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Zebrafish Embryo: A Novel Vertebrate Model For Preclinical Studies of Bioscaffolds

Abstract

The use of zebrafish embryos instead of fully developed animals to test the biocompatibility and clinical effectiveness of new bioscaffolds is covered in the article. Zebrafish embryos are used as a transitional model between in vitro and in vivo research, reducing the need for animal testing. The study examines the many methods used to test the biocompatibility of bioscaffolds and drug release experiments using zebrafish embryos. Discussions also include the model's benefits and drawbacks as well as related legal and moral issues. In the future, it is anticipated that the zebrafish model will be used more frequently to assess the biocompatibility of bioscaffolds covered with nanoparticles. The article's main goal is to emphasise how crucial it is to do preliminary biocompatibility and preclinical research on zebrafish embryos before moving on to clinical trials in translational research.

Keywords: Zebra fish, Embryo Toxicity, Bioscaffolds and Preclinical Studies

Introduction

Zebrafish as a substitute for in vivo testing both benefits and drawbacks. Although the chorion that surrounds the embryo offers protection, nanoparticles can build up on its surface and impede the barrier, killing the embryo. Dechorionation is a technique that can be used to prevent this, but it must be done carefully to protect the embryo. The particles must also pass through the epithelium; if they do not, they can be injected at a young stage of development.

It is a tried-and-true method to test the biocompatibility of injectable bioscaffolds using zebrafish embryos as a surrogate model (Wang et al., 2017). Zebrafish and humans only share 70% of orthologous genes, and their tissue responses may be different from ours. As a result, the validity of zebrafish as a model for evaluating the effectiveness of bioscaffolds intended for human use may be constrained. Prior to moving on with animal research, the zebrafish embryo model offers the exceptional advantage of bridging in vitro and in vivo procedures. In the early stages of preclinical testing, this method is especially helpful in reducing the necessity for animal testing. The zebrafish embryo model includes drawbacks that must be taken into consideration. Zebrafish embryos have a propensity to specific chemicals and ions; hence it is important to understand these qualities in order to apply the fish embryo toxicity (FET) model efficiently. For instance, ammonia and copper have secondary effects that may prevent any response to the material of interest in zebrafish embryos, which are particularly sensitive to both substances (Marlo et al., 2014). Due to interactions between surface charges, the chorion of the zebrafish embryo can also function as a barrier that prevents the aggregation of nanoparticles. This problem can be solved by dechorionation of the embryo, but it must be done carefully to prevent harm to the embryo. In conclusion, the zebrafish embryo model provides a link between in vitro and in vivo research methods and is a useful tool for evaluating the biocompatibility of injectable bioscaffolds. The model's limitations must be taken into account, though, and these include the requirement for a thorough understanding of the physical and chemical characteristics of the substance being examined as well as the sensitivity of zebrafish embryos to specific ions and chemicals.

Due to the zebrafish's great degree of molecular, genetic, physiological, and immunological resemblance to humans, it has become widely accepted as a trustworthy substitute for mammalian models like rats and mice. The study of bioscaffolds, nanomaterials, and drug carriers in response to medicines, bioactive substances, toxicity, and biocompatibility has demonstrated a strong link. This has streamlined the process for conducting clinical trials and decreased the likelihood that later testing will be unsuccessful.

An easy and effective way to research the toxicity of numerous substances during embryonic development is using zebrafish embryos. This model is especially useful because, in contrast to other animal models with maternal compartments or placental barriers, it is simpler to administer poisons or drugs to zebrafish embryos. In order to better understand chemical toxicity throughout embryonic development, zebrafish embryo toxicity studies have gained popularity.

Bioscaffolds and animal studies

More than 200 different cell types, including the immune system, are arranged into specialised organs and systems in the human body, which is a highly complex organism. Given this complexity, no in vitro or artificial model can accurately depict the countless biological processes that take place spontaneously and can be influenced by the introduction of substances. Predicting the biological impacts of materials, such as cutting-edge drug delivery systems, is challenging due to their rising complexity. As a result, in vivo studies are still required for the creation of pharmaceutical and medical devices, and the need for them may grow as bioscaffold technology advances. The zebrafish embryo model is a promising substitute that has previously shown its usefulness for evaluating materials at the nanoscale, helping to reduce the utilisation of full-grown animals.

Zebrafish embryo dechorionation has been demonstrated to improve the accessibility of toxicants and the consistency of toxicity outcomes in FET research. with a mix of proteolytic enzymes and mechanical manipulation, the chorion layer—the embryo's outermost protective layer—is removed with this method. Compared to non-dechorionated embryos, dechorionated zebrafish embryos are more sensitive to toxicants, and this greater sensitivity enables the identification of toxic effects at lower concentrations (Hill et al., 2005). Dechorionation can also make it easier to introduce substances like nanoparticles into the growing foetus for research on their potential toxicity and effects on embryonic development. Overall, the zebrafish embryo model is a good tool for determining the toxicity of substances and materials during embryogenesis and serves as a practical substitute for full-grown animal models. This is especially true when the fish have been dechorionated.

Zebrafish embryos are very permeable, making it simple to deliver chemicals by a variety of means, including water, microinjection, or direct injection. Zebrafish embryos have a unique chance to examine the impacts of chemicals or materials at different developmental phases, from early embryogenesis to late larval stages, due to their rapid development. Moreover, the transparency of zebrafish embryos allows for easy visualization of developmental processes and monitoring of the response to toxicants or drugs. As a result, zebrafish embryos serve as a good model for assessing the toxicity and biocompatibility of substances and medications.

Zebrafish reach sexual maturity at about three months old and continue to develop throughout their lifespan, which in a laboratory setting is typically two to three years

Interactions between zebrafish embryo and test material: The term "nanotoxicity" refers to the harmful effects of substances that are biocompatible at the macro- and microscales but poisonous at the nanoscale. When exposing zebrafish embryos to a substance, interactions may take place either directly if the substance is nanoscale and can enter the organism, or indirectly by ion discharge of the substance in an aqueous environment. Therefore, it is vital to rule out nanotoxicity when evaluating biocompatibility using the FET test. According to Elsaesser and Howard (2012), necrosis is frequently caused by nanotoxicity. The FET test can be used to evaluate the supporting and bioactive properties of materials for bioscaffolds with a beneficial effect as opposed to the conventional toxicity testing. This underlines the significance of using the FET test as a tool to assess a material's compatibility in a nanoscale setting. To guarantee that nanoparticles are used safely in biomedical applications, it is critical to have a better understanding of the potential harmful effects of these materials.

Biocompatibility studies performed with the FET

In recent years, the FET model has become increasingly popular as a method for assessing the biocompatibility of bioscaffolds. These studies have demonstrated that the FET model is both sensitive and specific in distinguishing differences in toxicity between similar materials (Hu et al., 2011). Zebrafish have been utilized in a variety of applications, including the evaluation of bioactive ions, drug delivery systems, xenografts, and hydrogel scaffolds. Additionally, zebrafish provide an opportunity to test the effects of bioscaffold degradation products. One of the main advantages of the FET model, as opposed to in vitro studies, is that it allows for the observation and assessment of toxic effects on the entire organism. The next section will outline several successful studies that have utilized the zebrafish embryo model.

Zebrafish husbandry and embryo culture

Adult zebrafish were purchased from a commercial aquarium store and kept in an aquarium tank that measured 30 cm in length, 25 cm in width, and 25 cm in height. The aquarium tank contained 10 L of water with a pH of 7, and was used to test the biocompatibility of extracted ECM. Every two days, the air stone and air pump that oxygenated the water in the tank were changed. The tank was home to both male and female zebrafish, which were fed dry food once daily and given a 14-hour light cycle and a 10-hour dark cycle. Zebrafish embryo viability and growth were observed throughout a lengthy culture period of five days.

Breeding of Zebrafish

The aquarium tank was covered with a net to gather eggs while keeping adult fish from getting to the eggs, which made it easier for zebrafish to breed. The breeding net was raised to stop adult fish from consuming the eggs, and no water filtration system was utilised in this tank to avoid filtering the eggs. For breeding, a 1:2 ratio of male and female zebrafish were kept in the dark all night. The eggs were retrieved and put in a sterile petri dish with E3 embryo media after pair-wise mating naturally produced the embryos. After that, dead and damaged embryos were removed by washing the embryos.

After Extraction of bioscaffolds/ECM in rearing media

The extraction of ECM of commercial and native chickens in rearing media was carried out as described by Tomic et al. (2021) with little modifications. Briefly, the ground ECM (200µg/mL) was continuously stirred @ 37°Cand 360 rotations per min (rpm) for 48 h.

Treatment of fish embryos with extracted ECM

The live embryos (n=60) were collected for further treatment to test the biocompatibility of ECM, as described by Tomic et al. (2021).

To test the effect of 1% Triton X-100 on the zebrafish embryos a representative 24 hours post fertilization (hpf) dechorionated embryos were taken into the diluent mixture of 1% Triton X-100 in E3 rearing media and observed under the stereo zoom microscope at 30X magnification.

To test the biocompatibility of ECM, as per Tomicet al. (2021), the dechorionated 24hpf embryos were made into different groups and the viability of the embryos was determined by looking forLethal endpoints like coagulation, tail detachment, and somite formation were evaluated as well as sub-lethal developmental endpoints like eye development, spontaneous movement, heartbeat and blood circulation, pigmentation, and edoema formation during the biocompatibility studies on zebrafish embryos. Teratogenicity endpoints, such as abnormalities of the head, face, arches, jaw, and overall retardation, were also assessed..Zebrafish embryos were grouped by restricting at least 10 embryos per batch trial. The 24hpf embryos have been dechorionated using fine ultra-forceps and needles.

Control group consisted of 24hpf zebra fish embryos was reared within the petridish in rearing media without skeletal muscle extracellular matrix over a period of 5 days and it was considered as Group I (n=60). Group II consisted of 24hpf zebrafishembryos was reared within the petridish in rearing media with extracted commercial chicken ECM over a period of 5 days (n=60). Group III consisted of 24hpf zebrafishembryos was reared within the petridish in rearing media with extracted native chicken ECM over a period of 5 days (n=60). Hence, it was tested whether extracted ECM of chicken skeletal musclewas toxic or nontoxic to zebrafish embryos by observing the lethality. The survivability or lethality of the 24hpf zebrafish embryos in each group was studied over a period of 5 days and the results wereanalyzed and interpreted

Statistically, the values of total body length (mm) were 0.39 ± 0.00, 0.39 ± 0.01 and 0.35 ± 0.01for control group (E3), C- ECM extract group and N- ECM extract group, respectively. There was a statistically significant difference in the mean values of total body length in all the groups.

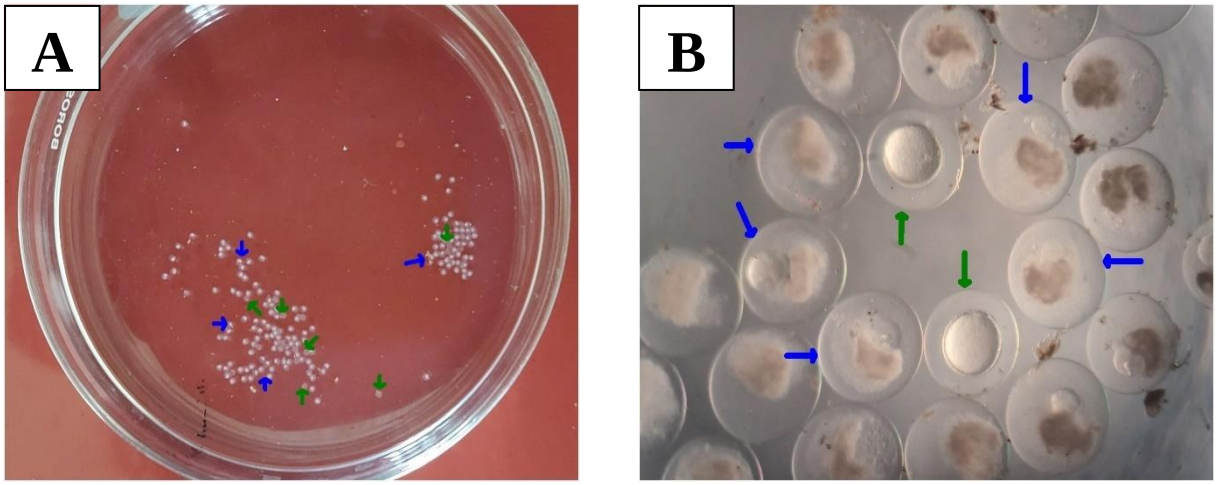

Figure 1: A-Photograph showing gross appearance of the fertilized zebrafish embryos and B- Stereozoom microphotograph image showing the live (green arrows) and dead embryos (blue arrows) (x30).

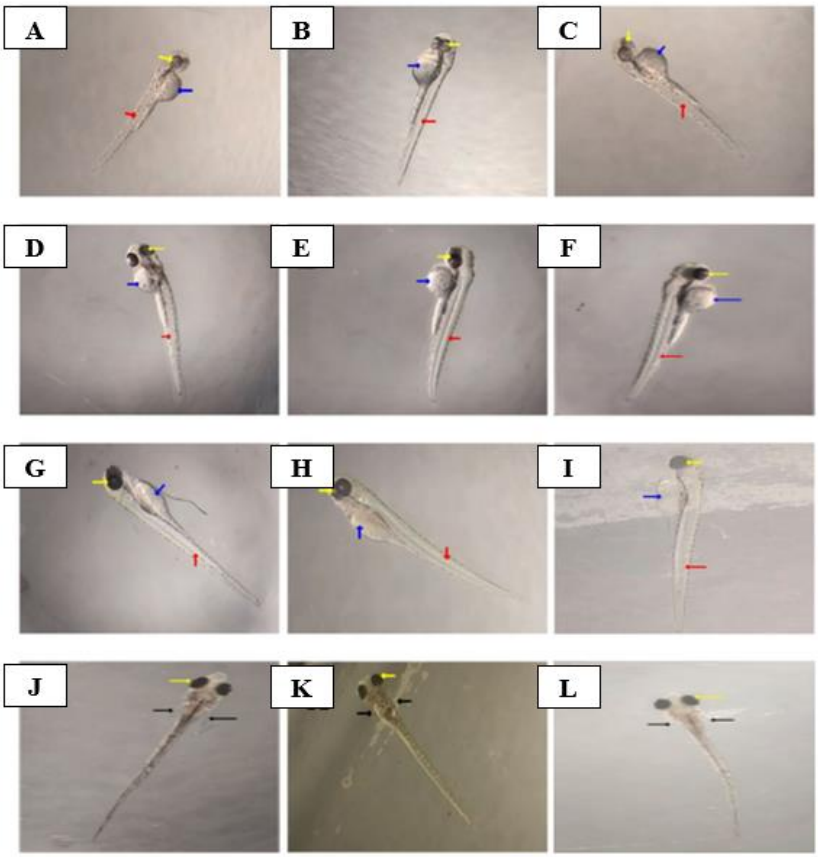

Figure 2: Stereozoom microphotographs of embryos at different post fertilization intervals

Note: Control group: A: 48hpf, D: 72hpf, G: 96hpf, J: 120hpf, Commercial ECM extract embryos group B: 48hpf B, E: 72hpf, H: 96hpf, K: 120hpf and Native ECM extract embryos group C: 48hpf, F: 72hpf, I: 96hpf, L: 120hpf. Red arrows - somites, blue arrows - yolk sac, yellow arrows - eye and black arrows - pectoral fins. (x30)

Upon exposure to the bioscaffolds extract to the zebrafish embryos over 5days as per OECD guidelines, there was no mortality in all the groups. The embryos were developed without any abnormalities like retention of yolk sac, malformation of head, face, arches or jaw nor any cardiovascular deformities. The embryos were active and freely swimming and had a good touch response. This suggested that the residue of harsh detergent in the ECM had no lethal effect on the embryos.

This also suggested that 1 % Triton X-100 was not detected as a residue in the ECM of both commercial and native chickens, implying that the total body length was closely related to the control group (Figure 1&2).

Conclusion

Zebrafish are advantageous in terms of cost, time, and infrastructure requirements, and they are also simple to handle. Thus, it is anticipated that the FET model will be applied to bioscaffold testing more and more. Zebrafish models should be employed more frequently to decrease the need for rodent research, according to a growing body of ethical, financial, and biological evidence.

The Federal Drug Administration has agreed to approve new drugs using zebrafish in toxicity and safety testing. This acceptance is anticipated to rise with the standardisation of administration methodologies, analytical techniques, and testing zebrafish strains. Zebrafish models are perfect for the development of NPs coated bioscaffolds as therapeutic agents because they have the potential for pre-clinical investigations as well. These models can contribute in the development of bioscaffolds for medical applications by helping to explore mitigation measures when a promising NP agent demonstrates toxicity.

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