

Advantages and disadvantages of 3D printing of fluorescent protein mixed with serum albumin by femtosecond laser direct writing

°(P)Daniela Serien¹, Hiroyuki Kawano², Atsushi Miyawaki^{1,2}, Koji Sugioka¹

RIKEN Center for Advanced Photonics¹, RIKEN Center for Brain Science²,

E-mail: daniela.serien@riken.jp

We study characteristics of microstructures made of fluorescent protein by femtosecond (fs) laser direct writing (LDW). In this presentation, we demonstrate to use the mixture of fluorescent protein with bovine serum albumin (BSA) as a precursor that provides both advantages and disadvantages for 3D printing of fluorescent protein.

In our work, we have so far successfully demonstrated 3D printing of pure protein using fs laser direct writing [1]. Although fs laser multi-photon cross-linking has been recently applied to fabricate microstructures of proteins, only limited types of proteins have been employed for fabrication [2]. Our recent contributions include the fabrication of fluorescent proteins emitting different fluorescence with colors of green [3, 4], blue [4], and red [4]. Since fluorescent proteins are a common tool to label or track cells for many biomedical applications, fabrication of their microstructures might render a useful addition to those biomedical applications.

In this presentation, we discuss the advantages and disadvantages when BSA is introduced into the precursor containing fluorescent protein molecules. We have observed that it is challenging to fabricate 3D microstructures from pure fluorescent protein alone. BSA provides a back-bone to the cross-linking processes occurring in the laser focal volume during the fabrication. Use of BSA facilitates a controlled 3D fabrication of fluorescent protein even with low concentration of fluorescent protein molecules in the precursor, while 3D fabrication of pure fluorescent protein requires high concentration of fluorescent protein and often is difficult to control. As disadvantages, we have observed that auto-fluorescence is generated and environmental pH-dependent fluorescence intensity change, which is typical for fluorescent protein, becomes less prominent due to incorporation of BSA molecules in the fabricated structures.

These results provide an early stepping stone to create deformable cell culture scaffolds with fluorescence tracking capability.

References

- [1] D. Serien, and Koji Sugioka, *ACS Biomaterials Science & Engineering* (2020), vol.6, pp. 1279-1287
- [2] D. Serien, and K. Sugioka, *review Opto-Electronic Advances* 1, 180008 (2018)
- [3] D. Serien, H. Kawano, A. Miyawaki, K. Midorikawa, and K. Sugioka, *Appl Sci* 8, 147 (2018)
- [4] D. Serien, M. Abe, M. Terakawa, H. Kawano, A. Miyawaki, K. Midorikawa, and Koji Sugioka, in preparation