Biochips with Tailored Volume Shapes Fabricated by Ultrafast Laser Processing for Cancer Research

Felix SIMA[1,2,*], Kotaro OBATA[2], Mirai HANZAWA[2], Hiroyuki KAWANO[2], Florin JIPA[2], Alexandra BRAN[2], Emanuel AXENTE[2], Koji SUGIOKA[2]
[1]CETAL, National Institute for Laser, Plasma and Radiation Physics, Romania
[2]RIKEN Center for Advanced Photonics, Japan
felix.sima@inflpr.ro

Lab-on-a-chip strategies are using miniaturized devices that enable cells to be cultured for subsequent studies in tridimensional (3D) spaces mimicking in vivo environment. Configurations relevant for specific tissues at adequate conditions of temperature and pH can be architected. This could allow live observation of cells with a high microscopic resolution over long time periods that are of great interest for *e.g.* cancer cell migration studies.
Ultrafast lasers, defined as lasers emitting pulsed beams with durations shorter than few picoseconds, are used nowadays for such Lab-on-a-chip devices. The extremely high peak intensity associated with ultrashort pulse width allows to induce nonlinear interaction such as multiphoton absorption with materials that are transparent to the laser wavelength. By focusing the ultrashort laser beams inside transparent materials one may confine the nonlinear interaction only within the focal volume, enabling 3D micro- and nanofabrication. We apply subtractive 3D processing technologies including femtosecond laser assisted chemical etching (FLAE) and picosecond laser assisted chemical etching (PLAE) to develop 3D microfluidic networks embedded in photosensitive glass microchips [1,2]. We have thus developed graded and hierarchical configurations with dimensions from hundreds of micrometres to hundreds of nanometres as relevant glass model platforms that mimic cancer cell intravasation-extravasation processes. The fabricated biochip provides 3D hierarchical architectures with nanoscale characteristics and an ultrathin (<100 µm) chip base for high-resolution live cell imaging (Fig. 1).

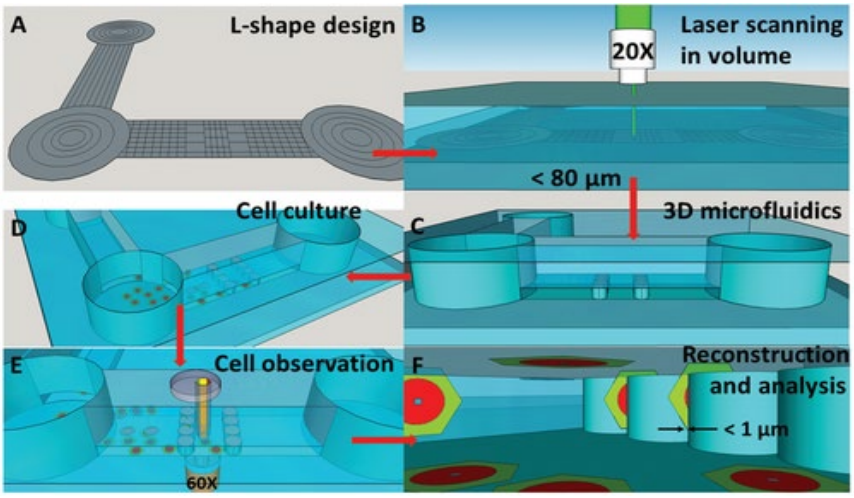

Fig. 1. *Sequential procedure of glass micro/nanofluidic biochip fabrication and study of cancer cell invasion and migration in the fabricated narrow constructive spaces.* ***(a)*** *2D design for laser direct writing scheme. The focused laser beam is scanned along the solid lines.* ***(b)*** *Laser direct writing process in the glass volume according to the design shown in a. To produce a 3D configuration, multiple layers were scanned according to the 2D model by shifting the focused laser beam along the beam axis.* ***(c)*** *3D micro/nanofluidic structures obtained after chemical wet etching and post-thermal treatment.* ***(d)*** *Cell culturing: loading cells into the micro-reservoir. (e) Time-lapse fluorescence observation of cancer cell invasion and migration within narrow constrictive spaces between pillars.* ***(f)*** *Image recording, reconstruction, and analysis.*

Nanochannels narrower than 1 µm with a height of 6.75 µm and a length of over 50 µm were developed inside the glass. Prostate cancer (PC3) cells were cultivated and grown inside the glass biochip, and migration and invasion of the cultivated cells in narrow spaces were then observed by high-resolution live cell imaging using confocal time-lapse microscopy. Such biochips proved capability

of offering both observation of collective prostate cancer cells migration over long time periods and individual visualization at unicellular and subcellular levels on the target cell [3] (Fig. 2).

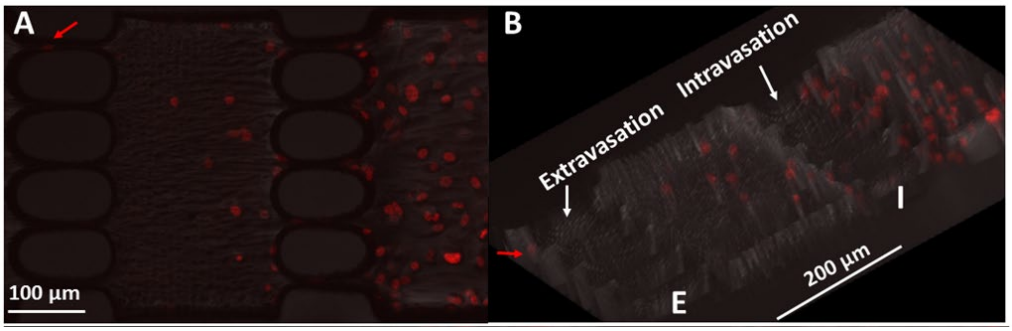

Fig. 2. *Cell migration and invasion in nanochannels: confocal fluorescence images of cell nuclei merged with optical images.* ***(a)*** *Top-down view and* ***(b)*** *reconstructed bird's-eye view showing that the first cell (indicated by a red arrow) is invading the second row of pillars (E-row).*

Miniaturized lab-on-chip glass platforms were further developed to simultaneously perform dosimetry measurements and evaluation of biological effects of ionizing radiation on cancer cells [4]. We designed and fabricated a tumour-on-chip model platform consisting of co-cultures of melanoma and melanocytes cells grown in a laser processed glass microenvironment that allowed to discriminate the radiation effect on cancer cells *vs.* normal tissue cells. This is of interest to validate potential benefits of new irradiation strategies over conventional radiotherapy methods.

References

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