Femtosecond Laser 3D Printing of CYTOP for High Resolution Live Cell Imaging

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Live imaging of cell behavior in three-dimensional (3D) micro- and nanoenvironment is important to investigate the mechanisms of immune systems or disease progression, and has been attracting much attention, especially in efforts to understand cancer cell invasion into other tissues, neutrophil migration at the sites of infection, and axon extension of neurons in brain development, for which microfluidic chips are the attractive tool [1]. A remaining problem of the current microfluidic chips is, however, the refractive-index mismatch between the materials used for chips and culture media containing cells (typically water), which makes it difficult to capture high resolution images. To solve this problem, we propose to use amorphous fluoropolymer CYTOP (AGC Corporation) as a platform of the microfluidic chips, because it exhibits not only excellent transparency but also a refractive index (1.334–1.340) very close to that of water (1.333) [2].

To fabricate the microfluidic chips, a 3D microstructure of SU8 was first fabricated by two-photon polymerization of SU8 using a femtosecond laser (wavelength: 515 nm, pulse width: 220 fs). The fabricated 3D structure was then used as a mold to be filled with liquid CYTOP, followed by thermal treatment for curing CYTOP. Finally, the SU8 microstructure remaining in CYTOP was removed by chemical treatment, resulting in fabrication of the 3D microfluidic structure inside CYTOP.

The fabricated microfluidic Chips, which consisted of six microchannnels with widths of 0.98-4.21 μm connected to two open microreservoirs at the both ends, were applied to live imaging of cells migrating in the microfluidic channels. Before seeding the cells, the microfluidic chips were immersed in a laminin solution for about 24 hours in order to enhance cell adhesion. Then, Human prostate cancer cells (PC3) were seeded from one side of the microreservoirs. A confocal microscope using a 60x objective (NA=1.20, water immersion) was used to observe PC3 migrating in the microfluidic channels after several days of incubation. We have confirmed that PC3 can enter and then pass through the microchannels with widths of 2.14-3.52 μm. Refractive index match between CYTOP and water enabled capturing clear images of cells even near the channel sidewalls. The fine structures such as nucleolus in the cell nucleus were also clearly observed. These results have demonstrated that the CYTOP microfluidic chips can provide ability of high-resolution live imaging of cells. Thus, the developed technique is expected to be used for fabrication of micro- and nanoenvironment platforms for super-resolution bioimaging.

References:

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