Using free radical scavenging activity to estimate bovine serum albumin reaction sites for 3D protein printing by femtosecond laser direct write

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We report preliminary findings of free radical scavenging activity impacting fabrication of pure proteinaceous microstructures by femtosecond laser direct write. We utilize a free radical scavenger, trolox, that consumes two free radicals per molecule. Relating the molar ratio of trolox to protein bovine serum albumin (BSA) to fabrication completion of line arrays, we approach an estimation of protein reaction sites involved in the fabrication process.

Amongst the 3D printing technologies, laser direct write of protein is an attractive method to create protein microstructures, since they provide complex biomimetic 3D microenvironment [1]. Due to the two-photon absorption, it is expected that the affected precursor material in volume becomes smaller than the focal volume [2]. Such microenvironment is considered relevant for cell culture studies to understand and manipulate cell behavior by protein-cell-interaction. Furthermore, protein binding selectivity in a complex 3D microenvironment might enable to enhance versatility of medical microfluidic devices. Recently, we have provided evidence that chemical cross-linking by radical chain reactions enables to create pure proteinaceous microstructures using photoactivator-free, pure protein precursor [3]. Last, we reported our qualitative observation that the laser induced cross-linking processes in pure protein precursor was able to be quenched.

Here, we present our recent progress using a standardized scavenger and quantify the fabrication result. Figure 1 shows a comparison of fabrication of line patterns formed on the glass substrate. With increasing molar ratio of the scavenger to BSA, the area coverage of fabrication is reduced. Because we know that two radicals are consumed per scavenger molecule, we can estimate active reaction sites of BSA during the laser direct write process. A preliminary prediction is that the threshold of reactive sites of BSA is approximately 130 amino acids. While we further need to explore stereochemistry and type of amino acids, we can note that the amount roughly matches the 153 amino acids uninvolved in helical structure of BSA.

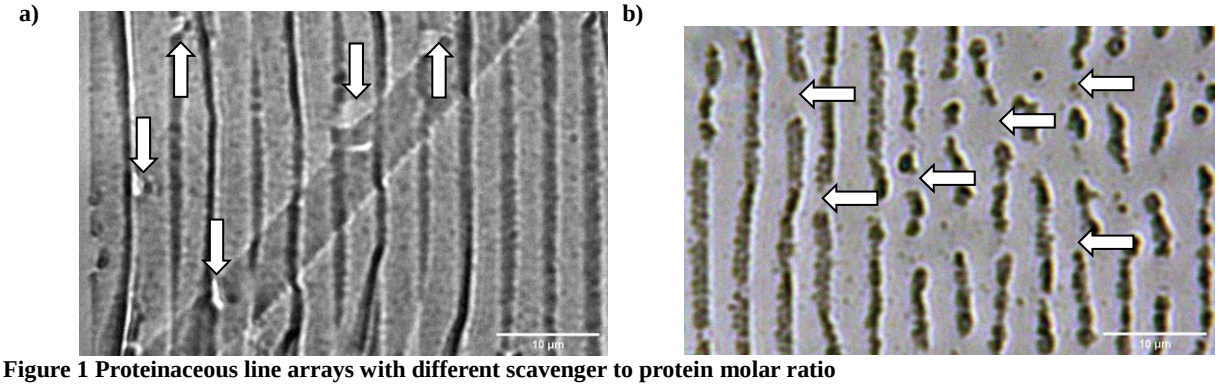

Figure 1 Proteinaceous line arrays with different scavenger to protein molar ratio
Fabricated with 524 nm fs-pulses at 1.5-2.5 mW average laser power and 15 µm/s line scanning speed. Each line was scanned with lateral shift of 4 µm. Optical microscope images show the top view. Line cross section is ellipsoidal and elongated along the z-axis out-of-plane. During the drying process however, the elongated sides of lines stick to the glass substrate and align in-plane. Where the lines fabricated incompletely, glass substrate is visible as light grey against the darker shades of grey that correspond to BSA. White arrows indicate examples of incomplete fabrication. a) 5:1 molar ratio allows well-connected near completed fabrication (99%), b) 40:1 molar ratio displays reduced connectivity and average fabrication completion is roughly 60%. Scale bars represent 10 µm.

References

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