Assessing spinal cord regeneration and therapeutic interventions using label-free multiphoton microscopy

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Unlike mammals, the axolotl salamander exhibits the unusual capacity to regenerate its spinal cord and other parts of the central nervous system even after severe injury. Label-free multiphoton microscopy (MPM) was used to assess the regeneration after spinal cord injury and the interaction with an experimental alginate-hydrogel implant.

Following the total resection of 1 mm spinal cord tissue, the resulting gap was filled with alginate-hydrogel or left without treatment (control). Cryosections of the injured area were prepared after 0, 2, 7, 14, 28, 56 d (n=10) and analyzed with MPM. Specifically, coherent anti-stokes Raman scattering (CARS) probed lipid-rich myelin via visualization of CH_2 vibrations, while two-photon excited fluorescence (TPEF) and second harmonic generation (SHG) displayed endogenous fluorophores and collagen, respectively [1]. Immunostaining for myelin basic protein, neurofilament and ionized calcium binding adaptor molecule 1 (Iba1) served as histological reference.

MPM visualized tissue components including myelin ensheathed axons (CARS) and meninges (SHG). Upon lesioning, we found profound changes within the area of injury that were characterized by a strong increase of TPEF-signal. The Iba1 staining characterized the majority of these TPEF-positive cells as activated microglia and macrophages.

The alginate-treated group showed a significantly higher number of TPEF-positive cells in all time points except for 56 d (e.g. 7 d: $P<0.0001$, mean: 77 and 179 cells). At 7 d post-lesioning, the formation and outgrowth of axons and meninges was significantly hindered compared to the control ($P<0.0001$ for both, difference of mean: 567 µm and 658 µm respectively). Moreover, growth of cells, axons or meninges into the alginate-hydrogel was not observed at any time point. Surprisingly, the intact structure of the spinal cord was likewise restored after 56 d in both groups with no differences in myelination, inflammation or meningeal status.

The regenerative context of the axolotl spinal cord proved to be a useful in vivo model to monitor the impact of novel implants. The combination with MPM provided high resolution morphochemical readouts, potentially suited for live imaging analysis. Consecutively, we will investigate different implants for spinal cord regeneration and further adapt our system for in vivo imaging situations.

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