TWO PHOTON POLYMERIZATION OF AN IMPLANTABLE MICROSCOPE OBJECTIVE FOR INTRAVITAL MICROSCOPY

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I. Introduction

Regulations for biomaterial testing rely on histopathological analyses of animal biopsies leading to ethical issues(1). In this context, intravital microscopy techniques allow to quantify *in-vivo* the foreign body reaction to the implant reducing the number of animals required to statistically validate the biomaterial(2,3). However, traditional windows chambers are invasive, lead to strong inflammatory reactions and fail in the reliability of long-term evaluations. Therefore, we developed a system of microlenses, coupled to a microscaffold, both incorporated in a miniaturised imaging window.

II. Materials and Methods

The device is microfabricated by two-photon polymerization (2PP) of a biocompatible photoresist called SZ2080(4). It is designed to act as an *in-situ* microscope objective with the aim to overcome the restrictions of *in-vivo* imaging related to tissue-induced spherical aberrations. The chip includes fluorescent beacons, allowing optical alignment for multiple observations. Firstly, we established the microlenses process of fabrication as 2PP of the outer shell followed by the UV bulk polymerization of SZ2080(5). We developed a dedicated protocol to fabricate both the microlenses and the 3D microscaffolds on the same chip. Then, we quantified the lenses dioptric power and magnification by coupling them to low numerical aperture objectives, to image stained cells cultured *in-vitro* both on flat substrates and inside the 3D microscaffolds.

III. Results

We proved the reliability of the microfabrication process and independently validated the microlenses morphological and optical quality and obtained a prototype of the integrated smart microstructured imaging window (Fig1.A-C). Remarkably, the fabricated microlenses allow to efficiently excite the fluorescence of labelled cells (Fig1.D-F) and collect high resolution, magnified images of them on a conventional two-photon scanning microscope (Fig1.G-H). The chip enhanced the possibility to reduce optical aberrations related to intravital imaging.

IV. Discussion

Thanks to these encouraging results, the chip will now be used for *in-vivo* observations of the host inflammatory response to the implant of biomaterials, in the aim (a) to reduce the number of animals sacrificed in biomaterial testing procedures and (b) strengthening the validation protocols in both a qualitative and quantitative manner.

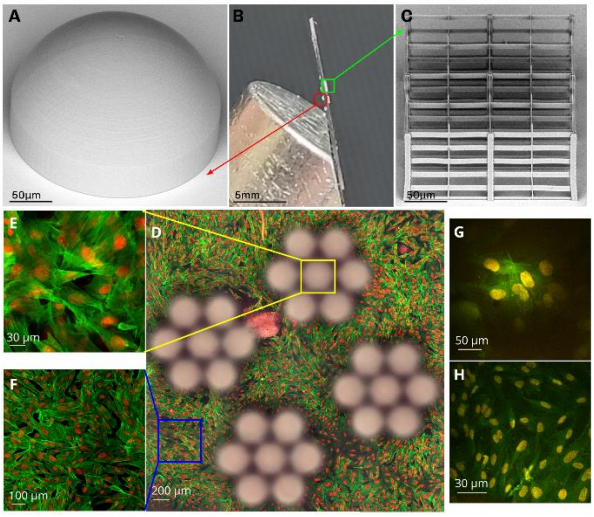

*Figure 1: Microlenses (**A**) and microscaffolds (**C**) fabricated on both sides of the device (**B**). Confocal images of stained human fibroblasts superimposed to the transmitted images of microlenses arrays (**D**), through a single microlens (**E**) and of cells at the glass coverslip focal plane (**F**). Fluorescence images of cells under two photon excitation through a single microlens (**G**) and at the glass coverslip focal plane (**H**).*

References

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