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Non-mammalian vertebrate embryos as models in nanomedicine

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Abstract

Various in vivo biological models have been proposed for studying the interactions of nano-materials in biological systems. Unfortunately, the widely used small mammalian animal models (rodents) are costly and labor intensive and generate ethical issues and antagonism from the anti-vivisectionist movement. Recently, there has been increasing interest in the scientific community in the interactions between nano-materials and non-mammalian developmental organisms, which are now being recognized as valid models for the study of human disease. This review examines and discusses the biomedical applications and the interaction of nano-materials with embryonic systems, focusing on non-mammalian vertebrate models, such as chicken, zebrafish and *Xenopus*.

Key words: Nano-materials; Zebrafish; *Xenopus*; Chicken; Embryos

By definition, nano-materials (NMs) exhibit dimensions at or below 100 nm.[1] At this scale, materials gain specific properties with respect to their bulk equivalents. Thus, NMs have a relatively larger surface area when compared to bulk material, and for this reason, they are more chemically reactive. Indeed, some materials are inert and become reactive only in their nano-scale form. In addition, the nano-scale has a marked effect on the strength and electrical properties because quantum effects dominate the behavior of the materials with respect to their optical, electrical and magnetic properties. For instance, carbon in nanotube form demonstrates magnetic properties,[2] and gold (Au) and silver (Ag)[3,4] nanoparticles (NPs) that exhibit tailored properties emit fluorescence. These striking characteristics combined with the ability to interact with biomolecules of a similar size have aroused interest in their potential for biomedical and clinical applications.[5,6] Various in vitro and in vivo biological models have been proposed for studying the interactions of nano-materials in biological systems. In vitro studies, which are based on cell culture, are rapid, efficient, and low cost. However, results from these studies provide an incomplete assessment of the interactions with the whole organism. Studies using in vivo systems carry a greater reliability, address the overall effect on the physiology and anatomy of the organism, and thus constitute a more immediately relevant platform for translational clinical studies. Although in widespread use, small animal models (rodents) are costly and labor intensive and have generated resistance in life science research from the anti-vivisectionist lobby. Such issues and concerns can be allayed using non-mammalian embryos for in vivo studies involving nano-materials. Developmental biology offers powerful models for the study of functional interactions because embryos are particularly sensitive indicators of adverse biological events. Moreover, embryos provide a useful platform to define the mechanism of action of any deleterious effects, which result from exposure to NMs.[7,8] Because highly coordinated cell-to-cell communications and molecular signaling are required for normal development, any perturbations by nano-materials will disrupt orderly embryogenesis, resulting in abnormal development that is manifested as morphological malformations, behavioral changes and may even cause embryonic death. In addition, it is known that many genes involved in developmental pathways are de-regulated in many human cancers and are thus useful subjects to assess therapeutic targets.[9-11]

This review addresses the use of embryonic systems to study the in vivo interactions with nano-materials, focusing on non-mammalian vertebrate embryo (NMVE) models that

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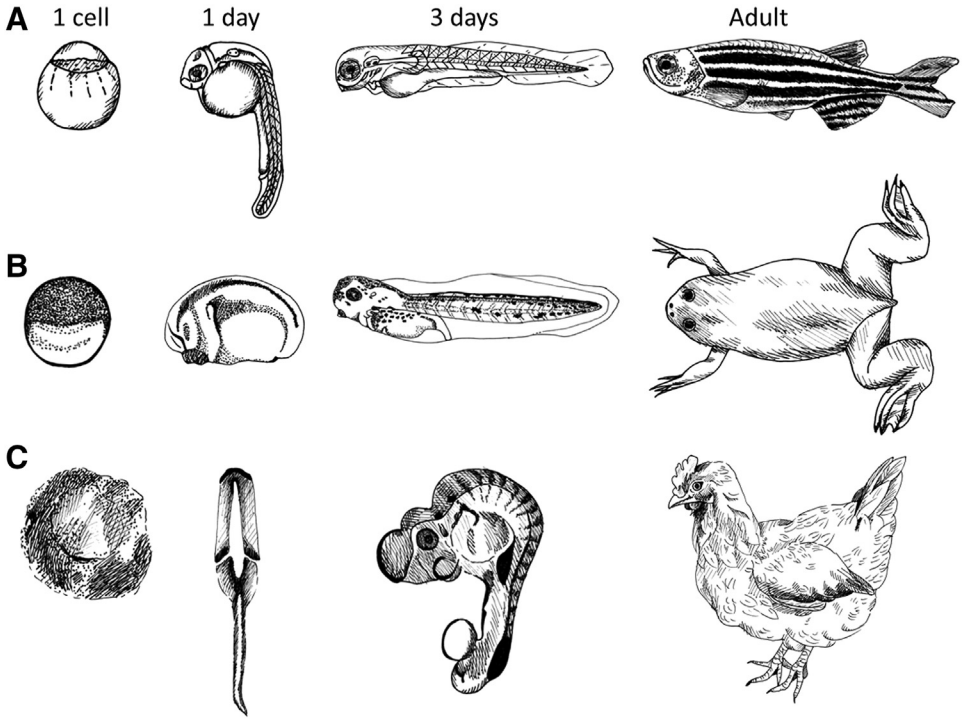

Figure 1. **(A)** Different developmental stages and adult stage of zebrafish (not to scale). One-cell embryo: 0.7 mm; female and male adults: 4-6 cm. **(B)** Different developmental stages and adult stage of *X. laevis* (not to scale). One-cell embryo: 1-1.2 mm; female adult: 13 cm; male adult: 8 cm. **(C)** Different developmental stages and adult stage of chicken (not to scale). One-cell embryo: 2-3 mm; female adult: 42-46 cm; male adult: 65-75 cm.

are most commonly used, namely the zebrafish (*Danio rerio*), frog *Xenopus laevis* and chicken (*Gallus gallus*) (Figure 1).

NMVE models

Zebrafish

The zebrafish has been increasingly used as a vertebrate model for assessing the toxicity of drugs and chemicals. Specifically, the zebrafish model has been shown to be effective in predicting adverse drug effects, with a good correlation between data derived from zebrafish analyses and data available from either human clinical trials or animal pre-clinical testing.[12] Thus, the zebrafish has become a useful model for rapid screening of the teratogenic and toxicological effects of drugs and nano-materials.[13-16]

The zebrafish produces a large number of embryos with each fecundation (hundreds), thereby providing the required statistical power for analysis, in addition to facilitating the collection of material for studies. Moreover, zebrafish exhibits a very rapid embryonic development with the first stages of development being completed within the first 24 hours post-fertilization (hpf) (pharyngula). The embryo fully develops at 120 hpf (larva) and the entire generation time is approximately three-four months, which is similar to that of rodents (10 weeks). The costs associated with zebrafish as a model system are significantly lower because zebrafish is much smaller (0.7 μm zygote to 4 mm) than mammals and requires less expensive husbandry. Compared to mammalian-based studies, zebrafish experiments require much less material to assess the nanomaterial–biological interactions and for toxicity studies. Moreover, the high fecundity and short developing time of zebrafish reduce the cost and completion times of these experiments, particularly for in vivo high-throughput screening studies. Because zebrafish embryos are transparent during the developmental stage, they enable a direct non-invasive assessment of the growth of their internal organs, morphogenetic tissue movements, cellular interaction and subcellular dynamics.[17-19]

Another favorable feature of zebrafish is that its genome is closely related to that of humans. Thus, a remarkable similarity in the molecular signaling processes, cellular structure, anatomy, and physiology has been observed among zebrafish and other high-order vertebrates, including humans.[20-22] Because the fundamental processes of vertebrate development are highly conserved across species, primary developmental mutations identified in zebrafish have close counterparts in mammals, including humans.[23] Most importantly, the zebrafish research community has developed a range of resources, including mutant strains, cDNA clone collections, and complete genome sequencing (www.zfin.org, the zebrafish model organism database). Finally, different transgenic zebrafish lines can be easily generated.[24-26] Taken together, these features render the zebrafish an effective model for the improved understanding of human diseases.[23]

X. laevis

X. laevis is one of the principal animal model systems used in developmental biology since 1950 and much of our knowledge regarding the mechanisms of early vertebrate development are