

Skin epithelium of zebrafish may work as an airway epithelia analogue model to evaluate systemic effects of micro- and nano-particles

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Fish have been used for haemostasis research for more than a decade. The look at evolutionary aspects revealed that beside parallels some details in this process are different compared to mammalian animals (1). In this issue of *Thrombosis and Haemostasis* McLeish et al. (2) evaluated zebrafish as a model for haemostasis research. Although the zebrafish currently is not the most important model for cardiovascular research with biomedical relevance, it is very popular in developmental and molecular biosciences. It is a well known fact that the zebrafish has many advantages compared to other model animals due to its tiny size, transparency, easiness to breed, short life-cycle and the possibility to apply genetics. Furthermore, the formation of the cardiovascular system occurs in a similar way in all vertebrates. It has been shown that early function of the heart and vasculature is similar in all investigated vertebrates with increases in cardiac output, stroke volume, blood pressure and a decrease in vascular resistance paralleling embryonic growth (3). Moreover, in lower vertebrate including zebrafish the molecular biological mechanisms of primary haemostasis are quite well understood (4). Therefore, it is possible to use model animals like zebrafish for cardiovascular and haemostasis research.

One has to keep in mind that a model primarily is a good model for the animal

itself or for the phylogenetic group it represents. This is also true for zebrafish, but possible differences to other animals may be of high importance for currently undiscovered or difficult-to-investigate mechanisms especially in mammalian animals. In contrast to mammals, zebrafish are able to repair an injured heart without functional impairment (5). Even mutations or treatments affecting the cardiovascular system at early stages often do not affect survival even several days post hatching and this enables the observation of processes which are impossible to observe in larger species. One reason for this is the fact that in tiny zebrafish larvae oxygen diffusion distances are small enough for the animal to be independent of blood flow for oxygen delivery (6). However, the tiny size of these animals also harbours disadvantages. Standard techniques (e.g. measurement of haematocrit or blood flow) for physiological characterisation cannot be applied in this millimetre-sized animal.

In recent years, the development of new methods and technologies (for review see Schwerte and Fritsche [7]) opened up the possibility of including embryos and larvae in research of cardiovascular physiology. The availability of transgenic animals which produce fluorescent proteins in blood vessels (8), cardiac muscle (9) and blood cells (10) in combination with use of high-speed cameras and laser scanning microscopes led to new imaging techniques for developmental physiological examination. Using these techniques, cardiovascular physiology and its underlying mechanisms can be studied in detail and even large scale screening for active compounds affecting these systems became possible.

In contrast to many other animal models, the number of physiological studies on zebrafish is lagging far behind those on topics like development or molecular biology. Physiological characterisation of the cardiovascular system is of importance for

the understanding of developmental plasticity in this popular model animal. The cardiovascular responses to environmental perturbations have been examined in detail in many species of fish and amphibians (11).

However, for embryonic or larval stages the relationships between the development of cardiovascular function and environmental, genetic or epigenetic effects are only beginning to be understood. While many pharmacological studies using the zebrafish model have been published, its use in toxicological studies is quite limited. In recent years technological usefulness of manufactured nanoparticles has been discussed in parallel to data about their possible toxicological effects. The accompanying paper in this issue of *Thrombosis and Haemostasis* by McLeish et al. (2) highlights that zebrafish can be used as a genetically tractable model to study the toxic effects of manufactured and combustion-derived nanoparticles on particle-induced haemostasis. In their study the authors used 2- to 3-day-old zebrafish larvae which have short diffusion distances (<1 mm) and non-functioning gills (2). This makes the skin the main organ for gas exchange, and the authors suggest that skin epithelia of zebrafish embryos at this stage may in fact be more analogous to airway epithelia, which is the main "entrance" for these particles in mammals. Surprisingly, it is shown in their study that even particle skin contact and not particle uptake across skin epithelium and gut mucosal barriers was required for particle-induced haemostasis. Skin damage mediated by oxidative injury caused toxic nanoparticle-induced haemostasis in the caudal artery and the capillary bed of the caudal vein. These findings are of high biomedical importance, and future studies should be focussed on how toxic particles like combustion-derived or manufactured nanoparticles mediate a systemic effect on blood flow and haemostasis even without

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penetration. Knowledge about this would enable the use of the well accepted zebrafish model for airborne and manufactured particle-induced systemic effects.

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