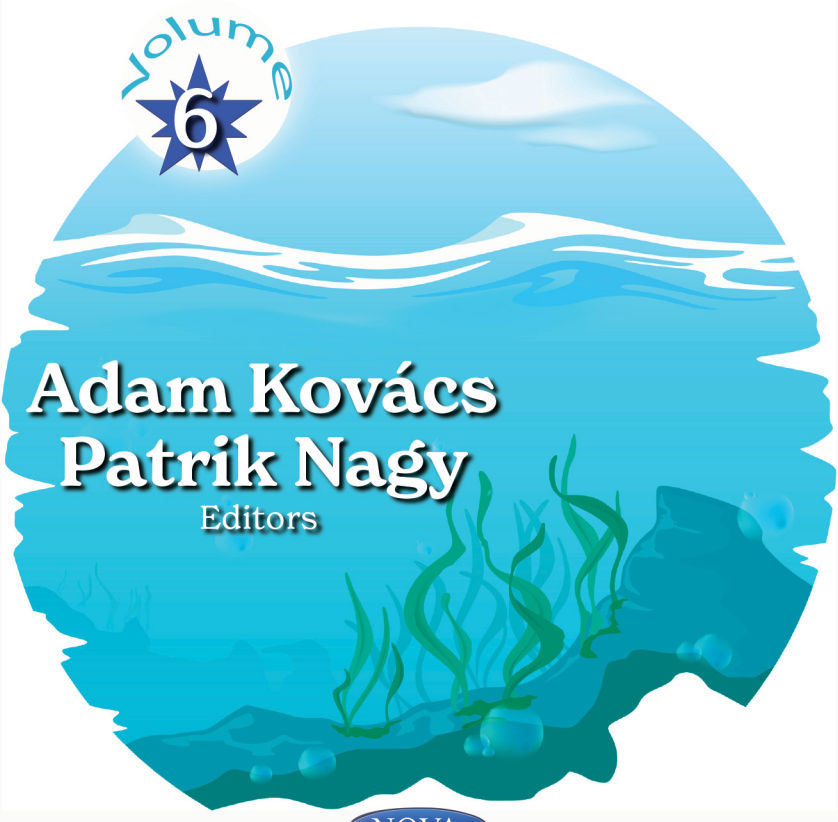
Advances in Marine Biology



Advances in Marine Biology

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and Patrik Nagy
Editors

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Contents

Preface

This volume contains six chapters that detail recent advances in marine biology. Chapter One highlights the usefulness of zebrafish for biological screening of hydrogels based on amino acid and acrylates and hydrogel scaffolds based on alginate, gelatin and 2-hydroxyethyl methacrylate. Chapter Two aims to critically review the effects of climate change on green mussels and the environmental factors that may influence mussel production. Chapter Three explores how certain oceanographical phenomenas or events affect local marine ecosystems and the cetacean communities in Indonesia. Chapter Four assesses the bycatch and discardment in benthic trawling in Northern Tunisia. Chapter Five covers the parasitic diseases that are common in rainbow trout, the ways to prevent and control these diseases, the treatment methods of the diseases, and the life cycles of the parasites. Lastly, Chapter Six reveals a comprehensive study of the nutritional content, fatty acid profile and shelf life of rainbow trout.

Chapter 1

Studies of Zebrafish and Their Behavior for (Meth)Acrylate-based Hydrogels and Hydrogel Scaffolds

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Abstract

Hydrogels and hydrogel scaffolds as polymeric biomaterials have gained great importance and potency in the medical and pharmaceutical fields. However, these types of polymeric biomaterials can cause negative effects on living species during usage. Thus, it is essential to identify hydrogels and hydrogel scaffolds with the least adverse effects on aquatic

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organisms. To determine the toxicity of these polymeric biomaterials, we investigated the effects of hydrogels based on amino acid and acrylates and hydrogel scaffolds based on alginate, gelatin and 2-hydroxyethyl methacrylate on zebrafish embryos using the fish embryo toxicity test (FET). In summary, all tested hydrogels and hydrogel scaffolds displayed the least toxic impact, thus suggesting adequate environmental biocompatibility for the generation of hydrogels and hydrogel scaffolds. This review highlights the usefulness of zebrafish for biological screening of hydrogels based on amino acid and acrylates and hydrogel scaffolds based on alginate, gelatin and 2-hydroxyethyl methacrylate.

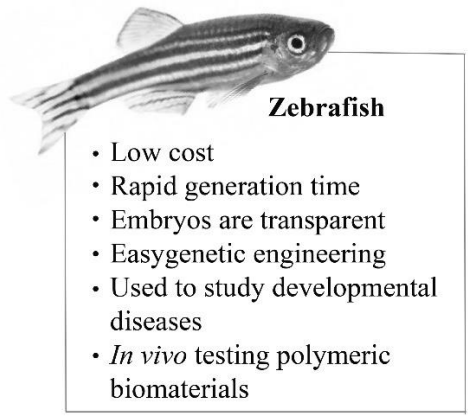
Keywords: polymers, hydrogels, scaffolds, alginate, gelatin, 2-hydroxyethyl methacrylate, apatite, *in vivo* biocompatibility, zebrafish

Introduction

Recent scientific studies have been indicated that animal models with the ability to regenerate tissue are more suitable options for regenerative medicine studies in several types of diseases, compared to models without regenerative abilities (Zang et al. 2018; Zang et al. 2015; Usai et al. 2020). Hence, the zebrafish has been introduced as a model that possesses the capacity to regenerate different organs and tissues, and fine regeneration in zebrafish has been broadly investigated in the regenerative medicine field. Additionally, the zebrafish is a suitable model for studying a variety of different situations. It has been used for developmental studies because of the special characteristics of its larva. The features of the zebrafish make it a desirable animal model, the advantages of zebrafish and recent research shows that zebrafish is a promising animal model for personalized developmental and degenerative diseases. It turns out that zebrafish can have a leading role in regeneration studies of endocrine diseases and provide a good perception of underlying mechanisms (Arjmand et al. 2020).

Animal testing has long been used in scientific researches to study complex biological phenomena that cannot be investigated using two-dimensional cell cultures in plastic dishes. With time, it appeared that more differences could exist between cell cultures and animal models and even more when translated to human patients. Innovative models became essential for developing more accurate knowledge. Tissue engineering can be used to provide some of those models, but it mostly relies on the use of prefabricated scaffolds on which cells are seeded. The self-assembly protocol has recently

produced organ-specific human-derived three-dimensional models without the need for exogenous material. This strategy will help to achieve the 3R principles.



Scheme 1. Advantages and applications of the zebrafish model in biomedical research.

Zebrafish are selected as an important vertebrate model organism for developmental biology, neurobiology and toxicology due to their small size, rapid generation time and optically transparent embryos (Xu and Zon 2010; Kabashi et al. 2011). Zebrafish embryos can develop to fully functioning juveniles by 120 h, which allows for a high-throughput screen of developmental related disease at low cost (George et al. 2011). These zebrafish-specific advantages confer an ideal model for rapid and large-scale toxicity screens (Scheme 1).

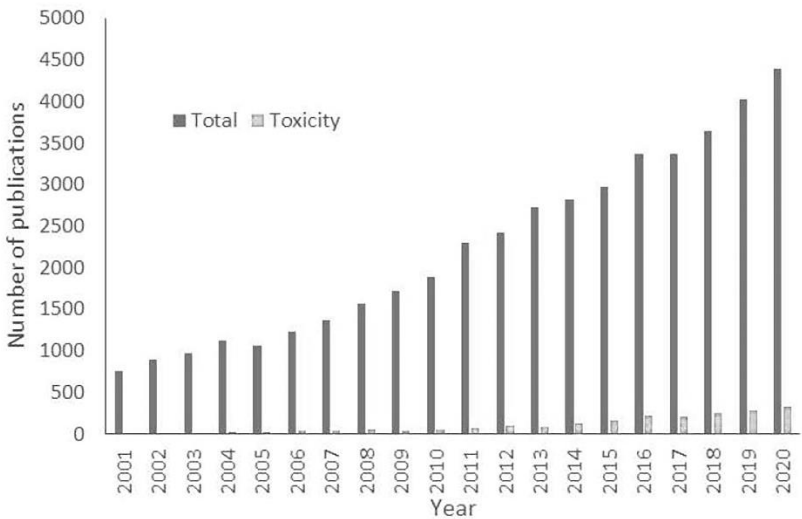
Zebrafish, a vertebrate model, presents specific properties that make it a suitable choice for investigating in different fields of biomedicine. Unlike several animal models, the zebrafish has the ability to regenerate its organs, such as fins, central nervous system (CNS), heart, pancreas, liver, and kidney. Regarding this given capacity, it can be used for different models of injury in cardiovascular, neurological, and metabolic diseases (Zang et al. 2018; Zang et al. 2015). Additionally, the zebrafish is used as an animal model in personalized medicine for studying different diseases like cancers, as avatars due to its beneficial advantages (Usai et al. 2020; Costa et al. 2020). Moreover, zebrafish were used to investigate one of the unique features in humans, the gut microbiota, to find the associations between metabolic disorders and intestinal microbiota (Faillaci et al. 2018).

Evaluation of toxicity of chemicals, compounds and/or materials is the most important step in researches, which can lead to the discovery of a new agent(s), that can be used in clinics for the treatment of a variety of diseases. *In vitro* studies with human cells and tissues can be very useful for metabolism and mode of action studies (MacGregor et al. 2001), while *in vivo* experiments on live animals can provide considerably more information including pharmacokinetics, impact on development and specific effects on different organs. Although limitations of *in vivo* studies where animal trials did not predict severe human toxicity have been recognized (Van Norman 2019), the use of animals in science has significantly benefitted humans. In this review, we will highlight the importance and advantages of the small freshwater fish, *Danio rerio*, and important studies on the toxicity of new biomaterials for this model system.

Zebrafish was first described by a Scottish physician Francis Hamilton in 1822, found in the river Ganges near the state of Bihar in north-eastern India (Hamilton 1822). The name of the species has undergone a few changes to its current Latin name *Danio rerio* (some recent synonyms were *Cyprinus* and *Brachydanio*). The first time zebrafish was mentioned in the context of research was in 1972 by American molecular biologist George Streisinger, who suggested zebrafish as a model for studying vertebrate development due to several important qualities. These qualities include the production of a large number of embryos (50 to 1000 per fertilisation) accessible at the earliest stages of development, the optical clarity of the developing fish, and a diploid genome allowing genetic studies (Houart 2001). In comparison to other *in vivo* model systems currently in use, the zebrafish has numerous advantages including a fully sequenced genome, high fecundity, short generation time, rapid embryonic development (24 h), and external fertilization (Teame et al. 2019). Unlike other animal models, zebrafish embryos and the fish in the early stages of development are fully translucent, and all organs can easily be visualized using a simple optical microscope. Blood circulation and vessels are also fully visible and approachable for laboratory manipulation. Furthermore, the maintenance of these animals in the laboratory is affordable, doesn't take a lot of laboratory space and growing of a reproductively capable male/female is less than 3 months under appropriate conditions (Teame et al. 2019). There are many different strains of zebrafish available for research, and some of the commonly used in biomedical research are AB, Casper, Ekkwill, Nadia, Wild Indian Karyotype, wild-caught, and Tubingen. Zebrafish strains are available for research purposes through the Zebrafish information link, a

database of genetic and genomic data (https://zfin.org/action/feature/wildtype-list).

Zebrafish is an extensively studied vertebrate model organism, judged by the steadily increasing number of scientific publications over the last 20 years (Figure 1). New techniques have unquestionably contributed to the rising popularity of this model, with some of the latest research on zebrafish, based transcriptomics (Silva et al. 2021), mRNA tracking (Holler et al. 2021), CRISPR-Cas9 (Zhang et al. 2018) and advanced microscopy (Høgset et al. 2020). Results from zebrafish studies have implications on numerous human diseases, including neural disorders, cancer infectious diseases, cardiovascular diseases, kidney diseases, diabetes (Zang, Shimada and Nishimura 2018), blindness (Leyk et al. 2017), deafness (Cairns et al. 2021), hematopoiesis (Chalin et al. 2021), and muscle disorders (Kürekçi et al. 2021). Human diseases studies on zebrafish are considered approximately accurate giving the fact that by direct comparison of the zebrafish and human protein-coding genes reveals that 71.4% of human genes have at least one zebrafish orthologue (Howe et al. 2013).



Source PubMed database.

Figure 1. The number of scientific publications with zebrafish as a model system in general and zebrafish toxicity.

Although only a fraction (up to 7%) of the total number of published papers regarding zebrafish, publications describing toxicity studies using this

model are also increasing (Figure 1). The zebrafish developmental toxicity assay has recently become a mainstay of high-throughput analyses. It should also be noted that zebrafish are also being utilized in high-throughput screening for drug discovery (Wiley, Redfield and Zon 2017), both for the discovery of new compounds and the identification of new, unrecognized targets (MacRae and Peterson 2015; Patton, Zonand Langenau 2021).

In developmental toxicity zebrafish assays (Buschmann 2013), generally multiple zebrafish embryos (20 to 50) are exposed to a range of chemicals and/or range of concentrations for five days, until the full organ development. Embryos at the 6 hours post-fertilization (hpf) stage are suitable for treatment (Kimmel et al. 1995). Toxicity is usually monitored by evaluating lethality, teratogenicity, or other phenotypic changes (Table 1), according to Organisation for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals Test 236 (OECD 2013). At the end of the exposure period, acute toxicity is determined based on a positive outcome in any of the observations recorded, and the LC50 is calculated (Buschmann 2013).

These toxicity assays became also a standard component of the United States Environmental Protection Agency's (US EPA) Toxicology Testing in the 21st Century (Tox21), both ToxCastTM Phase I (Padilla et al. 2011) and Phase II (Truong et al. 2014; Volz et al. 2015). It was shown that the results of the zebrafish ToxCast TM screens show good correlations to mammalian toxicity assays. Zebrafish LC50 values for acute toxicity correlating with rat LC50 inhalation values, and zebrafish Lowest Observed Adverse Developmental Effect Dose (LOADED) values correlating to the results of rabbit dermal and rat oral exposures (Ducharme et al. 2015).

Alginate/collagen hydrogels containing therapeutic, pro-angiogenic *fibroblast growth factor 2* (FGF-2), and formulated as microspheres, is a promising and clinically relevant vehicle for therapeutic angiogenesis. By titrating the amount of readily dissolvable and degradable collagen with more slowly degradable alginate in the hydrogel mixture, the degradation rates of the biomaterial controlled release kinetics of embedded proangiogenic FGF-2 can be adjusted. Furthermore, elaboration of a microsphere synthesis protocol allowing accurate control over sphere size, also a critical determinant of degradation/release rate. As expected, alginate/collagen microspheres were completely biocompatible and did not cause any adverse reactions when injected in mice. Importantly, the amount of pro-angiogenic FGF-2 released from such microspheres led to robust induction of angiogenesis in zebrafish embryos similar to that achieved by injecting FGF-2-releasing cells. These

findings highlight the use of microspheres constructed from alginate/collagen hydrogels as a promising and clinically relevant delivery system for pro-angiogenic therapy (Ali et al. 2018; Lawson and Weinstein 2002; Lee et al. 2009; Rouhi et al. 2010).

Table 1. Lethal and teratogenic effects observed in zebrafish embryos at different hours post-fertilization (hpf)

Category	Developmental endpoints	Exposure time (hpf)			
		24 h	48 h	72 h	96 h
Lethal effect	Egg coagulation[a]	●	●	●	●
	Lack of heart-beat		●	●	●
Teratogenic effect	Malformation of head	●	●	●	●
	Malformation of eyes[b]	●	●	●	●
	Malformation of sacculi/otoliths[c]	●	●	●	●
	Malformation of chorda	●	●	●	●
	Malformation of tail[d]	●	●	●	●
	Scoliosis	●	●	●	●
	Yolk edema	●	●	●	●
	Yolk deformation	●	●	●	●
	Growth retardation		●	●	●
	Hatching			●	●
	Growth retardation[e]	●	●	●	●

[a] No clear organs structure is recognized

[b] Malformation of eyes was recorded for the retardation in eye development and abnormality in shape and size.

[c] Presence of no, one or more than two otoliths per sacculus, as well as reduction and enlargement of otoliths and/or sacculi (otic vesicles).

[d] Tail malformation was recorded when the tail was bent, twisted or shorter than to control embryos as assessed by optical comparation.

[e] Growth retardation was recorded by comparison with the control group of embryos in regards to development or size (before hatching, at 24 hpf and 48 hpf) or body length (after hatching, at and onwards 72 hpf).

Using the brain injury model of adult zebrafish, it was demonstrated that the damaged mobile function of injured zebrafish was better rescued by neural stem cells (NSCs) embedded in the chitosan-cellulose nanofiber (CS–CNF2) hydrogel that had better self-healing properties. We speculated that the CS–CNF2 hydrogel injected in the injured zebrafish may heal more quickly to occupy a proper space for tissue remodeling. Hydrogel injection was reported to facilitate the remodeling of myocardial infarcted areas and subsequent

tissue repair (Lu et al. 2009; Johnson and Christman 2013). Brain injury also requires space for tissue remodeling (Cheng et al. 2005). The excellent space remodeling effect as well as the facilitated nutrient transport may contribute to the positive correlation between the self-healing property and neural regeneration by the hydrogels.

The influence of hydrophobically substituted quaternized hydroxyethyl cellulose polymers (SL) on the acute ecotoxicity of organisms living in the aquatic environment was studied (Simões et al. 2021). The ecotoxicity of four SL samples with different degrees of hydrophobic substitution (SL-5, SL-30, SL-60, SL-100 in mg L^{-1}) was investigated for seven species: *Vibrio fischeri*, *Raphidocelis subcapitata*, *Chlorella vulgaris*, *Daphnia magna*, *Brachionus calyciflorus*, *Heterocypris incongruens*, and *Danio rerio*. Hazard concentrations were derived using mean effective concentrations, realized from species-sensitive distribution curves. The influence of particle size, zeta potential and rheological properties were investigated. According to the results, the relatively low zeta potential of SL made them unstable in suspension. *Raphidocelis subcapitata*, *C. vulgaris* and *B. calyciflorus* were the most sensitive to all hydrophobically substituted samples indicating a possible imbalance of the lowest trophic levels. Accordingly, SL-5 is suggested as the eco-friendliest and is recommended to be used in the production of care products, to the detriment of the other three tested variants. The lowest level of hydrophobic substitution was found to be the least toxic variation.

A new method for harmful algae removal based on CaO_2@PEG- loaded water-soluble self-branched chitosan was investigated (Lin et al. 2021). This new method is much simpler than the complicated treatments and costly instruments in commercial use. The one-step removal of algae from water by (CP-SBC) system can be performed without additional instrumentation demonstrating better flocculation performance than commercial flocculants. This is due to self-branched chitosan flocculation properties (induced by CaO_2@PEG) because of the enhanced bridging and sweeping effect of branched chitosan. Zebrafish test and algae activity test showed remarkable biocompatibility of CP-SBC. CaO_2@PEG-loaded self-branched chitosan can be used to spontaneous float the flocs after flocculation by sustainably released O_2, as an “Air flotation system”. Besides, CP-SBC can improve water quality by minimizing dissolved oxygen consumption and reducing total phosphorus concentrations.

Polyethyleneimine (PEI) is a positively charged polymer very important as an effective *attachment factor* for weakly anchoring cell lines and primary cells. PEI coatings were studied as materials capable to increase the ability of

some cell lines and primary cultures to attach to culture plates (Vancha et al. 2004). PC-12 and HEK-293 cells plated on dishes coated with poly-ethyleneimine showed strong adhesion and homogeneous distribution. It is important to emphasize that mechanical interaction between a cell and its extracellular matrix polymers, such as polyethyleneimine, influence and control cell behavior and function. PEI can be used to increase adherence and allow axonal outgrowth from zebrafish retinal explants. Studying the effects of PEI coating on the transfection of loosely attaching cell lines it was determined that polyethyleneimine affords strong attachment of weakly anchoring cell lines and primary cells. When PEI is applied for lipofection protocols the procedure is more reliable and the yield of expressed products with PC-12 and HEK-293 cells is increased.

A method to investigate the biocompatibility of 3D printed devices by monitoring the viability and development of zebrafish embryos overextended culture was developed (Macdonald et al. 2016). The authors concluded that many of the untreated photopolymers used in commercial 3D printers are harmful to zebrafish culture. So VisiJet Crystal, classified as a USP Class VI material that was previously certified for use in animals, did not show favourable biocompatibility in zebrafish embryos test.

Pretreating photopolymer Fototec 7150 upgraded its compatibility with zebrafish culture, and it has been confirmed that pretreating photopolymer Fototec 7150 microfluidic devices can be used for biological applications (Macdonald et al. 2016). According to the results of this investigation, it is essential to test the biocompatibility of each photopolymer to enable biocompatible products in the 3D printing of microfluidic devices. The authors describe a method of treatment of the microfluidic devices which allows preserving zebrafish embryos development.

A one-step solid dispersion method was applied to prepare quercetin (Que) micelles using calculated amounts of Que and poly(ethylene glycol) monomethyl ether-ε-caprolactone copolymer (MPEG–PCL) (Wu et al. 2013). Micelles were investigated for their therapeutic effect on both cisplatin-sensitive (A2780s) and cisplatin-resistant (A2780cp) human ovarian cancer models. In the transgenic zebrafish models, stronger inhibitory effects of Que micelles were observed on embryonic angiogenesis, tumor-induced angiogenesis, tumor growth, and tumor metastasis. Furthermore, in comparison with free Que, Que micelles had improved anti-tumor and anti-metastasis activities.

The chetomin loaded micelles (Che-M) were prepared from monomethyl poly(ethylene glycol)-ε-caprolactone copolymer (PEG–PCL–PEG) and

chetomin (1:19 wt ratio) using a solid dispersion method (Wu et al. 2014). Micelles were obtained as an aqueous formulation to outbalance the hydrophobicity of chetomin. Che-M micelles can also form a thermosensitive hydrogel (Che-H) at copolymer concentrations higher than the critical gelation concentration. Che-M showed equal cytotoxicity with free chetomin, but the apoptosis-inducing effects of Che-M were more significant. In transgenic zebrafish models, Che-M dramatically inhibited embryonic angiogenesis, tumor-induced angiogenesis and tumor growth.

The zebrafish *in vivo* model was used to evaluate the effects of PEG-b-PCL nano-micelle on cardiovascular system development (Zhou et al. 2016). Although biodegradable poly(ethylene glycol) (PEG) and poly(ε-caprolactone) (PCL) (PEG-b-PCL) based nanomicelles, have been extensively studied since they are widely used as drug carriers, their impact on the cardiovascular system has not been sufficiently examined. Therefore, it is of great importance to investigate if PEG-b-PCL nano-micelles have a harmful influence on the cardiovascular system. The results of the zebrafish model investigation showed that PEG-b-PCL nano-micelle can trigger off embryo mortality just as embryonic and larval malformations depending on the dose administered. It can be concluded that this study indicates that polymeric PEG-b-PCL nano-micelle could appoint eventual hazards to cardiovascular development.

Macrophage cells are an integral factor in maintaining a stable state of the innate immune system. The potential to transform disease treatment using nanoparticles (NPs) tailored to accurately target macrophages have a great potential in chronic inflammatory diseases, cancer treatment as well as vaccine development. In the research of Crecente-Campo et al. (2019), authors try to elucidate the interaction of nanocapsules (NCs) with macrophages as a function of NCs size and shell composition, both *in vitro* and *in vivo*. Two different sizes of inulin and chitosan NCs were used to clarify the role of the size and shell composition of NCs in interaction with macrophages. Small NCs interacted more efficiently with macrophages than the larger ones, from which inulin NCs were significantly less toxic than chitosan NCs. Following *in vivo* administration (intravenous/intramuscular) to zebrafish, small NCs, regardless of their composition, spread out considerably faster and further than their medium size NCs.

To enable a scientific approach for the design and optimization of nanomedicines Tao and collaborators (Tao et al. 2020) synthesized (OPMs) micelles using triblock poly(ethylene oxide)/poly(propylene oxide) copolymer (PEO-PPO-PEO), and disulfide bond crosslinked PEO-PPO-PEO

copolymer (CPMs) to *measure* their elimination mechanisms *in vitro* and *in vivo*. Their integrity *in vivo* was visually investigated in zebrafish larvae across the entire living organism and at the cellular molecular level after intravenous microinjection. OPMs were rapidly disassociated and eliminated from blood circulation. CPMs were more stable and had a longer circulation in blood. Using the zebrafish model the authors were first to discover that disassociated polymeric micelles were eliminated through the hepatobiliary system. On the other hand, the CPM micelles were relatively difficult to eliminate.

Since its first report in the 1960s by Wichterle and Lim, hydrogels are increasingly being used as attractive and promising candidates for various biomedical applications. Thus, the assessment of the biological safety and possible biomedical application of hydrogels has become of primary importance for the suitable development of these biomaterials. Various *in vitro* and *in vivo* methods, due to their ethical and economical aspects are encouraged for the testing of new materials but despite that *in vivo* models are still needful. In the last decade, the use of zebrafish models significantly increased in different biomedical researches as well as for the faster and more economic testing of new biomaterials (Rothenbucher et al. 2019). Zebrafish have served as a model to evaluate the biological safety and biocompatibility of different synthetic and natural hydrogels for biomedical applications. Azman et al. synthesized the *Dioscorea hispida* starch-grafted polyacrylamide hydrogels by chemical polymerization method in basic solution and evaluated their beneficial use as disinfectants and biomaterials (Azman et al. 2015). The zebrafish embryo model was used to evaluate the biocompatibility of the *D. hispida* starch-grafted polyacrylamide hydrogel. Zebrafish embryo toxicity tests were performed as outlined in the Organisation for Economic Co-operation and Development (OECD) draft guidelines (Azman et al. 2015). The obtained data demonstrated that the 1:2 polyacrylamide-starch stock solution gave the highest embryo survival rate in comparison with 2:1 and 3:5 solutions; 0% of the embryos survive after 96-h exposure to the undiluted solution, while 88.89%, 77.89%, and 88.89% of embryos exposed to 1:100, 1:1000, and 1:10,000 test solutions survived after 96 h of observation and the zebrafish embryos were developing normally and displayed no signs of deformation that could be caused by the test solutions thus the hydrogel with 2:1 polyacrylamide: starch ratio shows an acceptable level of toxicity and excellent biocompatibility for biomedical application (Azman et al. 2015).

The polymeric drug carrier, which improves the uptake of the model drug valproate through the gastrointestinal tract, was synthesized by crosslinking of maleimide modified dextran (dextran maleimide), followed by reacting

dextran maleimide with poly(ethylene glycol) dithiol (Gao 2018). Finally, using β-cyclodextrins conjugated to a dextran-PEG network, cyclodextrins were conjugated to the dextran backbone to provide drug binding cavities. Toxicity assay using zebrafish embryos showed that by applying this macromolecular drug carrier the sensitivity of the zebrafish embryotoxicity assay toward the model drug valproate is significantly enhanced.

Hydrogels with self-healing behavior and the ability to withstand multiple damage-healing cycles were prepared by Balitaan et al**.** (2020)**,** using acrylamide-modified β-chitin (Am- β-Chn) derivatives cross-linked with alginate dialdehyde derivates (ADA). These hydrogels were obtained from sustainable natural resources and had excellent wound healing properties. The zebrafish model was used to examine how these hydrogels affect the healing of full-thickness cutaneous wounds. Indirect application of the hydrogels on full-thickness wounds of zebrafish led to significantly accelerated wound contraction.

An advanced hydrogel surface modification with bioactive molecules by modifying poly(ethylene glycol) (PEG)-based hydrogel (poly trimethylene carbonate)/acryloyl cyclic carbonate-PEG-poly trimethylene carbonate/ acryloyl cyclic carbonate (PTMAc-PEG-PTMAc, PTAE) copolymer) as a carrier of neural stem cells for the treatment of central nervous system disorders was achieved (Yang et al. 2017). The copolymer was modified by Arg-Gly-Asp-Cys (RGDC) and hyaluronic acid (HA). The best results were obtained with the Gel-1 (30% RGDC and HA 33%), which increased the survival and differentiation rates of NSCs and showed low toxicity to NSCs, zebrafish embryogenesis, as well as the liver and kidneys in rats with spinal cord injuries at 1, 4, and 8 weeks.

The self-healing hydrogel was prepared for central nervous system treatment in the form of the semi-interpenetrating polymeric network of chitosan, using hyaluronan (HA) as an interpenetrant (Liu et al. 2020). These self-healing hydrogels are meant to be injected locally to fill tissue defects after gelation. *The healing efficiency* of hydrogels was investigated by the zebrafish traumatic brain injury (TBI) and rat intracerebral hemorrhage (ICH) models. Both models confirmed good biocompatibility and therapeutic effect of CH hydrogel. The TBI zebrafish model also revealed a better repair function of chitosan-HA hydrogel in comparison with the chitosan hydrogel. Besides, the chitosan-HA hydrogel induced lesser extents of the inflammatory responses (edema, leukocyte infiltration, microgliosis, and astrogliosis) and glial scar formation after implantation into the rat brain compared to the chitosan hydrogel.

Based on recent literature and the importance of translating scientific results regarding novel biomedical materials into the commercial sector, the increase of works describing the toxicity of materials using the zebrafish model is expected to rise. The most commonly analysed materials include nanomaterials, biopolymers and hydrogels (Table 2).

The expansion of nanotechnology resulted in the generation of a variety of nanoparticles suitable for biomedical applications (McNamara and Tofail 2017). More recent studies implicate that inadequate usage of gold and silver nanoparticles can lead to potential risks for human health and ecosystems. Recently a review has been published with collated data regarding plasmonic nanoparticles toxicity in the zebrafish model (d'Amora et al. 2021). Overall, it was shown that gold nanoparticles induced minimal or low toxicity, characterized by small changes in the different biological parameters examined in embryos and larvae. Adults presented signs of genotoxicity and damage at the histological level in the ovary and liver. On the other hand, silver nanoparticles caused strong developmental toxicity, including mortality, malformations in organs and behavioral changes. These findings were supported by numerous previously published works, where it can be concluded the greater toxicity of silver nanoparticles vs. gold ones.

Apart from metal-based nanoparticles, numerous studies covered the toxicity of coated nanoparticles including lignin NPs (Stine et al. 2021). Stine and collaborators tested chitosan-coated lignin NPs and proved that lignin NPs did not affect zebrafish development while coated with chitosan induced severe toxicity in direct contact in higher doses >650 mg/l (Stine et al. 2021). Nanoparticles with silica coating (SiNPs) were also examined in the direct assay in serial concentrations, where it was found that in lower concentrations (up to 50 µg/mL) there was no lethal effect but with the increased dosages mortality has increased significantly (Duan et al. 2013). On the other side graphene-coated nanomaterials when tested on zebrafish, damaged zebrafish embryos by influencing the embryo hatching rate and body length in a concentration-dependent manner. Although there were no obvious effects on embryo development, graphene was adhered to a surface of chorion on some tested embryos, wrapping them and causing hypoxia and hatching delay. In further development graphene aggregated in organs (eyes, heart, yolk sac etc.) which caused apoptosis and reactive oxygen species (ROS) (Ou et al. 2016).

Zebrafish model was used for toxicity evaluation of biodegradable and biocompatible polymer polyhydroxyalkanoates (PHA) (Li et al. 2016). This extensive study among other findings confirms that PHA is a safe, non-toxic material with no significant embryonic abnormality or mortality rates