MINI INVASIVE IMAGING WINDOW TO GUIDE AND IMAGE FOREIGN BODY REACTIONS IN VIVO

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INTRODUCTION

The standardized assessment of the fibrotic reaction to the implantation of a biomaterial is based on ex vivo histopathological techniques that require a great number of sacrificed animals. Intravital microscopy allows instead to improve the resolution of microscopy images and to reduce the number of lab animals, but this procedure currently implies unethical and highly invasive imaging protocols. To advance the field, we developed a miniaturized imaging window, the Microatlas[1], integrating a micro scaffold able to guide tissue regeneration in vivo. The Microatlas can be implanted subcutaneously in a living organism to allow tissue regeneration and time-lapse imaging at the interface with an implanted biomaterial. By using label-free two-photon microscopy within the Microatlas implanted in living chicken embryos and repopulated by the host cells and blood vessels, here we quantified the foreign-body reaction in the same tissue district, at subsequent time points during implantation (Figure1, a).

METHODS

We fabricated the scaffolds (of dimension 500x500x100μm^3) on glass coverslips (Ø:12 mm) by two-photon polymerization of a polymer called SZ2080 (Figure1, a). We performed the chorioallantoic membrane (CAM) assay in living chick embryos at 3, 4 and 7 days after implantation. The imaging window was inspected in vivo by two-photon label-free and confocal microscopy. Collagen I was visualized in second harmonic generation (SHG). Embryos were sacrificed to perform ex vivo inspections. The CAM was labelled with DRAQ5 and imaged by confocal microscopy or stained by hematoxylin&eosin and paraffin-sectioned.

RESULTS

In chick embryos, confocal and two-photon inspections at implantation sites demonstrated growth of tissue inside the scaffolds (Figure1, b) and neo vascularization (Figure1, c) with presence of a capillary density six times greater than in unimplanted control tissue. SHG showed the presence of a preferentially oriented layer of collagen-I, with a density comparable with control regions. Confocal microscopy allowed for the quantification of cell density, that showed an infiltration rate two-fold greater than in untreated tissue. We identified and counted infiltrated cell populations, including granulocytes, fibroblasts and endothelial cells.

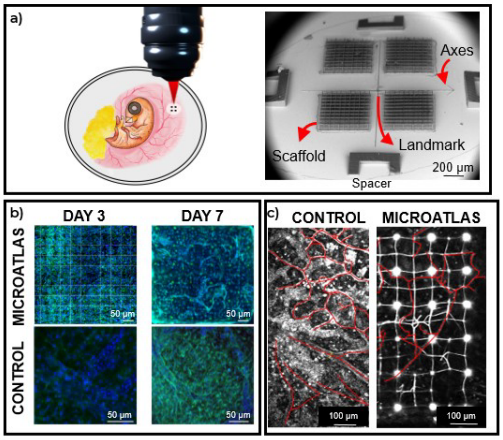

Figure 1: Panel(a) left, represents a sketch of the intravital acquisition setup. Panel(a) right, reports a low magnification SEM micrograph of the whole device with indication of the scaffolds, the spacers, and the frame of references. Panels(b) represents details of a Microatlas, imaged by confocal microscopy at DAY 3,7 after implant. Panel(c) shows segmented blood vessels within a control region and in the Microatlas at DAY 7 imaged by two-photon fluorescence microscopy.

DISCUSSION&CONCLUSION

The Microatlas was able to guide the host foreign body reaction to the micro scaffolds, in terms of cell repopulation, collagen generation and capillary formation. This miniaturized device has the potential to be used as a reliable and ethical imaging window for intravital quantifications, potentially replacing the current highly invasive window chambers. Also, with the Microatlas we can reduce the number of animals employed in preclinical studies, refine, boost the analyses, and replace expensive and lengthy analyses of cellular density, blood vessels sprouting, collagen and fatty infiltrates based on traditional histopathology.

References

1. Conci et al. Adv. Opt Mat. 2022

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