Fabrication of 3D Proteinaceous Microstructures by Femtosecond Laser Direct Writing: Reduction of Water Fraction to Hinder Cavitation Bubble Formation

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We discuss two strategies to reduce water fraction in aqueous systems usually required for microfabrication of proteinaceous structures by femtosecond (fs) laser direct writing (LDW). Microstructures made of protein have been created using a two-photon polymerization technique with fs LDW by photoinduced cross-linking of proteins [1-3]. Protein cross-linking can be induced when the laser intensity exceeds two-photon absorption threshold as well as the incident dose exceeds the polymerization threshold, while the higher laser intensity tends to generate cavitation bubbles. Cavitation bubbles are formed when laser-induced heat leads to expansion of the aqueous medium, as a common aspect in other laser-derived technologies [4]. Depending on the size of the formed bubble, damage to already fabricated structures or strong light scattering hinders the fabrication process. Here, we alter protein solution constituents to reduce the water fraction and notably even fabricate proteinaceous structures without water fraction by using a gently dried drop-cast solution. Each approach requires adjustments of the handling procedure. Notably, the dried drop-cast is easy to handle and fabricate, but is challenging to extract structures from the drop-cast when resolving the non-polymerized protein by washing. Thus, there is great potential in the partial or total removal of a water fraction in polymerization leading to overall easier fabrication and handling.

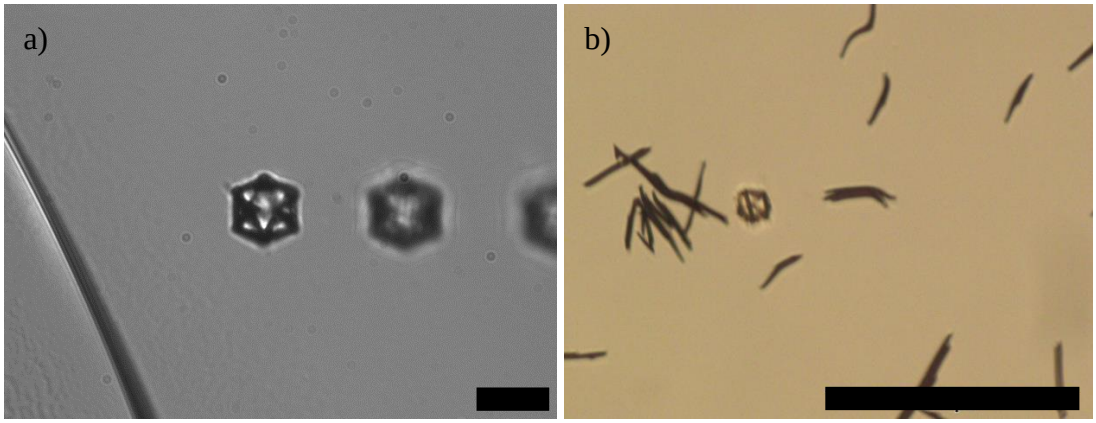

Figure 1 Bright field microscopic images of microstructures made of bovine serum albumin (BSA).
(a) Observation during fabrication of an icosahedron as 3D test structure, scale bar represents 20 μm.
(b) After resolving, the structures are detached, scale bar represents 200 μm.

Three-dimensional bovine serum albumin (BSA) structures were obtained by fs LDW in gently dried drop-cast (Figure 1a). Adding water to resolve the drop-cast that was not polymerized caused motion and detached the microstructures (Figure 1b). Fabrication in a dried drop-cast enables to reduce generation of cavitation bubbles and thereby enrich protein microstructure fabrication, but enhancement of adhesion and development of gentle resolving technique are required to maintain fabricated structures.

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