**MINIATURIZED IMAGING WINDOW FOR INTRAVITAL NONLINEAR MICROSCOPY**

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**INTRODUCTION:** Intravital microscopy techniques are currently performed using highly invasive window chambers. There exist no device providing a specific tracking geometry to reposition the field of view of the microscope for repeated analyses. We developed a miniaturized imaging window, the Microatlas, which can be implanted subcutaneously and allows repeated observation in vivo of foreign body reactions, for example to the implantation of a biomaterial. The device hosts a miniaturized scaffold able to guide cell migration. By applying two-photon fluorescence microscopy to the Microatlas, once implanted in vivo and repopulated by cells and blood vessels, it is possible to observe and quantify reactions in the same animal and tissue district, at different time points. Here, we grafted the Microatlas in living chicken embryos to conduct in vivo validation assays. Then, we employed white mice cadavers to pre-validate our device under thick tissues ex vivo.

**METHODS:** The Microatlas micro scaffolds were fabricated by two-photon laser polymerization on circular glass coverslips (Ø:5-12 mm), with a biocompatible photoresist, SZ2080. The micro scaffolds consist in several micro grids (500 μm x 500 μm x 100 μm). Reference structures were integrated to allow the microscope field-of-view repositioning at different time points (Fig. 1). The chicken embryo ex ovo culture was optimized and the optimal implantation time points were selected. The Microatlas was implanted and it was inspected in two-photon fluorescence and confocal microscopy. At each time point, the embryo was formalin-fixed, labelled with DRAQ5™ and imaged in confocal microscopy. The Microatlas was implanted subcutaneously in white mice cadavers at the posterior leg site and were observed with two-photon microscopy.

**RESULTS:** Confocal inspections at Microatlas implantation sites demonstrated growth of the recipient tissue inside the micro grids. Two-photon fluorescence acquisitions of label-free specimens showed the presence of a layer of collagen type I, localized mainly around or near the implanted Microatlas. Confocal microscope images allowed cell quantification of cell density (Fig.2) inside the Microatlas. The Microatlas implanted subcutaneously were detectable and observable along their whole height.

**DISCUSSION & CONCLUSION:** The Microatlas induced in vivo a quantifiable localized reaction inside its microscaffold, both in terms of cell repopulation and collagen generation as a probable foreign body reaction. Thus, our device can be used as a powerful imaging window for intravital fluorescence microscopy.

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