

Femtosecond Laser Direct Writing of Biopolymeric Nanocomposite for Biocompatible 3D Features

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Electronic Supplementary Information

Abstract Biopolymeric nanocomposites have attracted considerable attention because of their biocompatibility, biodegradability, and unique physicochemical properties. It is essential to manufacture three-dimensional (3D) biocompatible micro/nanostructures using biopolymeric nanocomposites. Herein, we demonstrate the high-fidelity fabrication of biocompatible 3D features with sub-50 nm resolution using femtosecond laser direct writing (FsLDW) of a biopolymeric nanocomposite composed of egg white and sulfonated graphene (S-graphene). The biopolymer nanocomposite acts as a negative photoresist suitable for water-based lithography. The introduction of S-graphene not only dramatically lowered the laser power threshold but also significantly modulated the morphology of the 3D features constructed by FsLDW. Microstructures with porous, rough, or smooth morphologies were obtained by optimizing the S-graphene concentration and laser scanning speed. The fabricated egg-white/S-graphene microstructures exhibited biocompatibility and environmental degradability. Egg white/S-graphene was also employed to fabricate diffractive gratings with superior optical quality. This study provides a promising method to manufacture biocompatible 3D features with controllable morphology, which has potential applications in biological and photonic fields.

Keywords Femtosecond laser direct writing; Biopolymeric nanocomposite; Egg white; Sulfonated graphene; Photoresist

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INTRODUCTION

Three-dimensional (3D) biopolymeric microstructures with biocompatibility and controllable morphology are important for fundamental research and practical applications.^[1,2] Biopolymers, such as collagen, chitosan, gelatin, and proteins, are attracting increasing attention for the construction of 3D biocompatible microstructures owing to their biocompatibility and biodegradability.^[3–7] Egg white is an attractive biopolymer owing to its biocompatibility, low cost, and antibacterial and anti-inflammatory properties.^[8] Due to its unique physicochemical characteristics, egg white has been applied in diverse fields, such as biosensors, bioinks, water purification, supercapacitors, cell scaffolds, and photoresists.^[9–15] The manufacturing of specific microstructures on egg whites is critical for their practical applications.

Conventional manufacturing methods for fabricating micro/nanostructures on egg whites mainly focus on electron beam and ultraviolet lithography, inkjet printing, vat-based

additive manufacturing, and EUV lithography.^[15–18] Electron beam lithography of egg whites has a resolution of 15 nm and requires a high-vacuum environment.^[15] Ultraviolet lithography is suitable for manufacturing egg white patterns over a large area, with the limitation of the prerequisite mask equipment. Inkjet printing is employed to process egg whites with a precision of approximately 5 μm and often needs to deal with the challenge of shape fidelity and cytocompatibility.^[16] EUV Lithography has been successfully performed on egg whites, resulting in a solubility switch in egg whites to obtain sub-100 nm patterns.^[18] As a result, egg whites have been proven to be a suitable resist for water-based lithography and serve as an eco-friendly alternative to oil-derived photoresists. Nevertheless, these fabrication strategies have limitations, such as the requirement of vacuum working conditions, time consumption, and 2D pattern processing capability. The fabrication of egg whites to obtain 3D biocompatible features with controllable morphology and high precision is essential for potential applications.

A femtosecond laser is a precise fabrication tool with the advantages of high resolution, masklessness, and real 3D fabrication capability.^[19–21] Femtosecond lasers have been employed to construct 3D micro/nanostructures and functional devices, including high-capacity optical disk memory, lithium

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niobate ferroelectric nanodomains, micro-supercapacitors, perovskite nanocrystals, microscale soft machinery, photonic crystals, sensors, 3D microfluidics, electronics, and micro ion traps.^[22–33] Although femtosecond laser-induced nucleation of egg white protein has been investigated, femtosecond laser fabrication of egg whites for designable microstructures remains elusive.^[34] Therefore, it is still challenging to fabricate biocompatible 3D features with specific morphology using femtosecond laser direct writing (FsLDW) of egg whites.

Herein, we demonstrate the FsLDW of biopolymeric nanocomposites for the fabrication of biocompatible 3D features with high precision and controllable morphology. The biopolymeric nanocomposite consists of egg white and sulfonated graphene (S-graphene) and acts as a negative photoresist employing water as the developer, eliminating the use of toxic resin and developer. The photoresist exhibits a feature size of 48 nm by FsLDW, indicating sub-diffraction lithography capability. The introduction of S-graphene not only dramatically decreases the laser threshold power but also influences the morphology of the cured egg white/S-graphene microstructures, resulting in the formation of a porous, rough, and smooth morphology. Arbitrary 2D and 3D features were constructed using FsLDW of the egg white/S-graphene photoresist. The fabricated egg-white/S-graphene microstructures exhibited biocompatibility and degradability, which have potential for biological applications. As a proof of concept, the fabricated egg-white/S-graphene microstructures can be employed to construct a diffractive grating. These results suggest that egg white/S-graphene photoresist can be employed in fabricating complex 3D microstructures for potential applications in biological and optical microdevices.

EXPERIMENTAL

Materials

Egg white was obtained from chick eggs purchased from a supermarket and used without further purification. Sulfonated graphene was obtained from the Suzhou High Technology Graphene Company (GCA-01) and used without further purification. Glass substrates were purchased from the Yanchengfeizhou Company. Goldfish liquid detergent was purchased from Sinopharm Chemical Reagent Co., Ltd. The glass substrates were sonicated in an aqueous solution of Goldfish liquid detergent to remove dust and organic residue. The substrates were then washed and sonicated in ultrapure water (18 M Ω , Mini Q) several times and dried by blowing N₂.

Preparation of the Egg White/Sulfonated Graphene Photoresist

Egg white/sulfonated graphene photoresist was prepared by dissolving sulfonated graphene in egg white with different contents by mixing overnight.

FsLDW Microfabrication of Egg White/S-graphene

FsLDW of egg white/S-graphene was performed using a home-built apparatus, as described in a previous report.^[35] In brief, the microfabrication system combined a 780 nm fs laser (120 fs, 80 MHz, Tsunami, Spectra Physics, USA) and an inverted optical microscope (Olympus, IX 71). The femtosecond laser pulse was focused by an oil-immersion objective lens (60 $\times$, NA=1.42, Olym-

pus) into the egg white/S-graphene photoresist in the cell, which was fixed on a 3D piezostage (P-563.3CL, Physik Instrumente) and scanned according to the designed program scales. After fs laser exposure, the cell was separated, and the glass substrate was dipped into ultrapure water to remove the uncured photoresist. Finally, residual water was removed by N₂ blowing to achieve the microstructures.

Characterization Techniques

The morphology of the egg white/S-graphene features obtained by FsLDW was characterized by field-emission scanning electron microscopy (FE-SEM, Hitachi, S-4800). The degradability of the egg white/S-graphene microstructure was investigated using environmental scanning electron microscopy (SEM, FEI, Quanta FEG 250). The cross-sectional morphology of the egg white/S-graphene microstructure was evaluated by helium ion microscopy (Orion NanoFab, Carl Zeiss, USA). The egg white/S-graphene grating was characterized by illuminating the specimens with a CW green laser (532 nm, Shaanxi' Richeng Technology Development Co., Ltd.), and the transmitted light was recorded on a flat screen. Diffraction patterns were recorded using a mobile phone camera (iPhone 12, Apple Company). Cell co-culture and fluorescence imaging: L929 cells were used to evaluate the biocompatibility of the egg white/S-graphene microstructure using FsLDW. The egg white/S-graphene microstructure and L929 were cocultured in DMEM (HyClone) at 37 °C under 5% CO₂ for 48 h. To evaluate cell survival conditions, fluorescent probe, MitoTracker Green (Life technology, λ_{ex} =488 nm), was used to stain cell mitochondria, and PI fluorescent probe (Invitrogen, λ_{ex} =561 nm) was used to stain dead cells. Fluorescent images were obtained using a laser confocal fluorescence microscope (Nikon, A1R MP).

RESULTS AND DISCUSSION

Fig. 1 depicts a schematic diagram of FsLDW of biopolymeric nanocomposites for 3D microstructures and cell culture. The biopolymeric nanocomposite was composed of egg white and sulfonated graphene. Egg white consists of 87.9 wt%–89.4 wt% water, 9.7 wt%–10.0 wt% proteins, 0.4 wt%–0.9 wt% carbohydrates, and 0.5 wt%–0.6 wt% inorganic salts^[36]. The proteins in egg whites mainly have a globular conformation, which is prone to aggregation under irradiation and/or heat.^[37,38] Therefore, egg white/S-graphene acted as a negative photoresist under femtosecond laser irradiation (Fig. 1a). During FsLDW lithography, a 780 nm fs laser beam is focused tightly onto the egg white/S-graphene photoresist and scanned on a 3D precise stage according to the predesigned program, resulting in solidified egg white/S-graphene microstructures (Fig. 1b). After FsLDW, the egg white/S-graphene microstructure was transferred to a petri dish for cell culture, as shown in Fig. 1(c). Finally, the L929 cells were seeded and co-cultured for 48 h. Fluorescence imaging was employed to study cell morphology and evaluate the biocompatibility of the egg white/S-graphene microstructures (Fig. 1d).

The fabrication characteristics of egg white/S-graphene by FsLDW were investigated by evaluating the dependence of the laser threshold power and linewidth of the solidified egg white/S-graphene features on S-graphene concentration. Figs. 2(a)–2(f) show SEM images of the nanowires construct-

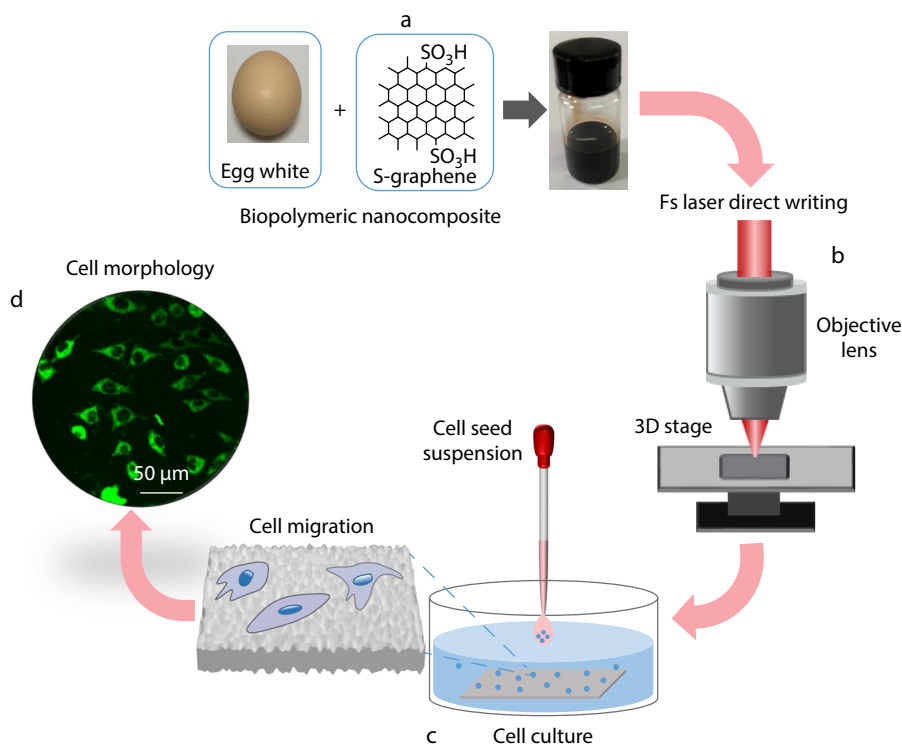


Fig. 1 Schematic diagram of FsLDW of biopolymeric nanocomposite for cell culture. (a) The biopolymeric nanocomposite is made up of egg white and S-graphene; (b) FsLDW of the egg white/S-graphene for 3D microstructures; (c) L929 cell cultured on the microstructure; (d) L929 cell morphology on the solidified egg white/S-graphene microstructure.

ed by FsLDW of egg white/S-graphene photoresists with different S-graphene concentrations. For egg whites without S-graphene, the laser threshold power is as high as 49.30 mW, and the fabrication process is unstable. The addition of S-graphene resulted in stable fabrication of egg whites and reduced the laser threshold power. For the S-graphene concentrations from 1% to 10%, the egg white/S-graphene photoresists can be readily fabricated in the liquid state. S-graphene improves the morphology of egg white/S-graphene nanowires fabricated using FsLDW. At the graphene concentrations of 1% and 2%, the nanowires were rough and discontinuous, as shown in Figs. 2(b) and 2(c). When the concentration of S-graphene increased above 4%, continuous nanowires were obtained with smooth surface and compact morphology, as shown in Figs. 2(d)–2(f). Although an increase in the S-graphene concentration improves the morphology of the nanowires, the narrowest linewidth fluctuates between 168 and 196 nm, as shown in Fig. 2(g). When the concentration of S-graphene increased from 0% to 10%, the laser threshold power decreased gradually from 49.30 mW to 2.04 mW, as shown in Fig. 2(h). The laser threshold power for egg white/S-graphene photoresist with 10% S-graphene was only 1/24 of that for bare egg white.

The decrease in the laser threshold power is mainly attributed to the thermal effect induced by quasi-continuous MHz-fs pulsed laser exposure of S-graphene.^[39,40] Although the temperature increment of graphene oxide exposed to an 800 nm fs laser pulse with a 1 kHz repetition rate was not observed, thermal accumulation of graphene oxide irradiated by a pulsed laser with 80 MHz repetition rate is non-negligible,

resulting in a focal temperature increase of approximately 100 °C.^[40] The temperature increase is sufficient for the denaturation of egg white because the required temperature for optimal cooking of egg white is approximately 85 °C.^[41] Sulfonated graphene, which exhibits a significant photothermal effect on NIR light and two-photon absorption capability at 800 nm,^[42,43] is expected to increase the temperature under exposure to a 780 nm fs laser beam with an 80 MHz repetition rate. The increase in S-graphene content would result in a greater temperature increase in the egg white/S-graphene photoresist under fs laser exposure, facilitating the solidification of the egg white. Therefore, the increase in the S-graphene content results in a dramatic decrease in the laser threshold power during the fabrication of egg white/S-graphene photoresist.

We systematically investigated the influence of the S-graphene concentration on the morphology of egg white/S-graphene microstructures fabricated by FsLDW. To evaluate the effect of S-graphene concentration, we fabricated cuboid features with a footprint of 15 μm × 15 μm × 4 μm using FsLDW of egg white/S-graphene photoresists with different S-graphene concentrations and scanning speeds. The laser powers for the fabrication of the egg white/S-graphene photoresists with the S-graphene concentrations of 2%, 4%, 6%, and 10% were fixed at 34.5, 30.1, 25.2 and 18.7 mW, respectively. For S-graphene concentration of 2%, structurally incomplete micro-cuboids with porous hierarchy and rough surface were fabricated (Figs. 3a, Figs. S1 and S2 in the electronic supplementary information, ESI). The integrity and uniformity of the porous microstructures worsened with an

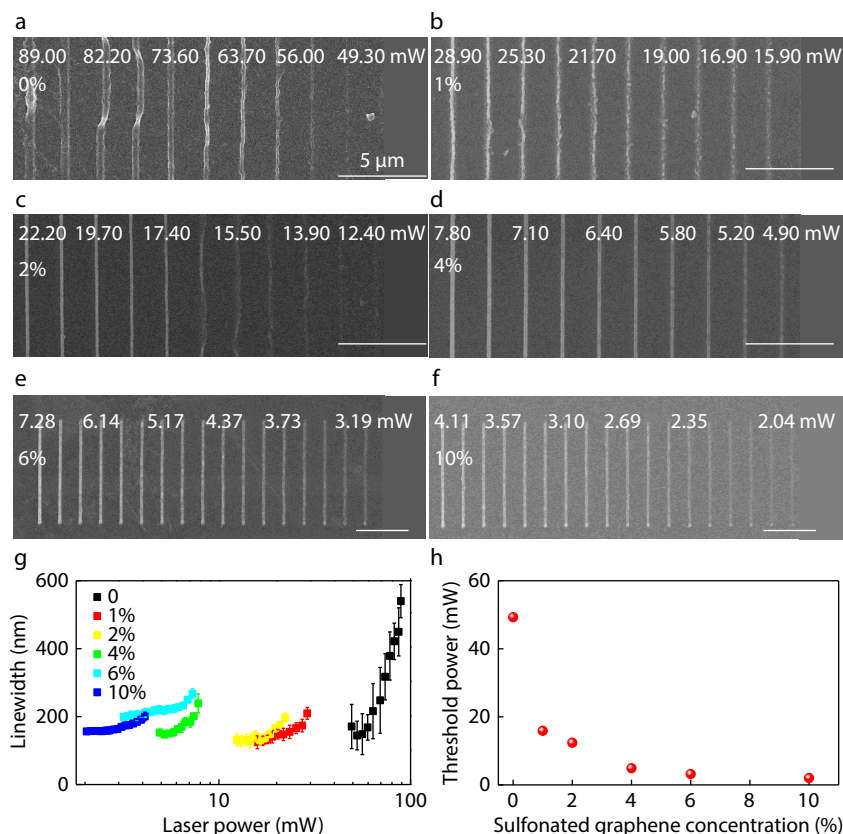


Fig. 2 The influence of S-graphene concentration on the fabrication characteristics of egg white/S-graphene by FSLDW. SEM images of nanowires constructed by FSLDW of egg white/S-graphene photoresists with S-graphene concentration of (a) 0%, (b) 1%, (c) 2%, (d) 4%, (e) 6% and (f) 10%, respectively. The scale bar is 5 μm . (g) The dependence of linewidth on the laser power in the FSLDW of egg white/S-graphene photoresists. (h) The influence of the S-graphene concentration on the laser threshold power in the FSLDW of egg white/S-graphene photoresists.

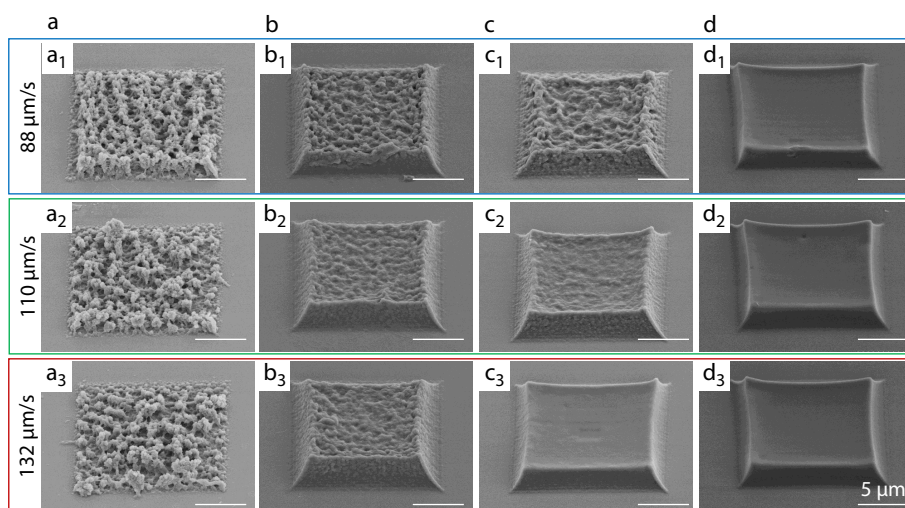


Fig. 3 The dependence of the morphology of solidified egg white/S-graphene microstructures on the concentration of S-graphene in the egg white/S-graphene photoresists. Titled SEM images of the cured features by FSLDW of egg white/S-graphene with S-graphene concentration of (a) 2%, (b) 4%, (c) 6% and (d) 10%. For (a₁–a₃), (b₁–b₃), (c₁–c₃) and (d₁–d₃), scanning speed changes from 88 $\mu\text{m/s}$ to 132 $\mu\text{m/s}$.

increase in scanning speed from 88 $\mu\text{m/s}$ to 132 $\mu\text{m/s}$. For S-graphene concentration of 4%, complete microcuboids with rough surfaces were constructed using FSLDW (Fig. 3b). The roughness of the surface gradually increased with an in-

crease in scanning speed from 88 $\mu\text{m/s}$ to 132 $\mu\text{m/s}$. For S-graphene concentration of 6%, micro-cuboids with morphology transformed from smooth to rough surfaces could be achieved by increasing the scanning speed. Micro-cuboids

with smooth surfaces were obtained at a scanning speed of 88 $\mu\text{m/s}$, whereas micro-cuboids with a rough surface were constructed by FsLDW with scanning speeds of 110 and 132 $\mu\text{m/s}$, as shown in Figs. 3(c₁)–3(c₃). For S-graphene concentration of 10%, micro-cuboids with smooth surfaces were fabricated using FsLDW with a scanning speed from 88 $\mu\text{m/s}$ to 132 $\mu\text{m/s}$ (Fig. 3d). As a result, the morphology of the egg white/graphene microstructures by FsLDW can be readily manipulated by controlling the concentration of S-graphene and the scanning speed of the laser spot, resulting in the formation of hierarchical features from rough and porous morphology to smooth and compact morphology. For rough and porous microstructures, the surface enhancement of Raman scattering (SERS) substrates can be achieved after the deposition of noble metals (Ag or Au) on the surface of the microstructures. Moreover, the morphological diversity of the egg white/S-graphene features by FsLDW is a perspective in the potential applications of constructing cell scaffolds to investigate the influence of morphology on cell adhesion and proliferation.

We demonstrated the ability to construct arbitrary 2D features using the FsLDW of an egg white/S-graphene photoresist. Fig. 4(a) shows the nanowire array on the support fabricated using FsLDW. The smallest linewidth of 48 nm ($\lambda/16$) was achieved, indicating the feasibility of sub-diffraction lithography. The high resolution is mainly attributed to the combination of the threshold effect of the femtosecond laser fabrication and shrinkage during the development process. During femtosecond laser exposure, the threshold effect localizes the reaction region below the optical diffraction limit. Meanwhile, the suspended feature on the support shrank during the development process owing to the removal of small molecules, which dramatically decreased the fabricated size. As a result, a high resolution of 48 nm could be obtained by femtosecond laser direct writing of the egg white/S-graphene. Figs. 4(b) and 4(c) show the nanodot array and nanocircle array. Furthermore, complex 2D features were fabricated, as shown in Figs. 4(d)–4(f). The microgear with nine gear teeth, the character “TPL” and the logo of organic nanophotonics were fabricated by FsLDW. The fabrication of arbitrary 2D microstructures proves the feasibility of pattern-

ing egg white/S-graphene using FsLDW with high resolution and designability.

Furthermore, we demonstrated the FsLDW of 3D egg-white/S-graphene microstructures. This is the distinctive merit of FsLDW for construct arbitrary 3D precise microstructures, which are difficult and time-consuming for traditional lithography methods. Fig. 5 shows the SEM images of a series of 3D microstructures fabricated by FsLDW of egg white/S-graphene photoresist. As shown in Fig. 5(a), a freestanding red cell microstructure with a diameter of 7 μm was constructed, which is similar to the size of a human red cell. The red cell microstructure was fixed vertically on the substrate without tilting, indicating sufficient mechanical properties of the solidified egg white/S-graphene microstructure. Fig. 5(b) shows the lizard microstructure fabricated using FsLDW. The lizard stands on four feet, and the main body is suspended with the feet and tail anchored on the substrate. Furthermore, the cellular scaffold microstructure was fabricated using FsLDW of egg white/S-graphene, as shown in Fig. 5(c). The surrounding columns are tilted toward the center owing to the surface tension and shrinkage of suspended nanobelts during the development process. We also constructed microtube arrays using the FsLDW of an egg white/S-graphene photoresist. The microtube was approximately 2 μm in height, with an outer diameter of 1.14 μm and an inner diameter of 550 nm. By carefully controlling the fabrication parameters, the outer diameter of the microtube can be varied from 1.14 μm to 0.87 μm , and the inner diameter can be tuned from 550 nm to 360 nm (Fig. 5d, Fig. S3 in ESI). The FsLDW of arbitrary 3D microstructures using an egg white/S-graphene photoresist proved the capability of constructing designable complex 3D microstructures.

We further investigated the biocompatibility of the egg white/S-graphene microstructure by culturing L929 cells on the microstructure. We fabricated egg white/S-graphene microsquares with a size of 400 $\mu\text{m} \times 400 \mu\text{m}$ and cultured L929 cells on the microsquares for 48 h. To evaluate the cytotoxicity of the egg white/S-graphene microstructure, confocal fluorescence microscopy was employed to distinguish between live and dead cells stained by MitoTracker Green ($\lambda_{\text{ex}}=488 \text{ nm}$) and propidium iodide ($\lambda_{\text{ex}}=561 \text{ nm}$). As shown in Fig. 6(b₁),

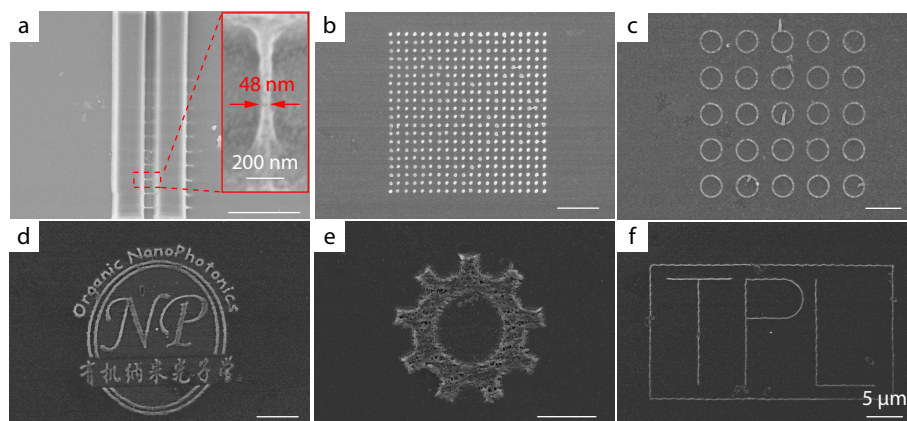


Fig. 4 FsLDW of 2D egg white/S-graphene microstructures. SEM images of (a) nanowire array, (b) nanodot array, (c) nanocircle array, (d) logo of organic nanophotonics, (e) microgear, and (f) character “TPL” (abbreviation of two-photon lithography). The scale bars are 5 μm . Inset in (a) is the magnified SEM image of the nanowire, showing the sub-diffraction lithography of 48 nm feature size by FsLDW.

the shaded area is the egg white/S-graphene microstructure. L929 cells grew well and showed a regular morphology on the egg white/S-graphene microstructure. Under 561 nm illumination, there are quite a few red fluorescence signals in the confocal fluorescence image, as shown in Figs. 6(b₂–b₄). Compared with the results of the control sample shown in Figs. 6(a₁–a₄), L929 cells grew well, and the PI fluorescent probe could not penetrate the cell membrane, indicating very few dead cells on the egg white/S-graphene microstructure. As shown in Fig. S4 (in ESI), the L929 cells on the microstructure exhibited a polygonal or spindle shape and spread well on the surface, indicating that they L929 cells survive well. As shown in Fig. 6(c), the cell viability of the L929 cells on the egg white/S-graphene microstructure was approximately 99.3%, similar to the value of 99.7% for the control sample. Therefore, the egg white/S-graphene microstructure is non-toxic to living cells and suitable for biological applications. Biocompatibility was further corroborated by the observation of L929 cell division on the egg white/S-graphene microstructure. As shown in Fig. S5 (in ESI), one kidney-shaped mother L929 cell attached to the egg white/S-graphene microsquare was divided into two spherical daughter cells,

which suggests that the egg white/S-graphene microstructure does not interfere with the normal cell division action of the L929 cell. The degradability of the egg white/S-graphene microstructure was investigated using environmental scanning electron microscopy (SEM, FEI, Quanta FEG 250), as shown in Fig. S6 (in ESI). After soaking in water at room temperature for 10 days, obvious cracks were observed on the surface of the microstructure with breaking on the edge. Upon further increasing the immersion time to 22 days, the cracks grew much larger, resulting in serious damage and degradation. Degradation of the egg white/S-graphene microstructure suggests that the egg white/S-graphene photoresist is eco-friendly.

Furthermore, we fabricated a diffractive optical device, that is, grating by the FsLDW of the egg white/S-graphene photoresist. The size of the diffractive grating was set to 100 $\mu\text{m} \times 100 \mu\text{m}$. Owing to the good reproducibility and stability of the photocuring of the egg white/S-graphene using FsLDW, a uniform diffractive grating microstructure with a periodicity of 5 μm was successfully fabricated. The uniformity of the egg white/S-graphene diffractive grating was confirmed by the optical transmittance micrograph and SEM image shown in

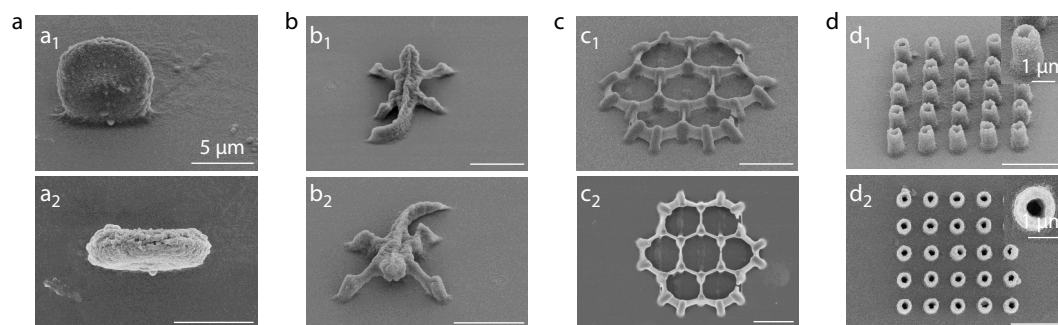


Fig. 5 FsLDW of arbitrary 3D egg white/S-graphene microstructures. (a₁) Tilted and (a₂) top view SEM images of red cell microstructure, (b₁) tilted and (b₂) top view SEM images of lizard microstructure, (c₁) tilted and (c₂) top view SEM images of cell scaffold microstructure, (d₁) tilted and (d₂) top view SEM images of microtube array microstructure, respectively. The scale bars are 5 μm . Insets in (d) are the magnified SEM images of the microtube. The scale bars are 1 μm .

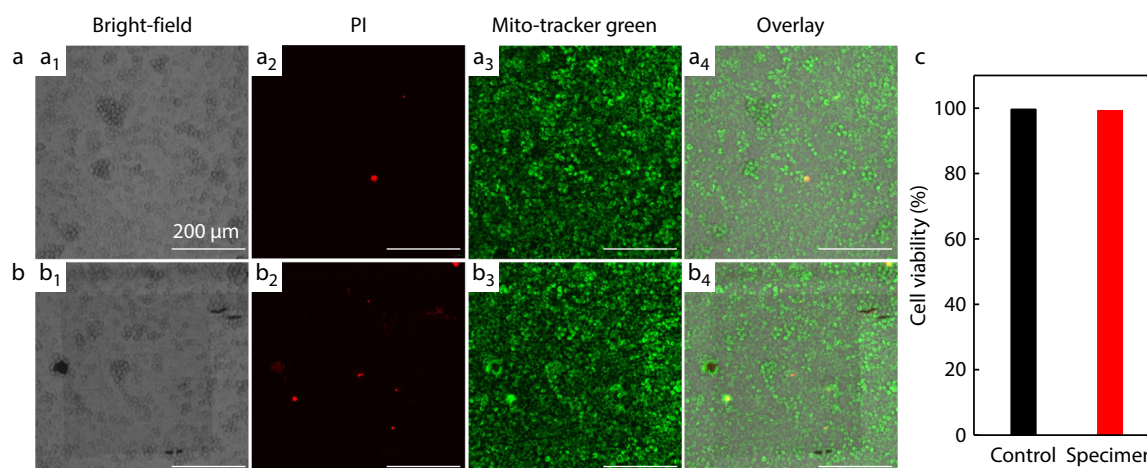


Fig. 6 Biocompatibility of the egg white/S-graphene microstructure by FsLDW. Confocal fluorescence images of L929 cells co-cultured with (a) control and (b) 400 $\mu\text{m} \times 400 \mu\text{m} \times 4 \mu\text{m}$ egg white/S-graphene microstructure for 48 h. (a₁) Bright-field image, (a₂) PI stained image, (a₃) Mito-tracker green stained image, and (a₄) overlay image of the control sample. (b₁) Bright-field image, (b₂) PI stained image, (b₃) Mito-tracker green stained image, and (b₄) overlay image of the 400 $\mu\text{m} \times 400 \mu\text{m} \times 4 \mu\text{m}$ egg white/S-graphene microstructure. (c) Cell viability of the control and egg white/S-graphene specimen.

Figs. 7(a) and 7(b). The diffraction order can be theoretically estimated by Eq. (1):

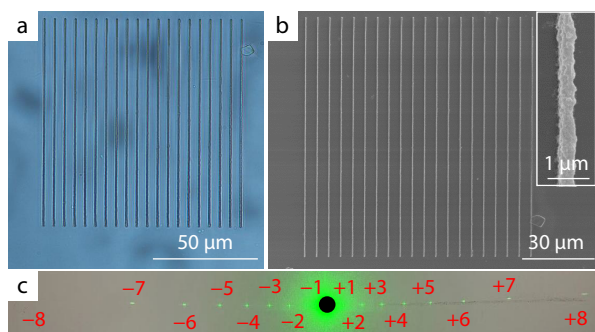


Fig. 7 The egg white/S-graphene grating fabricated by FsLDW. (a) Optical microscopy image of the egg white/S-graphene grating microstructure with a periodicity of 5 μm; (b) SEM image of the egg white/S-graphene grating microstructure. The inset is the magnified SEM image of the egg white/S-graphene grating microstructure. (c) The diffraction pattern of the egg white/S-graphene grating microstructure illuminated by a 532 nm CW laser. The red number refers to the diffraction order of the egg white/S-graphene grating microstructure. The zero order of the diffraction pattern is covered for clarity.

$$m\lambda = d\sin\alpha \quad (1)$$

In Eq. (1), m refers to the diffraction order, λ is the wavelength of the incident light, d is the period width of the grating, and α is the diffraction angle for different orders. According to Eq. (1), the maximum diffraction order 9 can be obtained for the egg white/S-graphene diffractive grating with a periodicity of 5 μm. The diffraction results for the egg white/S-graphene grating are shown in Fig. 7(c). In response to the 532 nm CW laser beam illumination, diffracted laser beam spots up to the 8th order can be recognized, while the 9th order diffraction spot is too weak to be recorded by the mobile phone camera. Meanwhile, the 1st order diffraction spot was very weak and could only be recognized in the magnified image (Fig. S7 in ESI) because of the high brightness of the zero-order diffraction spot. The agreement of the diffraction order between the experimental results and the theoretical calculation verifies the high quality of the egg white/S-graphene grating fabricated using FsLDW.

CONCLUSIONS

In summary, we demonstrated a versatile strategy for constructing 3D features with controllable morphology by femtosecond laser direct writing (FsLDW) of biopolymeric nanocomposites made up of egg white and S-graphene. We obtained egg white/S-graphene with S-graphene concentrations from 1% to 10%, which can be served as a negative photoresist. The increase in S-graphene concentration would not only significantly decreased the laser threshold power but also modulated the morphology of the fabricated egg white/S-graphene microstructures. The morphology from the porous to the smooth surface of egg white/S-graphene microstructures can be achieved by precisely controlling the scanning speed and S-graphene concentration. The FsLDW of egg white/S-graphene for 2D and 3D microstructures was successfully achieved, with

the smallest feature size of 48 nm. The constructed egg-white/S-graphene microstructures exhibited superior biocompatibility and degradability, which are critical for biological applications. Furthermore, we demonstrate that egg white/S-graphene is suitable for the construction of an optical microdevice such as a diffractive grating. This study proposes a versatile method for the fabrication of morphology-controllable biocompatible 3D features, and provides potential applications in the fields of cell scaffolds and optical microdevices.

Conflict of Interests

The authors declare no interest conflict.

Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at <http://doi.org/10.1007/s10118-025-3519-z>.

Data Availability Statement

Data supporting the findings reported in this study can be obtained from the corresponding author upon reasonable request.

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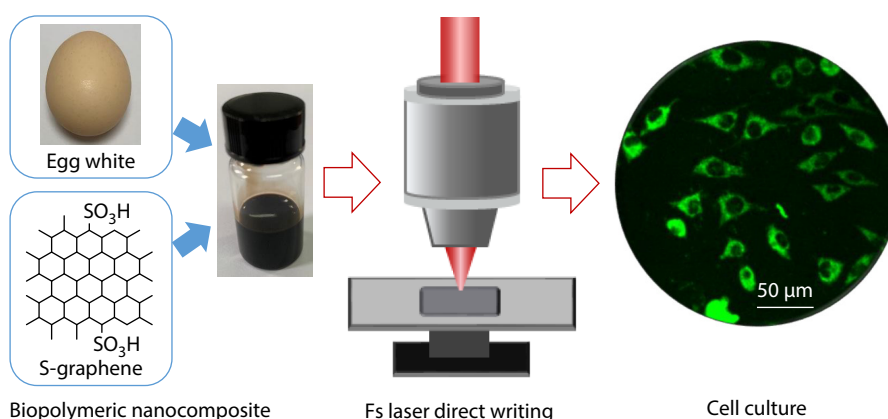
Graphical Abstract

Femtosecond Laser Direct Writing of Biopolymeric Nanocomposite for Biocompatible 3D Features

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We demonstrate femtosecond laser direct writing of 3D microstructures with sub-50 nm resolution using a biopolymeric nanocomposite composed of egg white and sulfonated graphene. The egg-white/sulfonated graphene microstructure has been proven to be biocompatible. This strategy is a perspective for potential applications in biological and photonic fields.



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