Optimized Two-Photon Polymerization Strategies for Fabricating Large, High-Density Micropatterned Scaffolds for Retinal Cell Culture

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Abstract

Advances in additive micro-manufacturing have opened new opportunities for engineering biomimetic scaffolds that can guide cellular organization, stimulate mechanobiological responses, and support tissue regeneration. Among these techniques, Direct Laser Writing (DLW) via two-photon polymerization (2PP) has emerged as a powerful tool due to its capability to fabricate complex three-dimensional microarchitectures with sub-micron resolution. Despite its potential, the geometrical fidelity of 2PP-printed structures remains highly dependent on both the digital design strategy and the selection of fabrication parameters, particularly when targeting large, highly dense micropatterned scaffolds intended for biological applications. In this work, we conduct a comprehensive and systematic study to identify optimal design and fabrication protocols for producing millimeter-scale 3D scaffolds with high-density micropatterning suitable for cell culture and tissue engineering. By using the Photonic Professional GT2 system (Nanoscribe®) and the proprietary photoresist IP-Visio, we investigated four distinct design pathways generated by combining two different CAD geometries with two writing approaches—namely, “contour” and “solid” trajectories. For each strategy, we evaluated and optimized a set of key process parameters, including writing speed, hatching and slicing distances, scaffold overall dimensions, and the hatching strategy. Quantitative assessments of the printed structures revealed that the design choice and scanning trajectory strongly influence pillar morphology and channel uniformity. Through systematic optimization, we identified fabrication protocols capable of producing micropatterned scaffolds with highly accurate geometrical features, particularly in terms of hexagonal pillar cross-sections and inter-pillar channel width—two essential characteristics for achieving consistent reliable cell–material interactions. By using the optimized protocol, we fabricated square scaffolds with side lengths of approximately 4 mm, each incorporating nearly 48,000 hexagonal pillars arranged in a dense periodic lattice. These structures were subsequently seeded with cells from the ARPE-19 retinal cell line, chosen for its relevance in retinal tissue regeneration studies. Preliminary biological observations confirmed efficient cell attachment, viability, and spatial organization across the micropatterned architecture, demonstrating that the enhanced design and fabrication workflow results in scaffolds capable of supporting sensitive cell types. Overall, this study establishes practical guidelines for producing large-area, high-resolution 2PP scaffolds with excellent geometrical fidelity, offering valuable insights for applications in regenerative medicine. The optimized methodologies presented here provide a robust foundation for future developments in engineered microenvironments tailored to study biological processes and guide tissue repair.

Keywords:

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