Zebrafish Model System to Investigate Biological Activities of Nanoparticles

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Abstract

Globally, the zebrafish as an experimental animal has been welcomed, and utilization increased successively. Zebrafish is a common name of *Danio rerio* fish. It belongs to the Cyprinidae family, within the order of the Cypriniformes. The fish is named for the five uniform, parallel blue-colored long narrow bands on the body side. The stripes are extended up to the tail end of the fish. The successful implementation of zebrafish as a trial animal majorly depends upon the key features like its genotypic and phenotypic similarities to human and their easy maintenance at laboratory scale. There is much similarity between the major organ systems like nervous, cardiovascular, and digestive systems of human and zebrafish. It is possible to identify and study the physiological and pharmacological responses of drugs and other bioactive compounds with therapeutic value. The zebrafish model is a powerful, well-established research platform for the testing of activities of new drug molecules. The nano-sized materials have opened up various possibilities in a variety of industrial issues and scientific endeavors. Nanomedicines are an effective way of drug delivery systems as they enhance drug absorption by improving the solubility characteristics of the drug. Bioactive nanoparticles can be easily and successfully studied on both embryos and adult zebrafish. Nanoparticles having biological activities like anti-convelsant,

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anti-melanogenic or other activities which affect the cardiovascular system, nervous system, reproduction system, etc. have been successfully studied on zebrafish as an experimental animal model. The zebrafish also plays an important part in toxicological studies of the nanomedicines. The zebrafish has proven its extensive promises as an in vivo animal model for screening of nanomaterials. The zebrafish can be employed in the process of drug development at the stage of pre-clinical testing. Presently, research is focused on the biological activity testing and toxicological testing of newly developed medicines especially chemotherapeutic agents or nanoparticles used in the treatment of cancer.

Keywords
Zebrafish · Nanoparticles · Genotypic · Phenotypic · Bioactive compounds

9.1 Introduction

Globally, the utilization of zebrafish as an experimental animal has been increased successively. Zebrafish is the common name of *Danio rerio* fish. It belongs to the Cyprinidae family, within the order of the Cypriniformes (MacRae and Peterson 2003). It is a small, freshwater fish. It is a tropical fish and can endure a temperature range of around 24–29 °C. It is native to Southeast Asia and found in the rivers of countries like India, North Pakistan, Nepal, and Bhutan. It commonly lives in streams, lakes, canals, and moving water to stagnant water bodies, including grasslands (Chakraborty et al. 2016b). Zebrafish is popular as aquarium fish and has been introduced in aquariums in the United States and Japan. This species is also popular for decorative purpose. The application of zebrafish as an experimental model was reported in 1955 for the first time. Since then to date, the use of zebrafish had an expanding growth (Fig. 9.1).

Fig. 9.1 Representation of life stages of zebrafish (Saleem and Kannan 2018)

9.2 Morphology of Zebrafish

The fish is named for the five uniform, parallel blue-colored long narrow bands on the body side. The stripes are extended up to the tail end of the fish. Male fish have torpedo shape and gold-colored stripes in between blue-colored stripes. While female fish have silver-colored stripes in between the blue-colored stripes. Females have a whitish belly and larger in size than male (Brundo and Salvaggio 2018). The zebrafish grows up to 6.4 cm (2.5 in.) approximately. Adult zebrafish are 3–5 cm in length. Due to their small size, they can be maintained easily in massive amount in the laboratory as experimental animal for research purpose. Most zebrafish live for 2–3 years in confinement. They can live up to 5 years in ideal conditions (MacRae and Peterson 2003).

Zebrafish embryonic growth has been thoroughly distinguished. The embryos themselves are clear in appearance during the first few days of life because chorion is translucent. After 30–72 h of post fertilization (hpf), pigment deposition appears in the embryos of zebrafish. The cytoplasmic movements are triggered by fertilization. About 40 min of post fertilization, the first bifurcation of the fertilized egg happens. The first cleavage of the newly fertilized egg occurs about 40 min after fertilization (Brundo and Salvaggio 2018). But the speed of zebrafish embryos growth varies according to temperature. The larval stage of zebrafish is transparent, and as it grows to adult phase, stripes start appearing. The stripes develop along the body length and in blue shade. Male fish are outlined like torpedo. They are slimmer than female and have a pink or yellow tinge usually, while female fish are fatter as they carry eggs. They are less pink than the male. They have the ability to deposit ample of eggs during the entire year. So, this is an excellent laboratory model (MacRae and Peterson 2003; Haque and Ward 2018).

The embryo phase of zebrafish is a "stereoblastula" as embryo is developed by spiral cleavage and absence of blastocoel. The blastula stage is equal to 2.25–5.25 h after fertilization (hpf), while gastrula stage of zebrafish is equivalent to 5.25–10 hpf (Brundo and Salvaggio 2018).

The term "pharyngula" (24–48 h) was referred to the embryo that has matured to the phylotypic phase. At this period of life, zebrafish is easily compared to other vertebrates for morphologies (Mushtaq et al. 2013).

9.3 Zebrafish Evelopment

The zebrafish eggs are sturdy and evolve in the exterior of the body. The optical microscopy of zebrafish showed visual analysis, in addition to fluorescent and other markers. So, it is easy to control and influence the zebrafish for research applications (MacRae and Peterson 2003).

Zebrafish have the ability to produce plenty of offspring with transparent embryo. The development of major organs of zebrafish occurs in the larval stage within few days post fertilization (dpf) as hatching eggs and production and development of the organs occur rapidly. The development of zebrafish is magnificently rapid, as they

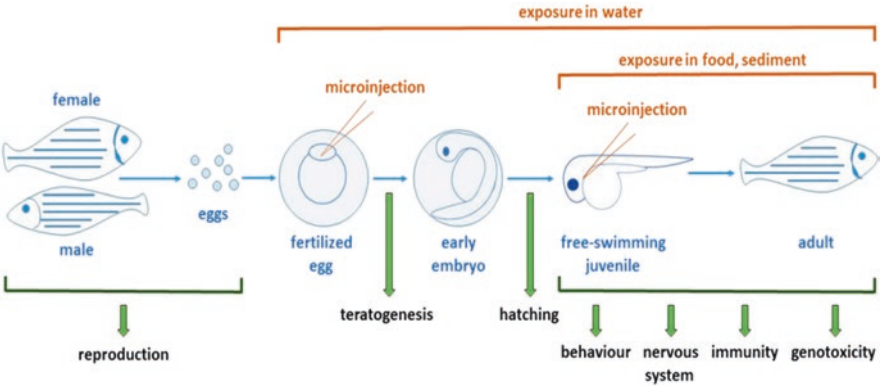

Fig. 9.2 Representation of zebrafish developmental stages and use of different stages in toxicity models (Haque and Ward 2018)

reach in adulthood in around 3 months with the well-established basic body plan by 24 hpf. The embryogenesis of well-established and completed within 72 hpf while fully developed organs by 96 hpf. This makes them susceptible to various toxicological applications through their whole lifespan (Fig. 9.2).

9.4 Zebrafish Animal: As a Perfect Experimental Model

The zebrafish (*D. rerio*) is a well-liked tropical fish pet. They are also a prime animal model for research in vertebrate development, genetics, and human biology and human disorders. The utilization of zebrafish as an investigational animal started in the 1960s. Zebrafish have features like a large family size, external development, and a relatively low cost of production and maintenance. These features make them useful in the laboratory animal model (Brundo and Salvaggio 2018). The successful implementation of zebrafish as a trial animal majorly depends upon the key features like its genotypic and phenotypic similarities to human and their easy maintenance at laboratory scale (Lin et al. 2013).

The zebrafish produces a large number of eggs. The life cycle of zebrafish is short with rapid development. The development of zebrafish occurs outside the uterus of female. These features contribute to the low cost of its production, maintenance, and care.

There are many benefits of utilizing zebrafish as a laboratory animal in research studies. Their fecundity rate is more as one female produces not less than 300 eggs. Their proficiency as an experimental animal is more (Lin et al. 2013). The adult zebrafish is tiny and has a length of 5 cm approximately. This is a critical feature for less space requirement. They can be maintained with no difficulty. It reduces housing space, housing requirements, and husbandry costs compared to the other animal models, making them cost-effective. The small size makes them easy to handle and use in laboratory experiments.

As the dimensions of the larva and adult zebrafish are tiny, it reduces the quantities of the dose of experimental or testing agent solutions. This cuts down the bulk of waste material destruction and also reduces requirement of quantities of glass wares, equipment, and chemicals used in laboratories for research purpose (Brennan 2014). As the embryos of zebrafish are small, more screening agents can be investigated simultaneously by using a multititer-well plate. As the maturation of zebrafish is quick, the trans-generational experimentation becomes easy.

The zebrafish remain transparent from the egg stage and fertilization to the embryo development. So, the development can be observed visually for the morphological development and other changes without any obstruction (Brundo and Salvaggio 2018). The unfavorable results of chemicals on the growth of the zebrafish organs can be easily assessed, with some magnification. The adverse effects can be quantified by measuring the size of the organs at every developmental stage. During mutagenesis, the identification of phenotypic traits is possible as the zebrafish have optical clarity. The advance techniques of immunochemistry allow the assessment of morphology (Sieber et al. 2017).

The genomic sequence of zebrafish and human has ~70% similarity. The progress of embryonic growth of zebrafish was reported in 1981. There was a significant improvement in genomics of zebrafish in the 2000s. In 2001, the series of mitochondrial genome of zebrafish was fully acquired. The DNA sequence of zebrafish consists of base pairs and protein coding, which are 1,505,581,940 and 26,247 in number, respectively. Their complete genomic sequence was published in 2013 (Das et al. 2013). Zebrafish is being employed as an experimental animal model for inheritable investigations. Zebrafish helps in many biological processes and muscular dystrophy. The researchers discovered the zebrafish as a wide range of resources and useful for studies like toxicity, DNA cloning, etc. (Mushtaq et al. 2013).

The zebrafish is a vertebrate animal and has been utilized to study many human diseases. They play cardinal part in understanding the mechanism and progression of many diseases like cancer. The application of zebrafish as an experimental animal has been expanded as being employed in preclinical studies, toxicology studies, and their applications (Brennan 2014). There is much similarity between the major organ systems like nervous, cardiovascular, and digestive systems of human and zebrafish. So, the developmental and physiological processes and the response to pharmacological agents are also similar (Amatruda et al. 2002). So, it is possible to identify and study the physiological and pharmacological responses of drugs and other bioactive compounds with therapeutic value. This makes zebrafish animal as a paragon model to analyze the in vivo characterization of a compound. The bioactive compounds can be diluted with distilled water and can be given to zebrafish. The compounds are easily absorbed by zebrafish through gills and skin and allow easy and rapid screening of many compounds per day. Majorly larvae and embryos are used as the experimental model (MacRae and Peterson 2003).

9.4.1 Benefits of Zebrafish

The benefits of the zebrafish (Fig. 9.3) can be summarized as follows:

- The zebrafish is a vertebrate, little in size, and robust animal model.
- The zebrafish are economical to maintain than other experimental animals.
- The cost of maintenance of animal housing and caring is comparatively low.
- The zebrafish breed tons of offspring at an interval of minimum of 7 days. This contributes to scientists with an adequate amount of study animals to carry out research investigation.
- Embryonic development of zebrafish is relatively fast (only 72 h).
- Its embryos are transparent and develop outside the body, so they can be easily observed.
- They provide genetic similarities with human.
- The progress of growth of internal organ systems of the zebrafish embryos can be easily examined as they are transparent.
- The early stages of life of zebrafish can be studied as the egg fertilization and development occur outside the mother's body.
- The zebrafish genetic structure has been thoroughly extracted to an excessive quality.
- The zebrafish have similar genomes to humans.
- The zebrafish have similar tissues and organ system as humans. As a vertebrate, they harbor several features almost identical with human systems.
- The zebrafish are able to repair heart muscle.

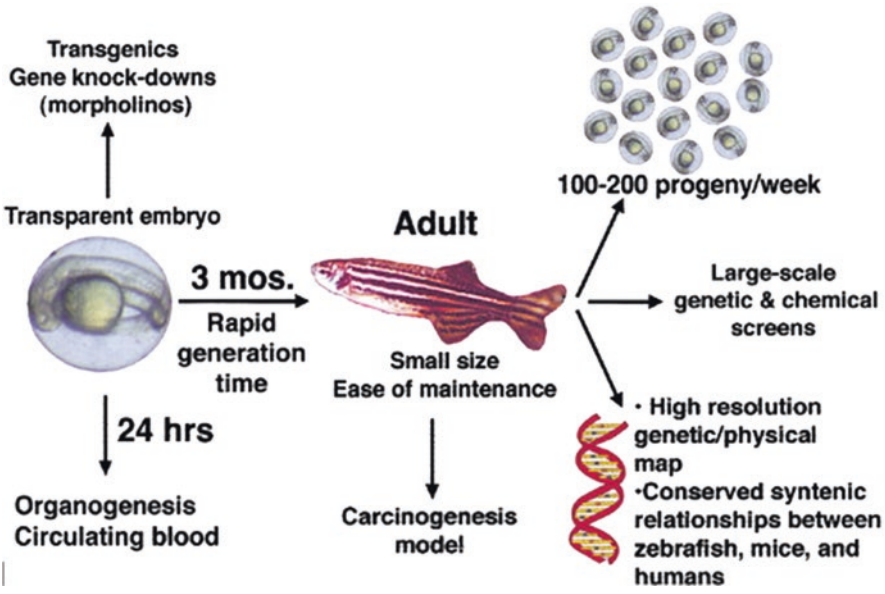

Fig. 9.3 Key benefits of zebrafish life stages (Amatruda et al. 2002)

9.5 Nanotechnology

The nano-sized materials have opened up various possibilities in a variety of industrial issues and scientific endeavors. Nowadays, nanotechnology encircles a major influence on the transformation. This is interpretive for multibillion-dollar commercial activities in industrial section (Sundararajan et al. 2016). The industrial sectors including engineering, disease diagnosis, drug delivery, biotechnology, etc. are using nanomaterials in their products. Nanotechnology is an upcoming solution for various industrial problems. It can be applied to all other science fields like chemistry, biology, physics, materials science, medicine, electronics, energy and environment, etc. (Hu et al. 2011).

Over the last few years, nanotechnology is a growing worldwide field for utilization of large variety of products. The progress in the field of medicine, engineering, and other technologies is remarkable. Nanotechnology is the science of design, development, interpretation, and implementation of materials at nanometer scale. Nanotechnology can be defined as a branch of science which deals with techniques, controlled at the level of nano-scale (Fabara et al. 2018). Nanotechnology is the study and applications of nano-scaled materials ranging from 1 to 100 nm (Fig. 9.4).

9.5.1 Nanomedicine

Nanomedicines are an effective way of drug delivery systems which include nanoparticulate carriers. The use of nanomedicines enhances the drug absorption by improving the solubility characteristics of the drug. The nanocarrier binds the drug

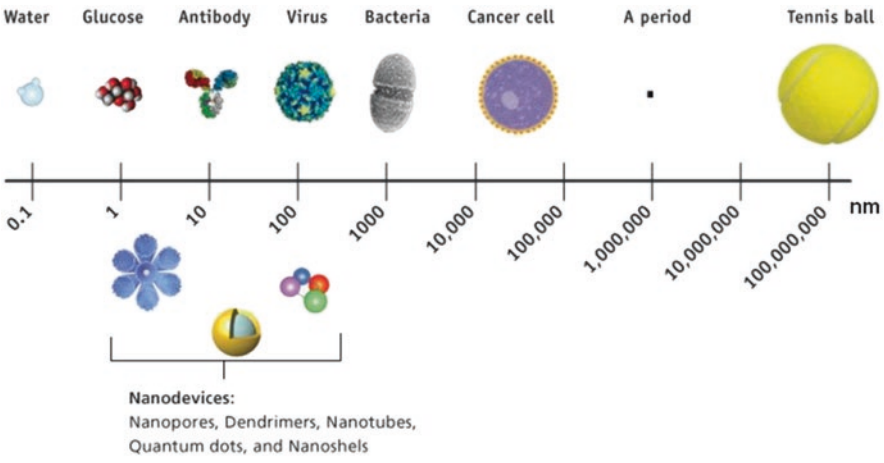

Fig. 9.4 Representation of nano-scale (Chakraborty et al. 2016a)

molecule to the specific target tissues. It minimizes the side effects (Celá et al. 2014). The physicochemical and pharmacokinetic properties of nanomedicines need to be optimized. Nanomedicines include nanoparticles, carbon nanotubes (CNT), metal nanoparticles, fullerenes, crystalline materials, nano-sized polymers, etc. (Liu et al. 2013). The nanomedicine formulations should be designed, developed, and optimized according to the properties like size, shape, chemical composition, surface charge, or modification (Harper et al. 2008). These formulations should also be tested for their half-life, absorption rate, bioavailability, cell specificity, stability, compatibility with biological environments, and toxicity study under in vivo conditions (Celá et al. 2014).

9.6 Nanoparticles

Nanotechnology deals with the design, development, and applications of nano-scaled materials. A nanoparticle is the particulate drug delivery system with nano-carriers. The size of nanoparticles ranges from 1 to 100 nm. Nanoparticles are very small-sized particles, providing large surface area for interaction of drug with the cell/tissue (Agrawal et al. 2018). The physicochemical properties of nano-scaled materials are different with the bulk compounds. On account of minute particle size and huge surface area, the nanoparticles facilitate easy absorption of drug (Bano et al. 2017). This enhances the bioavailability and reduces the requirement of dosing frequency and drug dose. They also improve the required pharmacological response and disease curing (Sundararajan et al. 2016).

The advantages of nano-particulate drug delivery are they can be used for targeting the diseased organ site. They provide enhanced therapeutic effect by enhancing drug absorption due to their nano-scaled size (Celá et al. 2014; Hu et al. 2011).

Table 9.1 Approved list of nano-pharmaceuticals (Choi and Han 2018)

Category	Marketed name	Name of drug	Indication
Liposomes	DepoCyt	Cytarabine	Brain cancer
	DaunoXome	Daunorubicin	Kaposi sarcoma by HIV
	Caelyx	Doxorubicin	Breast cancer, ovarian cancer, Kaposi sarcoma
	Abraxane	Paclitaxel	Breast cancer
Nanoparticles	Epaxal	Inactivated hepatitis A virus	Hepatitis A vaccines
	Zevalin	90Y-ibritumomab tiuxetan	Lymphoma
Nanocrystals	Emend	Aprepitant	Vomiting after surgery
	Rapamune	Sirolimus	Kidney transplantation rejection
Nanoemulsions	Norvir	Cyclosporine	HIV infection, kidney transplantation rejection
	Renagel/ Renvela	Sevelamer	Dialysis, hyperphosphatemia

Nanoparticles reduce the side effects due to drug toxicity on non-targeted organs. They provide safety and compatibility (Liu et al. 2013). Different types of nanoparticles include polymeric, inorganic, and solid lipid nanoparticles, nanocrystals, carbon nanotubes, fullerenes, liposomes, dendrimers, etc. (Mallakpour and Behranvand 2016). The physicochemical characterization and purity profile of nanoparticles can be derived by using various techniques (Epa et al. 2012). There are lots of opportunities and challenges in the development and laboratory testing of nanoparticles. Some of the nano-formulations are enumerated in Table 9.1.

The research on nanoparticle is an area of interest, because of a large variety of its implementations in biomedical, electronic, and other fields (Khan et al. 2017). These particles have applications in many fields like medical imaging, i.e., in disease diagnostic tools, nanocomposites, filters, drug delivery systems, and gene delivery. The main application of nanoparticles in cancer therapeutics is to determine physiological state of tumors and curing cancer by targeting the specific organ site (Salata 2004). Silver nanoparticles are becoming popular as the research shown its applications in numerous areas like integrated circuits, filters, sensors, biolabeling, antimicrobial toiletries fibers, cheap paper batteries, and antimicrobials (Asharani et al. 2008). Nanomaterials have an expansive range of applications in biomedical field. It includes antimicrobials, bio-detection, imaging and labeling, drug delivery, MRI agents, implants, cosmetics, and thermal spray coatings (Sundararajan et al. 2016).

9.7 Similarities of the Zebrafish to Humans

It is important to minimize the animal suffering and sacrificing during the research. The mouse is 83% similar to humans while chicken is of 64%. The zebrafish mimics the human body in 71%. All human organs are found in the body of zebrafish as they are vertebrate. While comparing the genetic characters, zebrafish have around 80% similar genes associated with human disease. Thus, zebrafish are similar to human anatomically and genetically. So, zebrafish is an ideal experimental animal and a popular choice for biomedical research (Sieber et al. 2017; Das et al. 2013).

There is ample amount of genetic information available to study the root causes of human disease. The genetic data available in public databases, and in medical records, help researchers to develop some novel treatments. But, as human genome can show only a statistical association between a particular gene and disease, this method has its limitation (Dooley 2000).

9.7.1 Human Disease Representation in Zebrafish Animal

In the latest years, the zebrafish are widely being used as recognized animal model for investigation of toxicity of nanoparticle and human diseases (Bradford et al. 2017). For toxicological studies, specific protocols should be used to use zebrafish as an animal model. For evaluation of toxicity of nanoparticles, correlation between

hatching efficiency and toxicity of embryo is a significant parameter (MacRae and Peterson 2003). Also parameters like hatching rate, developmental deformities, impairment in gills and skin, reproduction toxicity, behavioral abnormalities, mortality, etc. are used for the evaluation of toxicity of nanoparticles. Because of the unique features, zebrafish is becoming a popular animal model to study in vivo activities of nanoparticles (Chakraborty et al. 2016b).

The abnormal behavioral response of zebrafish is a sensitive indicator for toxicity study. The researchers proved that, after the chronic exposure of TiO_2 nanoparticles, the reproduction system of fish gets disturbed (Moreno-Olivas et al. 2014). To study the nanoparticle-induced toxicity, parameters like gills and skin disruption and disturbances in endocrine system are evaluated (Sizochenko et al. 2017). Some human diseases studied in zebrafish are listed in Table 9.2 as follows.

9.8 Nano-Particulate Drug Testing and Screening in Zebrafish Animal Model

The zebrafish are ideal for screening of chemical agents in their nano-particulate form as their embryos are transparent, rapidly developing, and produce externally (Sieber et al. 2017). Since the zebrafish have the permeability to small molecules, they can be used for screening and testing of drugs affecting on biological processes. Bioactive nanoparticles can be easily and successfully tested on both embryos and adult zebrafish (Bradford et al. 2017).

The zebrafish produces large quantities of eggs and embryos, and due to their small size, they can be fit in around 380-well plate. This allows testing of a number of compounds at a time, and screening can be done in a very less time frame (Sivamani et al. 2016). Automated screening techniques have been developed to minimize human error and to achieve high possible throughout. The automated techniques include embryo collection and preparation, delivery of testing compound, incubation, imaging, and analysis of results (Sieber et al. 2017). The automation allows the dissection of phenotype and evaluation of side effects. The rapid

Table 9.2 Studies of zebrafish animal models for human disease (Kari et al. 2007)

Disease	Responsible gene	Zebrafish model obtained by
Spinal muscular atrophy	Reduced levels of SMN protein	Morpholino targeting
Duchenne muscular Dystrophy	Mutation in the dystrophin Gene	Screening
Joubert syndrome	Mutations in the gene CEP290 (NPHP6)	Morpholino targeting CEP290
Severe congenital anemia	Defects in the 4.1R red blood Cell membrane protein	Screening
Erythropoietic protoporphyria	Disorder of ferrochelatase	Screening
Barth syndrome	Mutations in tafazzin	Morpholino targeting tafazzin
Nephrotic syndrome	Mutations in the PLCE1	Morpholino targeting PLCE1

development of zebrafish embryos is beneficial for drug testing and screening. So, in a short period of time, assays can be performed.

Some anticancer drug gives desired pharmacological response by interacting with a single specific target. The delivery design and development of such drugs are challenging. In zebrafish drug screening assays are reliable tool for pre-clinical safety evaluation and used routinely to evaluate toxicity (Santoro 2014).

The zebrafish provides advantages in structure-activity relationship and high-content screening. In the process of drug discovery and testing, the chemical compound selected for the screening can be modified for improvement in desired pharmacological response with minimum side effects (Mushtaq et al. 2013). The structural derivatives can be prepared and tested directly on zebrafish as animal model. By chemical modification in drug compound, improvements can be done and identified with in vivo testing. The zebrafish is becoming a prominent model organism for processes of drug invention that includes target identification, disease modeling, biological activities, and toxicology (Santoro 2014). The reference genes of zebrafish embryos used in toxicity studies are listed in Table 9.3 as follows.

9.8.1 Assessing Teratogenicity and Other Developmental Effects in Zebrafish Model

The eye development is disrupted, and pigment deposition was noticed with a simple microscope when the zebrafish embryos are exposed to gold nanoparticles, The visualization effects can be seen on pigmented cells, including RBCs, hatching, and mortality. The dose-dependent and time-dependent screening of silica nanoparticles toxicity was used in assessing the mortality rates and impacts on cardiovascular system (Chakraborty et al. 2016b).

Table 9.3 Reference genes of zebrafish embryos used in toxicity studies (Liu et al. 2018)

Gene symbol	Gene name	Accession	Function
18S rRNA	18S ribosomal	RNA generic	18S ribosomal RNA
eef1a1l1	Eukaryotic translation elongation factor 1 alpha 1, like 1	NM_131263.1	Factor for protein translation
actβ2	Actin, beta 2	NM_181601.4	Cytoskeletal structural protein
polr2d	Polymerase (RNA) II (DNA directed) polypeptide D	NM_001002317.2	Enzyme for transcription
Sdha	Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	NM_200910	Enzyme in tricarboxylic acid cycle
β2m	Beta-2-microglobulin	NM_131163	Beta chain of a major histocompatibility complex I molecular

Researchers studied the fetal alcohol syndrome on zebrafish. The embryos of zebrafish were exposed to ethanol at different concentrations and different time durations. They resulted in defects in the development. These developmental defects can be directly compared with the defects in human birth (Loucks and Ahlgren 2012). Scientists also studied teratogenic effects of nitrate and nitrite by using zebrafish as animal model. High levels of nitrates result in congenital defects or miscarriages in humans. As the embryo of zebrafish got exposed to higher level of concentration of nitrite, various birth defects were observed, while that of nitrate, no defects were observed. Even so, the zebrafish act as a beneficial model to quickly determine birth and developmental defects caused by exposure to teratogens (Keshari et al. 2016).

9.8.2 Genetic Analysis in Zebrafish Model

The genetic analysis consists of gene mutations and chromosomal alteration study. Because of chemical exposure, DNA is susceptible to damage. This can be studied in the different stages of life of zebrafish like embryo, larvae, and adult. The RAPD-PCR methodology is used to determine effect of nanoparticles on genetic analysis including genotoxicity of TiO_2 nanoparticles (Moreno-Olivas et al. 2014).

More sophisticated protocols have been used to study the transgenesis to enhance the zebrafish genomic resources. This established zebrafish as a human disease model (Carpio and Estrada 2006). The recently developed targeted genome editing techniques like CRISPR/Cas9, ZFN, and TALEN have been used in zebrafish model to reproduce pathological conditions similar to human and to investigate in vivo effect. The goal of genomic study on zebrafish is to determine new targets for drug therapy (Rissone and Burgess 2018).

9.8.3 Immune System Testing in Zebrafish Model

The nanoparticles are very sensitive toward the immune system. The nanoparticles with an inflammatory response are also associated with the activation of neutrophils and macrophages. Gold nanoparticles disrupt the pathways involved in immune response. While testing on zebrafish model, silver nanoparticles caused immunotoxicity (Chakraborty et al. 2016b).

During the embryogenesis of zebrafish, the specification of both B and T cells appears. Newly developed methods like ES cell gene inactivation allow the generation of specific mutants. Thus, the zebrafish became more resourceful as an experimental animal for immunological research (Yoder et al. 2003).

9.8.4 Reproduction Analysis in Zebrafish Model

The reproduction rate of zebrafish is high. The nanoparticles with activity affecting the reproduction system can be analyzed using zebrafish. The effect of such agents on egg production, fertilization, and embryo development can be tested. Instead of the tiny size of zebrafish, there are more similarities in reproduction system and its functions to mammals. This promotes their use as a model in infertility research (Hoo et al. 2016). The exposure of zebrafish to TiO_2 nanoparticles gives reduced production of eggs and increased mortality of embryo. While being exposed to silver nanoparticles, the maturation of zebrafish embryo enhances because of the elevation in levels of oxidative stress and apoptosis of follicle cell (Gondwal and Joshi 2018).

9.8.5 Nervous System Analysis in Zebrafish Model

The effect of nanoparticle activity on nervous system of zebrafish can be determined by evaluating some sensitive parameters such as spatial recognition, color preference, locomotion, etc. By observing these parameters, the changes in complex behavior of zebrafish can be observed. The development of the brain in zebrafish is at risk of oxidative stress due to excessive content of fats and proteins and low levels of antioxidants in cells. The oxidative stress occurs as the nanoparticles activate free radicals deposited on their surface. Commonly, neurotoxicity is observed in nanoparticles which lead to the degeneration of the nervous system (Win-Shwe and Fujimaki 2011).

The neurodegenerative diseases including Alzheimer's diseases, Parkinson's disease, Huntington's diseases, etc. have been studied on zebrafish as experimental model. Researchers evaluated the effect of extract of *Alpinia oxyphylla* fruit in ethanol on PC-12 cell line and zebrafish as an experimental animal model (Saleem and Kannan 2018). The extract has neuroprotective effect and been used in Chinese therapeutics traditionally. The outcome of the screening demonstrated that this extract blocked and repaired neuro-degeneration induced by 6-hydroxydopamine. It also reduced the locomotion activity in Parkinson's disease in zebrafish model study (Vaz et al. 2018).

9.8.6 Behavioral Analysis in Zebrafish Model

The specific nanoparticles can alter the behavioral system of the zebrafish. The locomotor activity of zebrafish can be seen altered with the use of quantum dots of cadmium telluride (CdTe). The color preferences get altered because of the use of silicon dioxide (SiO_2) nanoparticles. The exposure of zebrafish to the titanium dioxide (TiO_2) nanoparticles enhances the neuron apoptosis and proliferation of glial cell. Also gene expression alteration can be seen in zebrafish model (Moreno-Olivas et al. 2014).

9.8.7 Anticonvulsant Activity in Zebrafish Model

The extract of a longa is used for the treatment of epilepsy (Krausz et al. 2015). The anticonvulsant effect of this extract can be studied on the zebrafish animal model. For the study, seizures were induced in the animal with the chemical pentylenetetrazol. The insertion of this extract exhibited antiepileptic effect (Alafiatayo et al. 2019; Nieoczym et al. 2019). Similarly, antiepileptic drug was studied by exposing the zebrafish to an increasing concentration of the drug. This resulted in the increase in seizure as increase in concentration of pentylenetetrazol-induced seizures (Gupta et al. 2014). Scientists studied the effect of an anticonvulsant drug, i.e., pterostilbene (PTE), in the larvae of zebrafish. This showed that there were no any noteworthy changes in neuromuscular power and movement (Nieoczym et al. 2019).

9.8.8 Melanogenic Activity in Zebrafish Model

Easy examination of phenotypic pigmentation process is possible in the larvae of zebrafish. So, it is considered ideal for the melanogenic-activity-related studies. The study of hanginine A activity suggested that it promotes the anti-melanogenic activity. The treatment of zebrafish with arctigenin showed that there is a decrease in pigment deposition in zebrafish after 15 days of fertilization. In another study, the extract of *Eurya emarginata* produces compound rengiolona which has anti-melanogenic activity. It was observed that there is an inhibition of pigment deposition on the body of zebrafish after the depletion of amount of melanin (Santos et al. 2016).

9.8.9 Anti-Inflammatory Activity Evaluation Using Zebrafish Model

To study the anti-inflammatory activity, the zebrafish was infected with *Staphylococcus aureus*. Then the zebrafish were treated with grape extract containing dihydrofolate reductase activity. The significant decrease in the inflammatory activity was observed.

The investigation of anti-inflammatory activity of essential oil extracted from *Rosmarinus officinalis* L. (OERO) was carried out on zebrafish as an experimental model. This study on zebrafish resulted in inhibiting the inflammatory process. In another study, abdominal edema in zebrafish animal model induced by using carrageenan was treated with methylprednisolone. This resulted in significant inhibition of carrageenan-induced inflammation.

9.8.10 Antithrombotic Activity Evaluation Using Zebrafish Model

The zebrafish can be evaluated for rare genetic blood diseases (Rissone et al. 2018). Zebrafish can be treated as animal model to examine the compounds isolated from plant extracts having anti-thrombotic activity. These compounds were examined together with other known compounds, to investigate the anti-thrombotic activity on zebrafish. Among these compounds, the eriodictyol indicated to be a potent anti-thrombotic agent which hinders the formation of thrombus. Researchers carried out studies to evaluate anti-thrombotic properties of hawthorn leaves. An extract of hawthorn leaves was prepared in ethanol and tested on zebrafish as animal model. The inhibitory activity of the drug was investigated by aggregation of platelets and anti-thrombus assessments using a zebrafish model (Gao et al. 2019).

9.9 Future Prospects

The zebrafish also plays an important part in toxicological studies of the nanomedicines. The zebrafish has proven its extensive promises as an in vivo animal model for screening of nanomaterials. The zebrafish can be employed in the process of drug development at the stage of pre-clinical testing. The future studies are necessary to determine new targets of testing agents.

They are able to survive without a fully functional cardiovascular system. If the cardiac muscles get damaged, the zebrafish has ability to develop the muscles newly. So, with cardiac muscle development property of the zebrafish, it will become common animal model for evaluating drugs used in the treatment of cardiovascular diseases (Zakaria et al. 2018). A broader research is required for novel targets of testing compounds, which are responsible for the effects on the development of the cardiovascular system.

9.10 Conclusion

The zebrafish have genotypic and phenotypic similarities as humans. The zebrafish offers advantages like rapid development, simple maintenance, egg collection, and low cost of production. The zebrafish model is a powerful, well-established research platform for the testing of activities of new drug molecules. They are vertebrate animals mimicking the human body. The zebrafish offers advantages mainly as low cost of production, maintenance and utilization as an animal model, rapid development and rapid in vivo analysis. With these features, the use of zebrafish in testing of biological activities is becoming frequent and more popular.

The zebrafish has become an experimental animal for evaluation of activities of bioactive extracts and constituents with medicinal activities derived from plants, natural resources, etc. So, the study of small molecules like nanoparticles and natural products can be carried out with zebrafish model. The main attraction of increased use of zebrafish is that a variety of tests can be carried out on this animal. The assays

of bioactive compounds derived from the plant extracts can be carried out on zebrafish. Presently, research is focused on the biological activities such as testing and toxicological testing of newly developed medicines especially chemotherapeutic agents or nanoparticles used in the treatment of cancer. The zebrafish animal model is inexpensive and more systematic. It completes the study much rapidly. By using advanced techniques, the zebrafish are becoming a remarkable possible option of other vertebrate animal models for investigating toxicity studies of nanomedicines.

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