Quantification of the foreign body reaction by means of a miniaturized imaging window for intravital nonlinear microscopy

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Abstract

Brand new biomaterials, intended to be used on humans, must undergo *in vivo* quantification standardized, expensive and unethical procedures mainly based on histopathological analysis, from dissections, as defined by the ISO 10993 normative set. The aim is to prove the biomaterials biocompatibility. There exist no methods based on intravital microscopy able to satisfy the normative quantification requirements both reducing the number of employed animals and related costs. We developed a miniaturized imaging window, the Microatlas, which allows subcutaneous repeated observations *in vivo* of the foreign body reactions, for example to the implantation of a biomaterial. Confocal and two-photon microscopy inspections at Microatlas implantation sites demonstrated growth of the recipient tissue inside the microgrids both with micro vascularization formation and collagen generation. In conclusion, the Microatlas guided *in vivo* a quantifiable localized reaction inside its microscaffold, both in terms of cell repopulation, collagen and capillary formation as a probable foreign body reaction.

Introduction

To gain the authorization of being used on humans, brand new biomaterials must undergo *in vivo* quantification standardized, expensive and unethical procedures (defined by the ISO 10993) to prove their biocompatibility. These ones are mainly based on histopathological analyses in large number of mammalians, subcutaneously implanted, through different timepoints. Currently there exist no methods based on intravital microscopy able to satisfy the normative quantification requirements, with the aim to reduce the number of employed animals and related costs. We developed a miniaturized imaging window, the Microatlas, which can be implanted subcutaneously and allows repeated observation *in vivo* of the foreign body reactions, for example to the implantation of a biomaterial. The device hosts a miniaturized scaffold able to guide cell migration close to the desired target. 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 the foreign body reactions in the same animal and tissue district, at different time points. Thus, we can reduce the number of employed animals in subcutaneous validation protocols, refine and boost the conducted validation analyses and replace old and outdated quantification processes in term of cellular density, blood vessels sprouting, collagen and fatty infiltrate generation. Here, we grafted the Microatlas in living chicken embryos to conduct *in vivo* validation assays.

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Materials and 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 (Figure 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 by two-photon fluorescence and confocal microscopy. At each time point, the embryo was two-photon imaged first, then formalin-fixed, labelled with DRAQ5™ and imaged in confocal microscopy.

Results

Confocal and two-photon inspections at Microatlas implantation sites demonstrated growth of the recipient tissue inside the micro grids both with micro vascularization formation. Two-photon fluorescence acqui

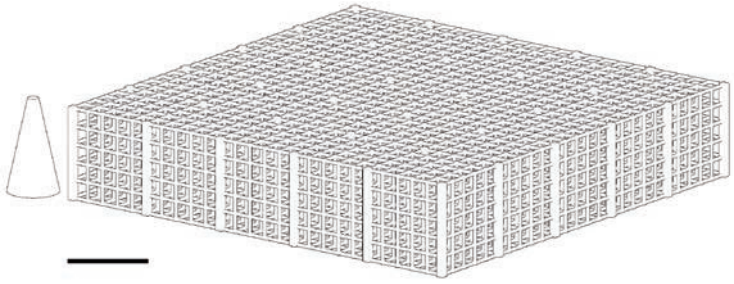

Figure 1. Representation of a Microatlas micro scaffold. Scale bar 100 μm.

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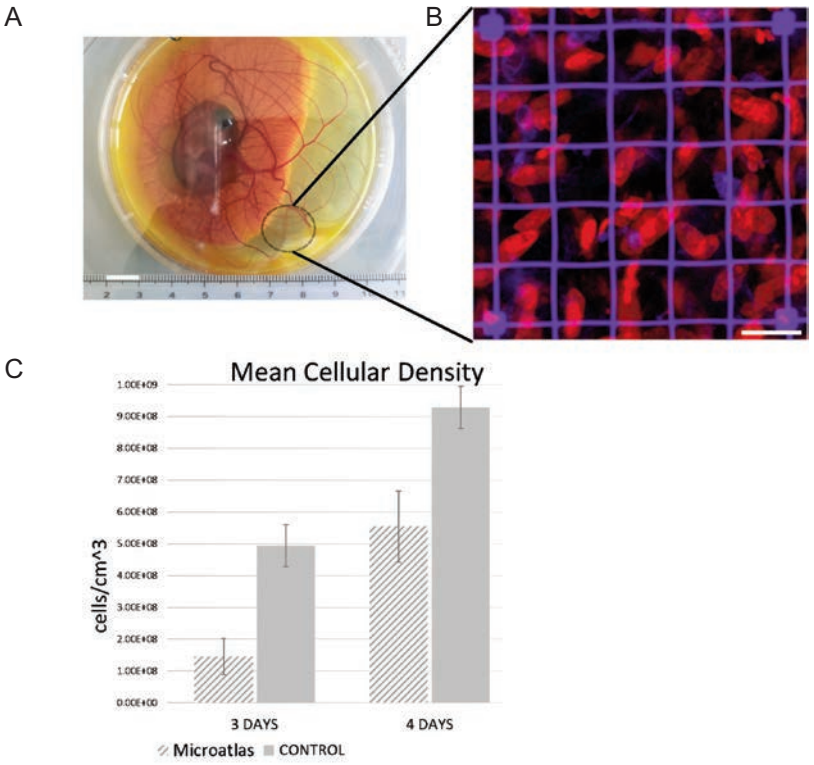

Figure 2. A) Chick embryo at incubation day 11, scalebar 1 cm. B) cells imaged inside the Microatlas, scalebar 20 µm. C) cellular density trend inside the Microatlas microgrids.

sitions of label-free specimens specifically showed the presence of a layer of collagen type I, localized mainly around and inside the implanted Microatlas. Microscope images allowed quantification of cell density, collagen formation and neo-vascularization rate (Figure 2) inside the Microatlas as required by the ISO10993-6.

Conclusions

The Microatlas guided *in vivo* a quantifiable localized reaction inside its microscaffold, both in terms of cell repopulation, collagen generation and capillary formation as a probable foreign body reaction. Thus, our device can be used as a powerful imaging window for intravital fluorescence microscopy with the capability to quantify *in vivo* the reaction to biomaterial implantation.

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