Organ-specific accumulation of Superparamagnetic Iron Oxide Nanoparticles (SPIONs) in the apple snail Pomacea canaliculata

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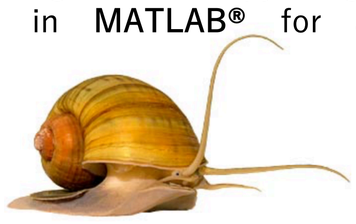
Introduction

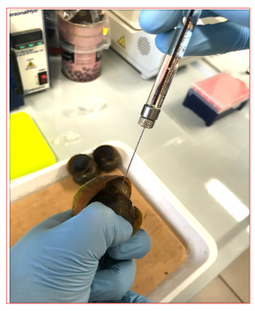
The development and testing of new nanomedicines have to be performed in the frame of European and Italian legislations, both recommending the reduction of animal experimentation, with special reference to vertebrates, like rodents.

Here we present a protocol finalized to the quantification of Superparamagnetic Iron Oxide Nanoparticles (**SPIONs**, size 14-80 nm) in target organs of a new research organism, the **freshwater snail** *Pomacea canaliculata* (Pc), which is easy to breed, manipulate and presents size and longevity similar to mice. After foot injection of two preparations of SPIONs (TCD#1 or TCD#2), organs were collected, histologically processed and stained for evidencing accumulated SPIONs in the **anterior kidney** (**AK**) and **heart** (**H**). The analysis of light microscopy images was performed through a script implemented in **MATLAB®** for accurate and automatic quantification.



Methods

Injection (SPION-free vehicle, TCD#1, TCD#2)



After **24 h** incubation

Dissection

- **Anterior kidney (AK) and heart (H)** fixation with aqueous 4% paraformaldehyde
- Paraffin embedding and serial cutting (7 µm slices)
- Staining with **Perls' prussian blue**

Image collection and analysis

AK accumulates SPIONs distributed by the circulating hemolymph

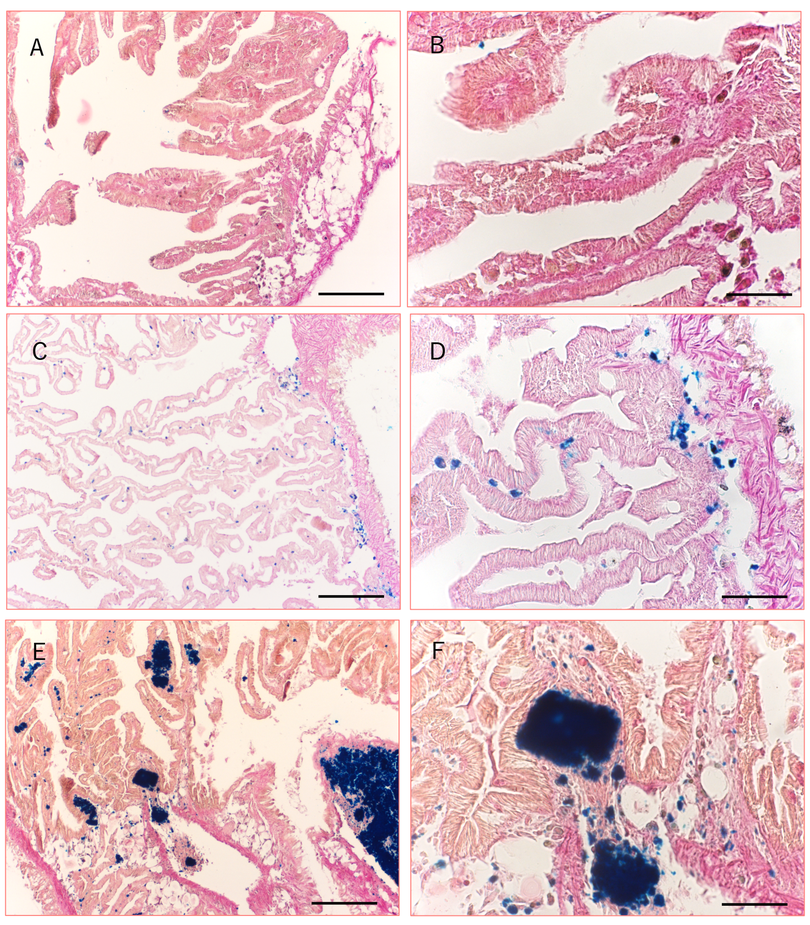


Fig. 1 AK stained with Perls' prussian blue after injection of a **SPION-free vehicle solution** (A,B); 60 mg/kg dose of **TCD#1** (C,D); 60 mg/kg dose of **TCD#2**. A, C, E: magnification 100x, bar = 200 µm. B, D, F: magnification 400x, bar = 50 µm

SPIONs carried by the circulating hemolymph crosses the H with scarce involvement of cardiac muscular cells

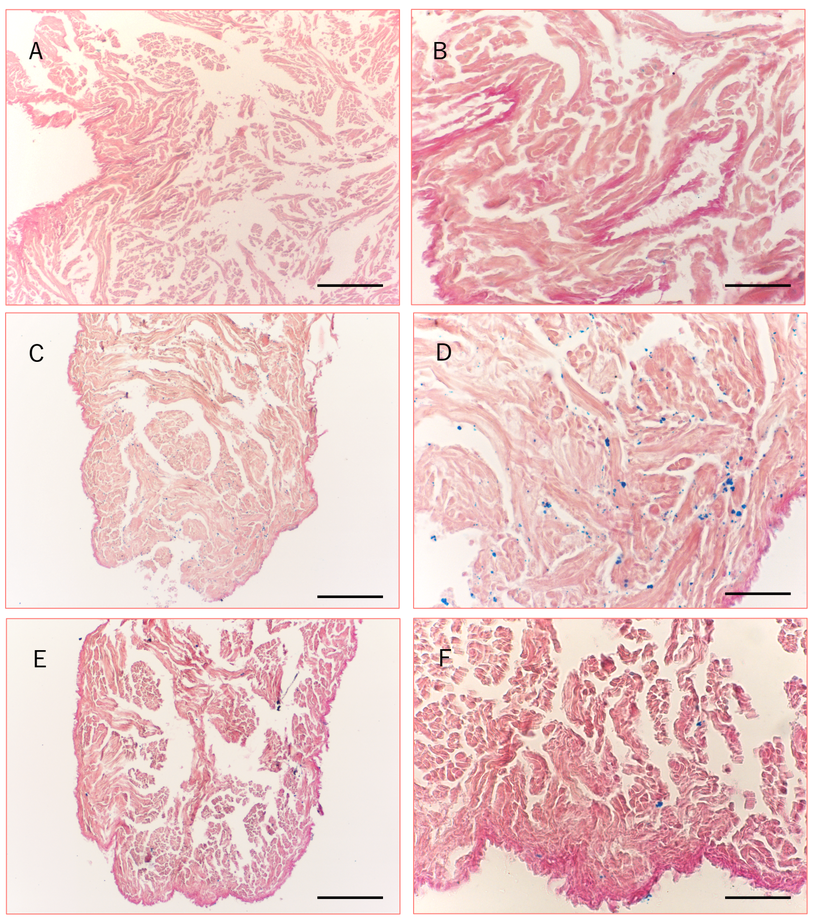


Fig. 2 H stained with Perls' prussian blue after injection of a **SPION-free vehicle solution** (A,B); 60 mg/kg dose of **TCD#1** (C,D); 60 mg/kg dose of **TCD#2**. A, C, E: magnification 100x, bar = 200 µm. B, D, F: magnification 400x, bar = 50 µm

MATLAB® analysis confirms histological data about accumulation of SPIONs in AK and not in H

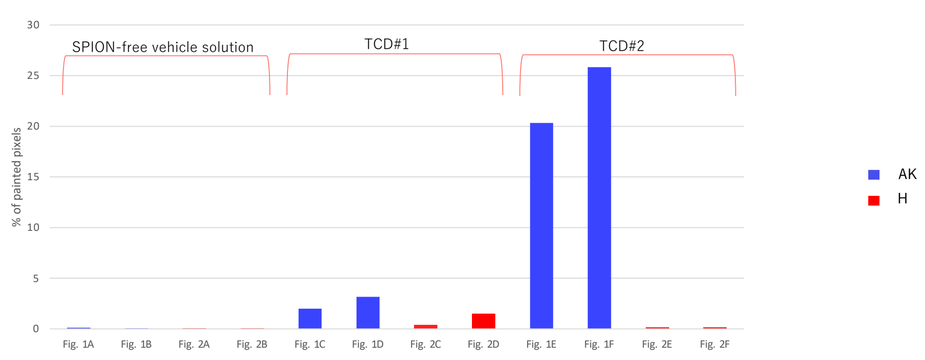


Fig. 3 Percentage of blue pixels in Figs 1 and 2 automatically detected by a script implemented in MATLAB®

Histological observations and automatic quantifications indicate that SPIONs are present in AK and H of *Pc*, 24 h after the injection. Our preliminary data suggest that, in absence of snail mortality, AK retains more SPIONs than H, in line with the biological roles of the two organs. In all, we provide the protocol and the preliminary proof of concept data for the adoption of *Pc* as a possible alternative to rodent *in vivo* models in preliminary studies on **bioaccumulation** and **bio-safety** of nanoparticle-based drugs.