International Journal of Bioprinting

RESEARCH ARTICLE

Synthesis of biocompatible BSA-GMA and two-photon polymerization of 3D hydrogels with free radical type I photoinitiator

Supplementary File

1. Femtosecond laser direct writing optical setup

The femtosecond laser direct writing optical setup consists of five parts: a femtosecond laser source, a laser alignment part, a three-dimensional (3D) motorized stage (Physik Instrumente), a real-time observation system, and the software control system (**Figure S1**). The femtosecond laser direct writing system uses a near-infrared Ti: sapphire femtosecond laser beam with a central wavelength of 780 nm, a pulse width of 80 fs, and a repetition rate of 80 MHz. The laser beam is tightly focused onto the photoresist by an oil immersion objective lens (60×, numerical aperture 1.42, Olympus). The laser power is precisely controlled by an attenuator. Before fabrication, the photoresist is dropped between two cover glasses. The photoresist is scanned in 3D by a computer-controlled *XYZ* motorized stage.

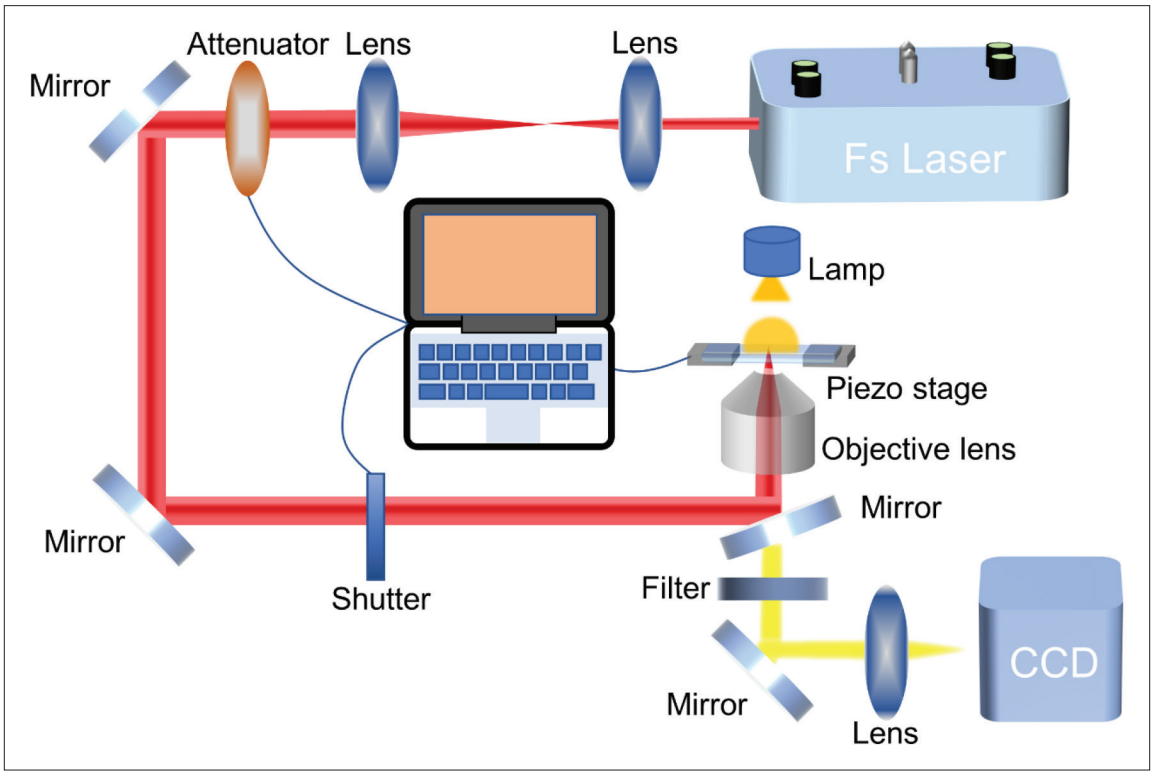

Figure S1. Schematic diagram of femtosecond laser direct writing optical setup.

2. Molecule structure characterization by ^{1}H-NMR

Figure S2 shows the ^{1}H-NMR images of BSA and the samples of Lys: GMA = 1:1, Lys: GMA = 1:1.5, and Lys: GMA = 1:2. The ^{1}H-NMR spectra were corrected for the baseline with a polynomial fit and adjusted for phase. The BSA powder has an aromatic group peak for tyrosine at δ = 7.1 ppm and a primary amine group peak on lysine at δ = 2.9 ppm (Figure S2A). In contrast to BSA, the other three samples modified by GMA showed new peaks, which are methacrylate group peaks on GMA at δ = 5.7 and δ = 6.1 ppm, and a methyl peak on GMA at δ = 1.8 ppm (Figure S2B–D). Furthermore, it is obvious that the degree of methacrylation increases with the increase of the added GMA content from the integrated areas at δ = 7.1 ppm as well as at δ = 5.7 and δ = 6.1 ppm. The degree of methacrylation of BSA-GMA was defined by the percentage of the signal of the methacrylate group at δ = 5.7 and δ = 6.1 ppm versus the integral area of the proton signal generated by the aromatic amino acids at 6.6–7.4 ppm.

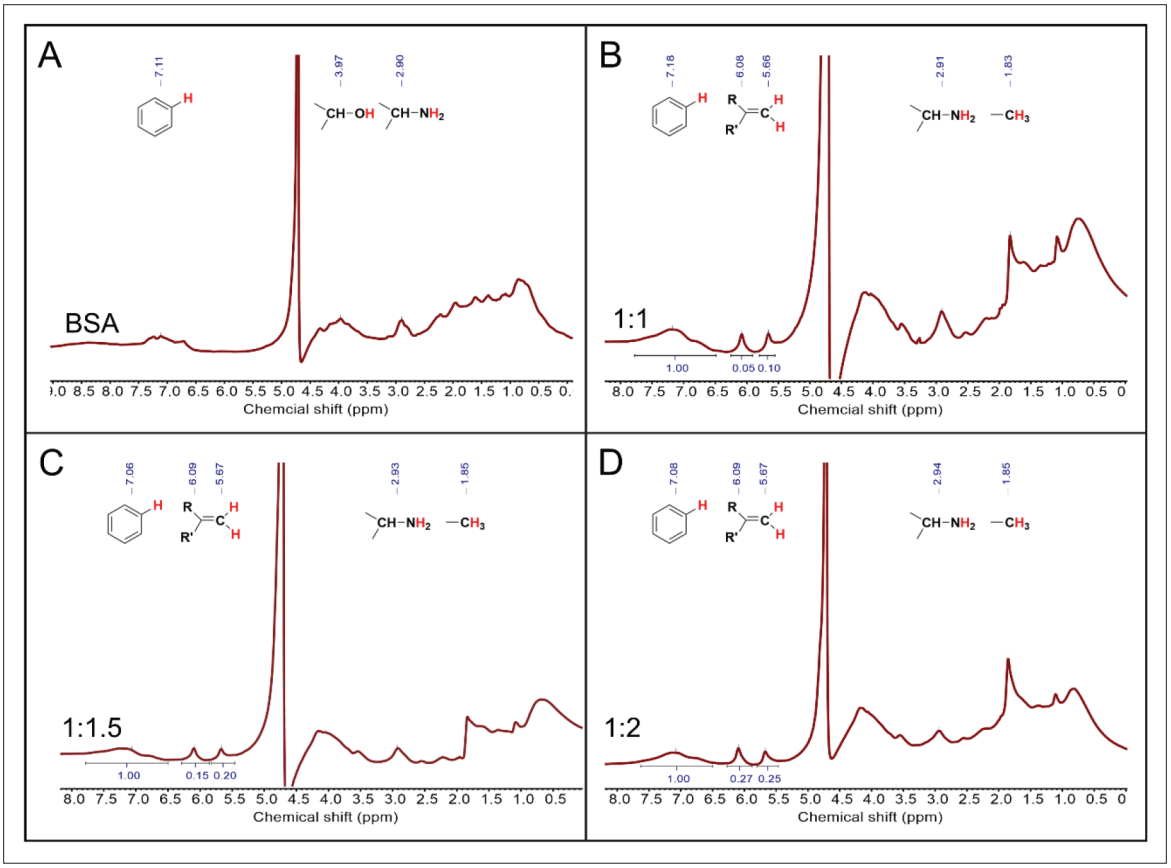

Figure S2. ^{1}H-NMR spectra of unsubstituted BSA and BSA-GMA with different degrees of methacrylation. ^{1}H-NMR spectra of (A) BSA, (B) Lys: GMA = 1:1, (C) Lys: GMA = 1:1.5, and (D) Lys: GMA = 1:2.

3. TPP property of BSA hydrogel

Figure S3 shows a series of line structures obtained by varying the laser power at a fixed scanning speed of 10 μm s^{-1} using 30 wt% BSA as monomer and 0.5 wt% LAP as initiator. The two-photon processing using the radical type I initiator LAP is poor for the pure BSA system. The TPP process of the BSA+LAP system only occurs in the laser power range between 20 and 23.7 mW. The precursor cannot be photopolymerized at the laser power below 20 mW. On the other hand, the precursor is easily exposed to aggregation and is not suitable for the processing of 3D structures at the laser power beyond 23.7 mW.

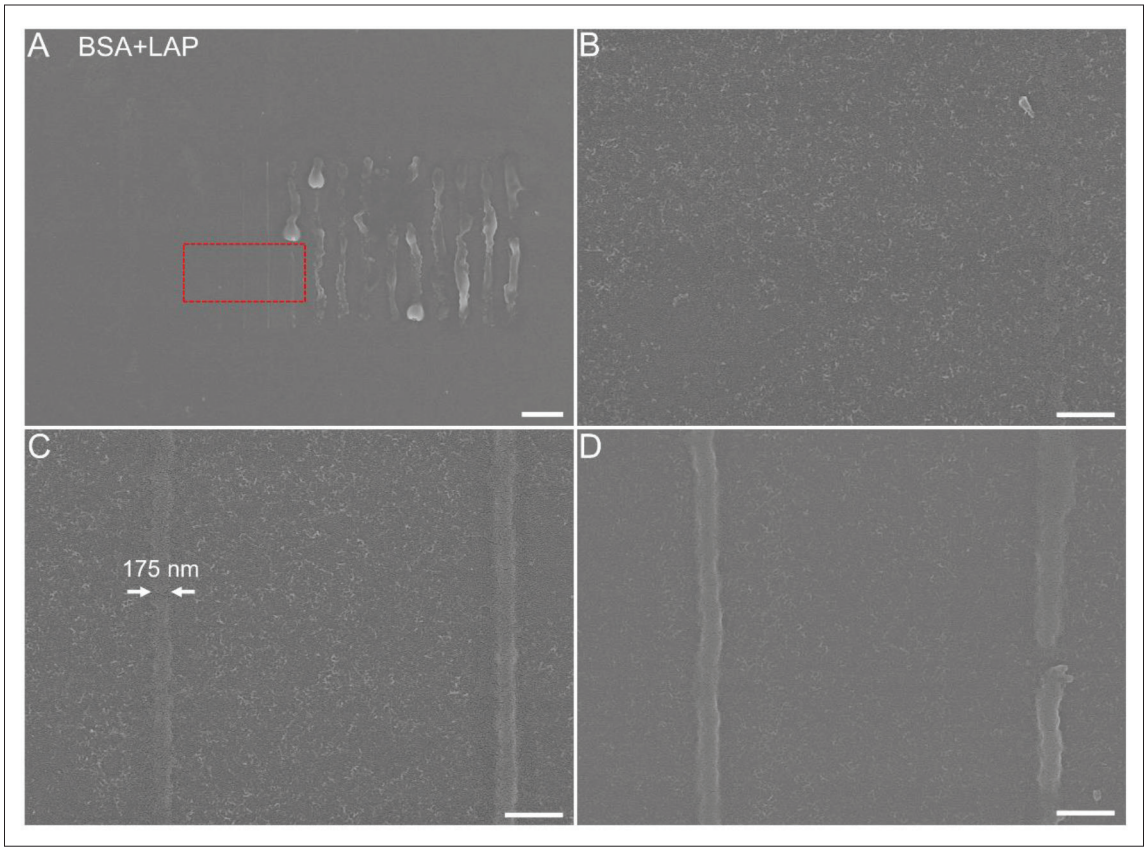

Figure S3. TPP lines of BSA+LAP photoresist. (A) SEM images of the polymerization lines of BSA hydrogels. Scale bar: 5 μm. (B–D) SEM images of the local magnification of the polymerization lines. Scale bar: 500 nm.

4. 3D optical section of the biocompatible BSA-GMA hydrogel cell scaffolds

The survival and spreading of cells on the scaffold are clearly visible from every single channel (**Figure S4**). A few dead cells were visible on the scaffold from the channel of the propidium iodide (PI). The growth and spreading of living cells were clearly observed from the channel of Hoechst and the channel of Mito-Tracker Deep Red.

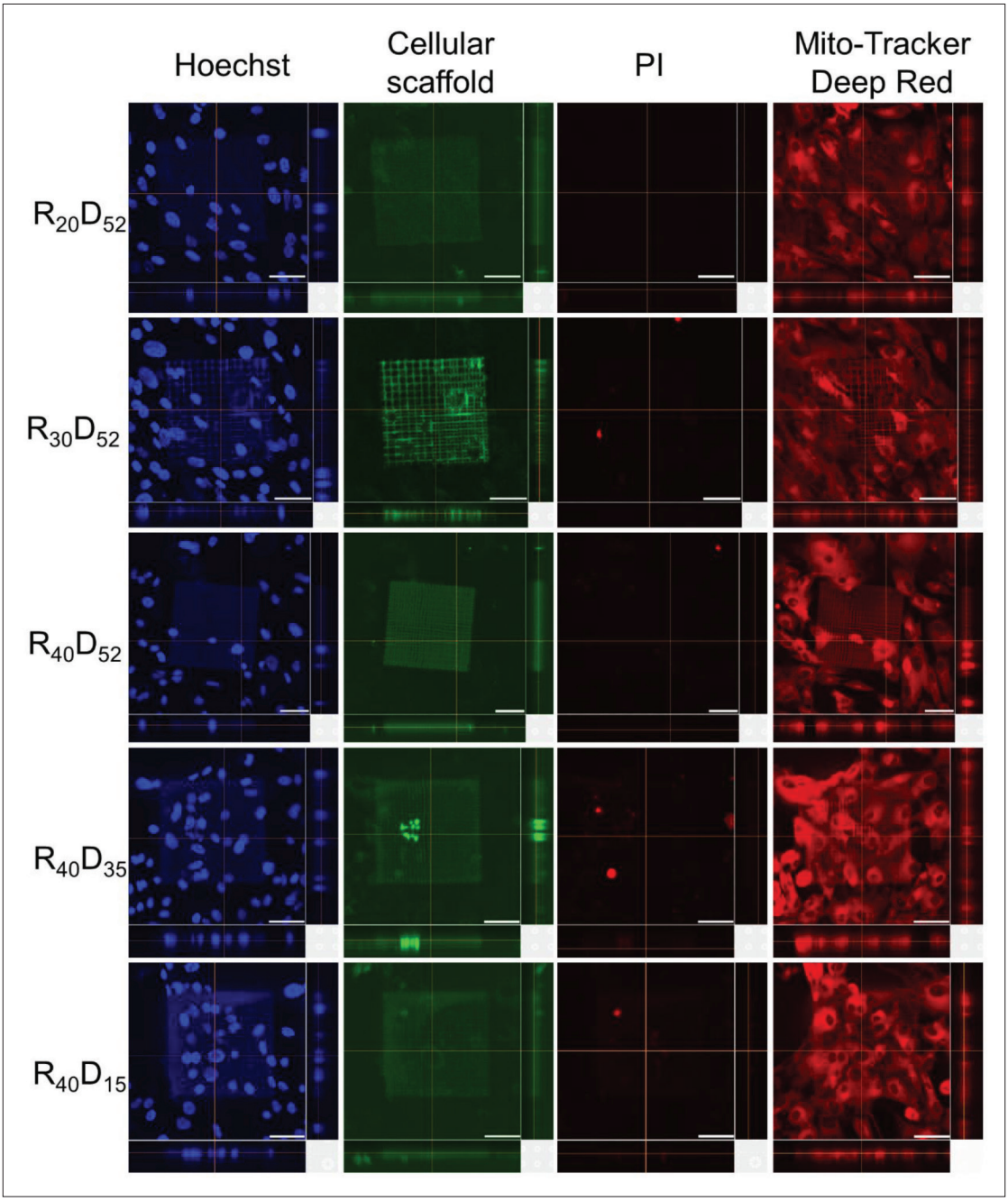

Figure S4. Confocal fluorescence images of 3D optical section of living cells on five hydrogel scaffolds for channels of Hoechst (405 nm), scaffold (488 nm), PI (561 nm), and Mito-Tracker Deep Red (640 nm). Scale bar: 50 μm.