

TRACKING STEM CELLS IN A INHERENTLY REGENERATIVE ENVIRONMENT

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Introduction: Regenerative potential in humans is very limited. Like other mammals we rely heavily on fibrosis and scar formation in response to injury. On the contrary, urodele amphibians (salamanders and newts) such as the axolotl (*Ambystoma mexicanum*) are champions of tissue regeneration among vertebrates mastering the ability to replace most tissues in addition to whole limbs, tail, jaw, etc. following damage or amputation. Regeneration in this species is taking place by dedifferentiation of cells to form a collection of stem cells, the regenerative blastema, that proliferate and regenerate lost tissue without scar formation. Modern regenerative medicine seeks way to adopt these capacities to regenerative therapies in humans. Though much effort is put into the development of stem cell therapies, there exists currently no satisfying technique for non-invasive follow up examinations of such therapies. The objective of this study was to non-invasively evaluate regeneration over time in a truly regenerative process, the regeneration of an axolotl limb, employing superparamagnetic iron oxide particles (SPIO) contrast agents for stem cell tracking in MRI.

Materials and Methods: Amputation of one hind limb of anaesthetised axolotls was performed to induce a regenerative response. The potential effect on cell viability of two commercially available SPIOs, Resovist (Bayer Schering Pharma, Hydrodynamic diameter 62 nm) and VSOP-C200 (Ferropharm, Hydrodynamic diameter 7 nm) and Resovist in conjugation with the transfection agent poly-L-lysine (PLL) was tested on cultures of axolotl blastema cells from 7 animals *in vitro*. PicoGreen-DNA quantification following 3 weeks of culturing was performed to quantify cell viability.

MRI-tracking of SPIO labelled blastema cells in the regenerating limb of 5 labelled axolotls was tested *in vivo* against 6 sham-operated animals for 84 days, using a clinically available 1.5 T system (Siemens), with a small radiofrequency loop-coil. Image data was processed by ImageJ and comparison of tissue signal intensity and rate of regeneration was performed using SAS JMP8.

Results: SPIO labelling with neither VSOP-C200, Resovist nor Resovist/PLL had any significant effect on blastema cell viability *in vitro*. Labelled tissue was clearly detectable *in vivo* 49 days after amputation using MRI (Fig. 1) and a significant decline in signal intensity of labelled limbs versus sham-operated limbs was evident 49 days after amputation, whereafter proliferative activity of labelled stem cells resulted in a dilution of SPIO concentration causing a signal loss (Fig. 2A). SPIO labelling displayed no significant effect on the rate of regeneration (Fig. 2B).

Discussion: SPIO labelling for MRI cell tracking has shown promising results for regenerative therapies using stem cells. This study contributes to broaden the potential of SPIOs to track regenerating tissue in an inherently regenerative model, facilitating the use of SPIOs in future pharmaceutically or genetically induced regenerative therapies. Additionally, this study concludes that SPIO labelling and MRI tracking of axolotl stem cells allow for non-invasive longitudinal studies in this model, increasing the potential to draw knowledge from the regenerative capacities of this species.

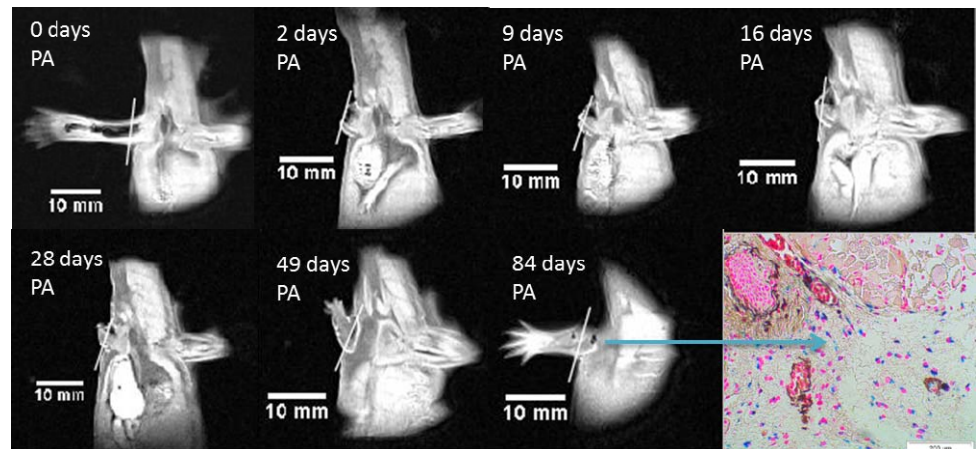


Fig. 1. The 84 day post amputational (PA) regenerative response of a SPIO labelled axolotl limb in MRI. Labelled tissue appears dark. Insert in lower right is a Prussian Blue histological verification of iron oxide particles (blue) still present 84 days PA proximal to the regenerate.

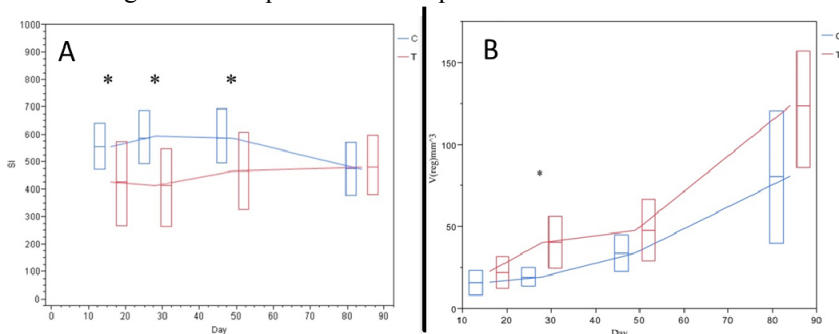


Fig. 2. A: Mean signal intensity (SI) of SPIO labelled (red) versus unlabelled sham blastemas (blue). SPIO labelling causes a significant signal intensity decrease until 49 days after amputation. **B:** Rate of regeneration in SPIO labelled (red) and unlabelled sham operated limbs (blue). SPIO labelling does not cause a significant decrease in the rate of tissue regeneration.