BS), jaw malformation (JM), opaque yolk (OY), pericardial edema (PE), stunted growth (SG), small head (SH), tail malformations (TM), and nondepleted yolk (YND). Scale bar = 0.5 mm (Bar-Ilan et al. 2009)

AgNP-treated embryos showed mortality and hatching delay. Furthermore, developmental toxicity like pericardial edema, slow blood flow, arrhythmia, twisted notochord, and body axis abnormality were the outcomes of AgNP treatment (Asharani et al. 2008; Shaw et al. 2016). Zebrafish embryos, when exposed to gold nanorods coated with cetyltrimethyl ammonium bromide (CTAB), were shown to induce delayed embryonic developments such as delayed eye, head and tail elongation development, pericardial edema, and tail deformities. These embryos were also found to induce mortality when exposed to CTAB (Mesquita et al. 2017). It has been observed that metal oxide NPs are also capable of inducing developmental and acute toxicity in zebrafish. Abnormal phenotypes like delayed epiboly and smaller head and eyes in zebrafish can be observed as a result of copper oxide NP exposure (Xu et al. 2017). Another metal oxide, namely ZnONPs, can cause toxic effects such as skin ulceration, hatching delay, and high mortality in zebrafish (Zhu et al. 2008). Toxicity to zebrafish embryos due to TiO_2NPs was also evaluated and found to affect the hatching time of embryos (Samaee et al. 2015).

7.3.2 Immunotoxicity

Application of zebrafish in the field of immunology has gained momentum in recent years. It has been observed that the immune system is sensitive to NPs, predominantly inducing an inflammatory response in addition to accumulation and activation of neutrophils and macrophages (Johnston et al. 2018). The process in which toxic substances destroy the function of immune system is known as immunotoxicity (Giannakou et al. 2016; Selgrade 2007; Xu et al. 2015; Jin et al. 2011) (Fig. 7.4). For instance, AuNPs have been proved to disrupt inflammatory and immune response pathways (Truong et al. 2013). In another study, an adult zebrafish was exposed to AgNPs and subsequently a gene expression study was performed in its liver tissues. The study proved that AgNP exposure resulted in immunotoxicity in adult zebrafish because of oxidative stress (Krishnaraj et al. 2016). ZnONP exposure also resulted in transcriptional changes of pro-inflammatory cytokines, interleukin (IL)-1β, and tumor necrosis factor-α and a significant upregulation in eleuthero embryos and a downregulation in zebrafish embryos. Therefore, ZnONPs have been proved to cause modulation of pro-inflammatory reactions (Brun et al. 2014).

7.3.3 Neurotoxicity

Zebrafish model has emerged as a sensitive and useful animal model for the assessment of neurotoxicity induced by NPs. The damage of nervous tissue and subsequent irregular activity of nervous system, when exposed to toxic substances, is called neurotoxicity and these toxic substances are known as neurotoxins (Segura-Aguilar and Kostrzewa 2006). A variety of NPs can activate free radical actions at their surfaces, thus generating oxidative stress at particle deposition and translocation site (Sato et al. 1998; Dellinger et al. 2001; Li et al. 2003). Specific behavioral

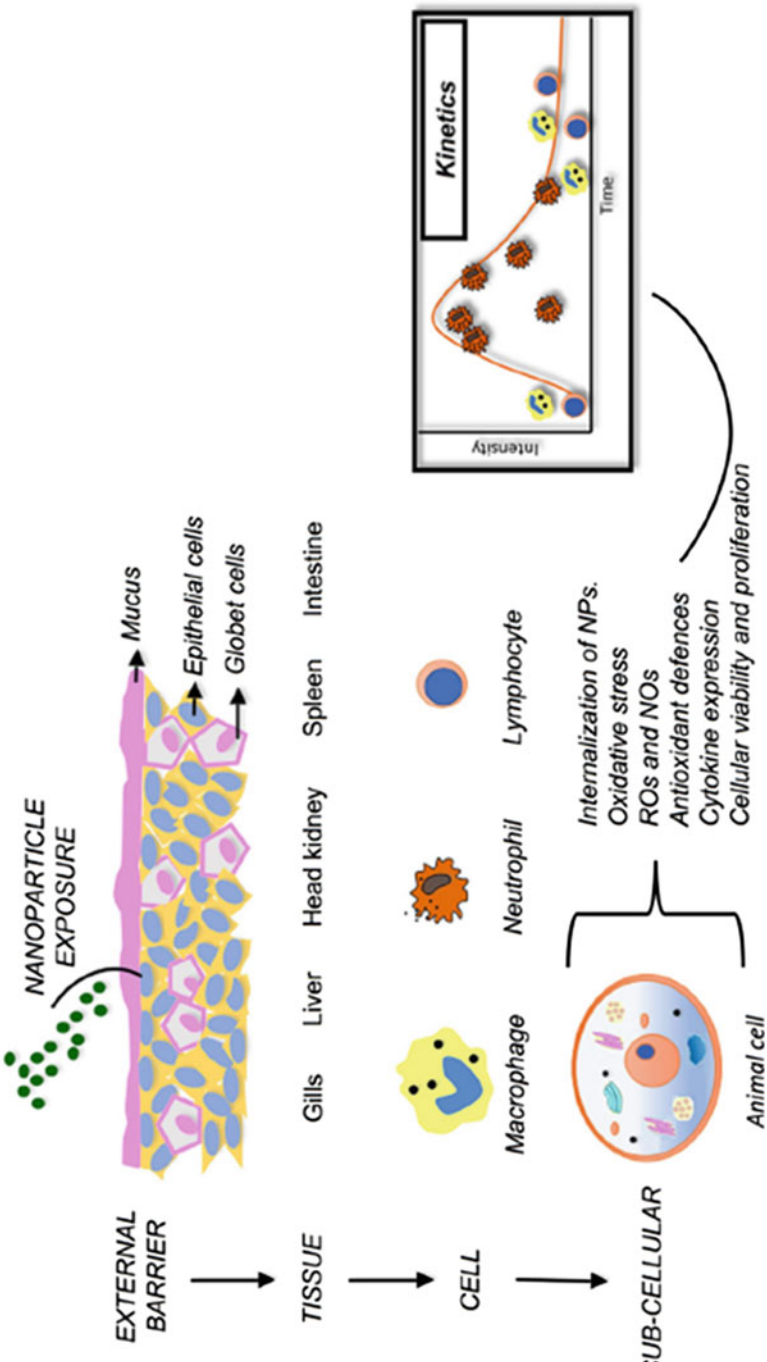

Fig. 7.4 Innate immunity as a bioindicator of health for teleost fish exposed to nanoparticles. Following NP exposure, fish immunity is evident at different levels (external barrier, tissue, cellular, and subcellular). Each provides unique insights into changes to homeostasis and, thus, can be used to detect nanoparticle-induced immunotoxicity (Torrealba et al. 2019)

effects for particular NPs are also seen. The brain tissues of juvenile zebrafish after 5 days of fertilization have been differentiated into telencephalon, diencephalon, midbrain, hindbrain, and rhomboidal ganglia. Behavioral toxicity of NPs such as learning, motion, and memory ability can also be evaluated using well-differentiated brain tissues of juvenile zebrafish. Furthermore, neurotoxicity of NPs to zebrafish embryos can also be evaluated using apoptosis of neurons, necrosis, morphological changes, and biochemical indicators. Neurotoxicity can be seen commonly in NPs that are capable of reaching brain and causing neurodegeneration (Win-Shwe and Fujimaki 2011; Chakraborty et al. 2009). Combustion-derived NPs have been proved urotoxic from in vivo and in vitro studies, due to the incidence of NP aggregation (Morimoto et al. 2010). For instance, silicon dioxide NPs resulted in altered color preferences (Li et al. 2014), whereas cadmium telluride quantum dots affected locomotor activity (Zhang et al. 2012). A size-dependent effect was observed on zebrafish due to polyvinyl pyrrolidone-coated AgNPs-PVP. The smaller AgNP-PVP sized 10 nm resulted in decreased locomotor activity, while hyperactivity was caused by the larger one (50 nm) under specific light conditions (Powers et al. 2011). Earlier studies have shown that TiO_2NP activates expression of genes like BDNF C-fos and C-jun. On the contrary, these NPs suppress the expression of genes such as NGF, p38, and CRE causing brain damage of zebrafish (Sheng et al. 2016). Alteration of neurotransmission and subsequent increase in brain acetylcholine esterase activity were caused by AuNP exposure (Dedeh et al. 2015). A delay in retinal neurodifferentiation with subsequent reduced locomotor activity was caused by CuONP exposure at high doses ($\geq$12.5 mg/L) (Sun et al. 2016). Exposure of FeONPs coated with dextran was also found to be neurotoxic to zebrafish. The toxic effects included higher levels of ferric iron in the brain, reduction in the exploratory performance, decreased acetylcholine esterase activity, and induction of casp8, casp9, and jun genes (De Oliveira et al. 2014).

7.3.4 Genotoxicity

Genotoxicity is the damage of genetic information inside a cell because of chemical agents which cause DNA damage, gene mutation, and chromosomal alteration (Bolognesi 2003). Genotoxicity is a major risk factor for carcinogenesis. Zebrafish model can be used to study various chemical-induced genotoxicities with the help of different techniques. Genotoxicity can be evaluated in embryos, larvae, or adult tissues and various techniques such as quantitative RAPD-PCR methodology for demonstrating dose-dependent genotoxicity of TiO_2NPs (Rocco et al. 2015) and comet assays for checking the effect of ferric oxide (Fe_2O_3) NPs can be used (Villacis et al. 2017). Moreover, RAPD-based methodology was used to assess genotoxic effects of gold NP on zebrafish (Dedeh et al. 2015; Geffroy et al. 2012). However, only fewer studies have been reported on the assessment of genotoxicity of NPs on zebrafish; hence, this area has to be studied extensively.

7.3.5 Cardiovascular Toxicity

Cardiac toxicological evaluation of NPs can be successfully performed using zebrafish embryos. Resemblance of zebrafish heart to human embryonic heart and direct observation of shape and rhythm of heart like heartbeats, cell activity in blood vessels, and blood vessel morphology using a microscope have greatly enabled efficient toxicity evaluation and toxicological research of NPs. Regular heartbeats in zebrafish commence at 36 h after fertilization. Monitoring and quantitative evaluation of cardiovascular damage on exposure to specific NPs have been effectively established using transgenic zebrafish lines. A study using transgenic zebrafish Tg (nacre/fli1: EGFP) revealed that CuONPs inhibit vasculogenesis through induction of apoptosis and reduction of vascular endothelial growth factor expression (Chang et al. 2015). The hematopoietic system of zebrafish is regulated by molecular pathways that are quite conventional. Particularly, the early development of cardiovascular system resembles that of humans. Therefore, AgNP toxicity in hematopoiesis was studied using a zebrafish model. Transcriptional responses of zebrafish embryos to AgNPs were revealed using microarray analysis. This analysis was performed at 24 h after fertilization. Gene ontology analysis revealed that AgNPs were responsible for downregulation of hemoglobin genes. It was also studied that erythrogenesis inhibition caused by AgNPs was cell specific and developmental stage specific. Further, it was found that this inhibition was caused mostly by AgNPs compared to their releasing ions (Cui et al. 2016).

7.3.6 Hepatotoxicity

The liver performs many important functions of body as it is the main metabolic organ of human body. Toxic effects of various chemicals can cause functional damage to liver and this may affect the normal functioning of body. The way in which the liver of zebrafish in its early developmental stages responds to toxic chemicals is similar to that of humans. Therefore, zebrafish model is ideal for studying NP-induced hepatotoxicity. Earlier studies have shown that when zebrafish embryos and larvae are exposed to CuONPs at high doses for a short period of time, hepatotoxicity and neurotoxicity, displaying as hepatic hypoplasia and delayed retinal neurodifferentiation coupled with decreased locomotor capacity, can be observed (Sun et al. 2016). Another study on the effects of oxidative stress and ZnOP damage on intestine, gill, and liver of zebrafish revealed that liver tissues were mainly targeted by oxidative damage. It was shown in the further study that ZnOPs produced higher OH radicals. The malondialdehyde, which is one of the biomarkers of oxidative stress, was increased in gills and liver of zebrafish (Xiong et al. 2011).

7.3.7 Reproductive Toxicity

Partial or whole life cycle tests of zebrafish can be used for testing reproductive toxicity of NPs. For instance, AgNP exposure resulted in oxidative stress, followed by germ cell apoptosis through mitochondrial dependent pathway. This finally led to damage of reproductive ability of zebrafish (Ma et al. 2018). In another study, AuNP (10–50 nm) exposure to adult female zebrafish gave rise to strand breaks in ovarian cells due to the ability of AuNPs to enter zebrafish ovaries (Dayal et al. 2016). Reproductive toxicity to zebrafish testis on exposure to TiO_2NPs was also studied. TiO_2NPs in higher doses were found to induce autophagy and necrosis in Sertoli cells and thus had a negative impact on testicular morphology and spermatogenic cells of zebrafish. It gave rise to mitochondrial degeneration with swelling and crista loss (Kotil et al. 2017).

7.3.8 Disruption of Gill, Skin, and Endocrine System

NP-induced toxicity also interrupts gills, skin, and endocrine system. Waterborne NPs mainly target gills of zebrafish. Silver ions (Ag+) produced by AgNPs show acute toxicity as they interact with the gills. Osmoregulation is affected in the gills, due to inhibition of Na^+/K^+-ATPase action and enzymes related to Na^+ and Cl^- uptake by Ag+ ions (Bury et al. 1999; Wood et al. 1999). Insoluble forms of CuNPs were also found to be very toxic and their suspensions may cause damage to gill lamellae (Griffitt et al. 2007). Moreover, NPs such as Ag-BSA enter embryo skin via diffusion or endocytosis, get deposited on the epidermis layer of larvae, and lead to skin abnormalities through apoptosis (Asharani et al. 2008). It was also suggested that TiO_2NPs cause an increase in the bioconcentration of lead, and lead to interruption of thyroid endocrine system in zebrafish larvae (Miao et al. 2015).

7.4 Nanotoxicology in Zebrafish

Nanotechnology has emerged as an interdisciplinary field which is linked to various subjects like physics, chemistry, biology, medicine, and toxicology (Weiss and Diabate 2011; Donaldson et al. 2004). Nanotechnology research primarily requires animal models to check nanotoxicity and zebrafish has the potential for the same as notable advancement has been made in the mentioned field using zebrafish (Jang et al. 2014). This section emphasizes on some recent studies and available data related to toxicity of NPs using zebrafish model.

7.4.1 Metal Nanoparticles

7.4.1.1 Gold

Unique properties AuNPs make it a preferred choice for various fields like cellular labeling, drug delivery, imaging and diagnostics for cancer, diabetes, and Alzheimer's disease (Li and Chen 2011). However, AuNPs may cause cytotoxicity in humans (Goodman et al. 2004; Gerber et al. 2013). Therefore, zebrafish has become a popular in vivo model for the assessment of toxicity caused by most commonly studied NPs (AuNPs) at present. Among all the engineered nanomaterials, AuNPs have the least empiric proof of adverse impacts on organisms, yet fewer number of investigations have been carried out to assess in vivo toxicity (Caballero-Diaz and Valcarcel 2014). In vitro assessment postulates some mechanisms such as genotoxicity, apoptosis, generation of ROS, leakage of toxic materials, interactions with lipids and proteins, mitochondrial damage, endocrine disruption, cellular morphology changes, and altered gene expression (Caballero-Diaz and Valcarcel 2014). There are a number of reported studies where embryos are exposed to 100 μL/mL of gold nanoclusters, but none showed toxic impact on mortality, gene expression, heart rate, hatching rate, and malformations (Chandirasekar et al. 2016). However, toxic impact was observed at relatively higher concentration, which does not have environmental importance. At 300 mg/mL, AuNPs showed 100% embryo mortality as an anticancer agent (Ramachandran et al. 2017). AuNPs were turned out to be less toxic toward embryos or adult zebrafish compared to other NPs such as Ag, Pt, and Cu (Ramachandran et al. 2018; Browning et al. 2019; Bar-Ilan et al. 2009; Asharani et al. 2010). But some studies reported toxic effect of AuNPs on zebrafish which may end up with embryonic lethality, neurotoxicity, developmental toxicity, and immunotoxicity (Truong et al. 2012; Kim et al. 2013). Presence of AuNPs (12 and 50 nm) in food leads to a variety of cellular malfunctions and genome modifications in adult zebrafish depending on size, exposure time, and concentration (Geffroy et al. 2012). Genome alteration in various adult tissues was observed when zebrafish was exposed to sediment containing 14 nm AuNPs for a longer period of time, which may be due to increase in oxidative stress (Dedeh et al. 2015). AuNPs were found to have more potential toxic effects than ionic Au if accumulated in tissues. Another work confirmed that 0–50 nm AuNPs could induce strand breaks in zebrafish ovaries (Dayal et al. 2017).

7.4.1.2 Silver

AgNPs are one of the most extensively studied NPs used as therapeutic agents, antimicrobials, and biosensors, in various cosmetic products and drug delivery systems (Czupryna and Tsourkas 2006; Yoon et al. 2007; Jin and Ye 2007; Prow et al. 2006; Perugini et al. 2002). AgNPs exert size-based toxicity which indicates that the dimension of NPs plays a crucial role in their toxicity profiling. A previous study established this fact by performing in vivo quantitative study in zebrafish to verify size-dependent transport and toxicity of AgNPs (Lee et al. 2012). In the abovementioned study, it was found that AgNPs having 30–72 nm diameter were

capable to diffuse into the zebrafish embryos through chorionic pores due to random Brownian motion and may produce more potent toxic effect. However, different size (3, 10, 50, and 200 nm) of AgNPs (synthesized) showed 100% mortality rate after 120 hpf when administered to zebrafish embryos irrespective of size (Bar-Ilan et al. 2009). Hence, size-dependent toxicity profile of AgNPs is conclusive. A number of toxicities were observed including damage to neuromast hair cells, reduction in heart rate, teratogenicity, and mortality when AgNPs were exposed to zebrafish during early development (Yoo et al. 2016). But another study concluded that low concentrations of 10–20 nm AgNPs (<5 mg/L) do not have much impact on normal embryonic development, but higher concentrations showed significant impact on the growth of ectodermal and mesodermal tissues, probably due to delayed or inhibited cell division (Xia et al. 2016). Immunotoxicity and oxidative stress were observed due to the localization of AgNPs in the gills and liver when an adult zebrafish was exposed to it (Krishnaraj et al. 2016). A number of AgNPs possess different shapes and are known to induce oxidative stress, but plate-shaped AgNPs were more prone to show toxic effect than spherical and wire-shaped forms (George et al. 2012; Abramenko et al. 2018). Interestingly, these effects were associated with the presence of surface defects rather than Ag shedding (George et al. 2012). However, reductions in oxidative stress in embryos or adults were observed when AgNPs were coated with cysteine (George et al. 2012) or sulfidation (Devi et al. 2015). Increase in embryonic toxicity of AgNPs was detected after exposure to simulated solar light (George et al. 2014). Collectively, this suggests complex interplay of factors, where a range of physiochemical properties underpin biocompatibility.

7.4.2 Metal Oxide Nanoparticles

7.4.2.1 Titanium Dioxide

Among all, TiO_2NPs are one of the most extensively manufactured and commercially applied nanomaterials due to its area of application from colorants in sunscreens to excipients of toothpastes, shampoos, soaps, etc. which projects enormous growth potential; presently global annual production stands at around 10,000 Tm (Noman et al. 2018; Drobne 2018). Low-dose TiO_2NP does not show major developmental abnormalities in zebrafish when embryos are exposed to it (Wang et al. 2014). But various studies have reported their capability to trigger premature hatching in a dose-dependent manner (Samaee et al. 2015; Clemente et al. 2014). As per some studies, higher dose of TiO_2NPs may trigger embryonic malformation and death (Chakraborty et al. 2009). Another study reveals that the capability of TiO_2NPs to absorb photons may trigger production of electron-hole pairs which can interact with water and oxygen molecules to produce reactive oxygen species that are poisonous to zebrafish larvae (Bar-Ilan et al. 2012). Prolonged exposure of adult zebrafish to TiO_2NPs for 6 months at low concentrations (<4 mg/L) was also linked with low toxicity, judged by mortality rate. However, higher concentration leads to accumulation of NPs in various parts of the fish, including the heart, liver, gill, and brain (Chen et al. 2011a, b) and exhibits genotoxic effects (Rocco et al.

2015). Exposure of zebrafish embryos to TiO_2NPs starting from fertilization to the free-swimming phase does affect hatchability, survival, and malformation rate. However, larval swimming parameters such as average velocity and maximum velocity were considerably altered, indicating that the behavioral endpoints were far more sensitive than other parameters like hatchability and survival (Bar-Ilan et al. 2012; Chen et al. 2011a, b). However, the foremost consequence of TiO_2NP exposure is neurotoxicity. Even low level of TiO_2NPs may damage brain by crossing the blood-brain barrier, causing neuronal differentiation and neurogenesis (Wang et al. 2014; Chakraborty et al. 2009). Long-term low-dose exposure of TiO_2NPs to adult zebrafish for 45 days showed alteration in behavior and histopathological variations in the zebrafish brain due to the reduction in neurotransmitter level which were linked to dose-dependent elevation in nitric oxide levels (Sheng et al. 2016).

7.4.2.2 Copper and Copper Oxide

Utilization of copper has seen an upward trend over the years due to its considerable demand in various sectors like electronics, petroleum lubricants, catalysis, sintering active agents, consumer products of the pharmaceutical industry, adsorbents for water purification, and biomedical industries (Adeleye et al. 2016; Dankovich and Smith 2014; Lee et al. 2016; Liu and Astruc 2018; Goel et al. 2014). Copper and its oxides have been utilized in many areas including biosensing (Mao et al. 2015), energy storage (Dar et al. 2015), and development of antibacterial agents (Chatterjee et al. 2014). However, these materials can simply discharge copper particles which are exhibited to initiate cellular damage by prompting oxidative stress. Assessment of toxic impacts of Cu-based nanomaterials is far more difficult, as the toxicity is not only caused by the dissolved copper ions. One examination uncovered that CuNP introduction on zebrafish embryos indicated that CuNP creates ROS in a concentration-dependent manner (Denluck et al. 2018). CuNPs deferred embryo hatching time and produced teratogenicity of larvae. Dose-dependent mortality in zebrafish embryos was observed when CuNPs were exposed to it, whereas higher concentration leads to death of gastrula-stage zebrafish embryos (Bai et al. 2010). A previous study revealed that CuNPs cause acute toxicity to zebrafish embryos followed by gill injury (Griffitt et al. 2007). A new work reported on earlier report further disclosed that CuNPs (25 nm, 1 mg/L) might induce significant transcriptional changes in the pro-inflammatory linked genes in the skin and intestine and raise the movement of neutrophils in the tail of zebrafish embryos (Brun et al. 2018). Mentioned statement revealed CuNP-induced dermal and intestinal inborn immune responses, which may indicate the possible adverse events of CuNPs at higher levels of biological organization. CuONPs are vastly used in numerous fields like batteries, gas sensors, high-temperature superconductors, agricultural biocides, photocatalysts, energy transfer fluids, and antimicrobial agents (Batley et al. 2013; Hou et al. 2017; Kim et al. 2012; Llorens et al. 2012). Therefore, extensive use and its production may cause possible threats to individual organisms and ecosystem too. The outcome of the potential toxicity assessment of CuONPs in zebrafish embryos and larvae (Bai et al. 2010) exposed that CuONPs have the capability to interfere in

embryo hatching in a dose-oriented way and produced amplified expression of the heat-shock protein 70 in zebrafish larvae when a higher dose was given (Lin et al. 2011). Additionally, administration of CuONPs in zebrafish embryos was discovered as a source of oxidative stress-mediated teratogenicity and this observation was primarily attributed to the particles themselves rather than dissolved Cu. Reactive oxygen species (ROS) may be generated due to the accumulation of CuONPs in embryos, which may further lead to cell apoptosis followed by production of deformed embryos (Ganesan et al. 2016).

7.4.2.3 Zinc Oxide

ZnONPs are considered as one of the most promising nanomaterials with widespread biomedical applications (e.g., anticancer and antibacterial therapy) (Mishra et al. 2017; Sirelkhatim et al. 2015) and possess characteristic properties like transparency, biocompatibility, high isoelectric point, and photocatalytic efficiency; they are frequently used in cosmetics, sunscreens, ceramics, photonics, and electrical appliances (Mirzaei and Darroudi 2017). Unfortunately, ZnONPs are categorized as "extremely toxic" and may cause severe threat to the environment and ecosystem (Kahru and Dubourguier 2010). Usually, the ZnONP-caused toxicity is primarily due to the dissolution of Zn^{2+} which can trigger various biological effects starting from lysosomal damage, mitochondrial perturbation, generation of ROS, initiation of pro-inflammatory responses, and lastly cell death (George et al. 2010; Xia et al. 2008, 2011). Zebrafish embryos and larvae show toxic effects such as retarded hatching, tail malformations, reduction in body length of the larvae, and tissue damage when they are exposed to ZnONPs at lower concentrations, but higher concentrations may lead to embryonic mortality (Zhu et al. 2008, 2009; Kteeba et al. 2017). Shape of the particle and surface coating play a significant role in experiencing ZnONP toxicity. Polymer-coated ZnONPs were considered to be more biocompatible compared to spherical ZnO, whereas leaf-shaped ZnONPs show extreme influence on hatching (Ong et al. 2014). Another research work on shape-based toxicity study of ZnONPs showed that nanospheres and cuboidal submicron particles were found to be less toxic than nanosticks in terms of hatching and overall mortality (Hua et al. 2014). Zhao et al. (2013) predicted the fundamental mechanism of ZnONP exposure-induced developmental toxicity which is linked to cellular oxidative stress, DNA damage, and altered actions of several critical defense enzymes (i.e., catalase, glutathione peroxidase, and superoxide dismutase).

7.4.2.4 Magnesium Oxide

MgONPs are commonly utilized in medicine, manufacturing, and anticancer therapy and as an antibacterial agent in the food industries. Extensive use of these NPs in our everyday lives results in unavoidable discharge and environmental exposure. Many researches have revealed variable toxicity of other metal oxide NPs. Exposure of MgONPs initiated increased mortality in zebrafish (Kovrižnych et al. 2013). Many researches evidenced concentration-dependent MgONP-induced cellular apoptosis and ROS. Dose-dependent alteration in hatching rate, malformations, and survival of

zebrafish embryos were observed due to the exposure of MgONPs (20 nm) (Ghobadian et al. 2015).

7.4.2.5 Aluminum and Aluminum Oxide

Other NPs like aluminum nanoparticles (AlNPs) and Al_2O_3NPs have been broadly utilized in the drug delivery systems, optoelectronics industry, electronics, and biomedical products. Al_2O_3NPs and Al_2O_3 bulk showed very little acute toxicity to zebrafish embryos and larvae (Griffitt et al. 2008, 2011).

7.5 Limitations of Zebrafish Model for Nanotoxicity Study

Zebrafish as an in vivo model for toxicity profile of nanomaterials is a well-accepted phenomenon. The extent of toxicity of these NPs was evaluated by noticing the functional defects and malformations in zebrafish. However, literature survey reveals numerous lacunae in assaying nanomaterial-based immunotoxicity. Moreover, it is very challenging to assess embryo-based nanotoxicity assays systematically due to the fast developmental stages witnessed in zebrafish. However, advanced technologies along with automation help in screening nanotoxicity using zebrafish embryos. A number of nanomaterials are used for the purpose of therapeutic intervention in the area of antimicrobial therapy and drug delivery. Therefore, it is necessary to figure out the pharmacokinetic profiling of these nanomaterials. However, it is a bit challenging to perform ADME assay in zebrafish model after nanodrug delivery.

7.6 Future Prospects

Zebrafish as an in vivo model for toxicity profiling of nanomaterials has shown enormous potential. At present, several advance molecular biology techniques and zebrafish model transgenic lines are available for this purpose. Several zebrafish microarrays along with huge genomic resources are currently accessible for the purpose of nanotoxicity evaluation. These extremely advance resources make zebrafish a flexible system for toxicogenomic studies of nanomaterial in the coming days. Evaluation of nanomaterial toxicity on zebrafish development with the help of proteins and gene expression studies has enormous potential. Although zebrafish as a high-throughput screening system utilizing larval stages was previously explored for the purpose of evaluating nanomaterial toxicity, still huge scope persists for nanomaterial toxicity assays.

7.7 Conclusion

At present, zebrafish presents itself as a smart vertebrate model for testing NP toxicity and biocompatibility. Furthermore, this animal model has been much cheaper, faster, easy to conserve, and able to test agents efficiently via various routes for more than a decade. Additionally, definite physiological influences can be evaluated at multiple developmental stages. With the help of advance and up-to-date technology, zebrafish can become a meaningful alternative than other mammalian models for evaluating toxicity of nanomaterial in the coming days.

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