Supporting Information

An Image-Guided Intrascaffold Cell Assembly Technique for Accurate Printing of Heterogeneous Tissue Constructs

Kevin F. Firouzian^{1,2,3}, Ting Zhang^{1,2,3*}, Hefeng Zhang^{1,2}, Yu Song^{1,2,3}, Xiaolei Su^{1,2,3}, and Feng Lin^{1,2,3s}

¹Biomanufacturing Center, Department of Mechanical Engineering, Tsinghua University, Beijing 100084, China
²Biomanufacturing and Rapid Forming Technology Key Laboratory of Beijing, Department of Mechanical Engineering, Tsinghua University, Beijing 100084, China
³111"Biomanufacturing and Engineering Living Systems" Innovation International Talents Base, Department of Mechanical Engineering, Tsinghua University, Beijing 100084, China
*Email: t-zhang@tsinghua.edu.cn

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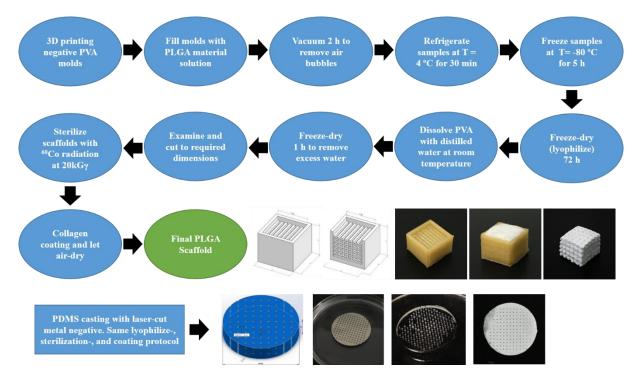


Figure S1. Scaffold fabrication processes. Flowchart highlighting main protocol(s) for creating 500 µm pore diameter poly(lactic-coglycolic acid) (PLGA) spatial-grid design scaffolds with interconnecting pore structure via water-soluble poly(vinyl alcohol) (PVA) negative casting molds and 200 µm pore diameter PLGA scaffolds with axial pore structure via poly(dimethylsiloxane) (PDMS) stamp casting. Negative casting molds are 3D printed with adequate line width and line-to-line spacing to ensure a positive PLGA scaffold with the same design. Scaffold are placed in a vacuum chamber for 2 h to ensure removal of air bubbles to avoid defects during freeze-drying (lyophilization). Samples are first refrigerated and then frozen to ensure gradual temperature decrease and uniform material solidification. Lyophilized of 72 h ensures complete drying and removal of harmful solvents. PVA negative is dissolved in distilled water (frequently changed), at room temperature, during constant stirring. Pre-sterilization, samples are examined and cut/shaped into desired dimensions to remove minor defect/imperfections. PDMS stamp casted scaffolds are made using the same processes albeit with pre-made, laser-cut, metal negatives instead.

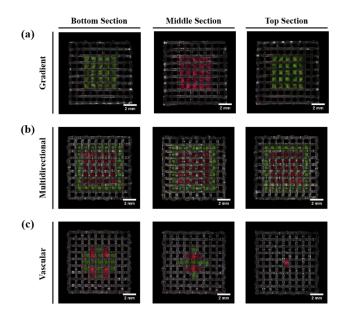


Figure S2. Complex intrascaffold assembly. (a-c) Highlighting four-layer sectional views of bottom-, middle-, and top sections of the gradient-, multidirectional-, and vascular complex structures made via complex intrascaffold assembly of fluorescent dye blended hydrogels within pre-made transparent polylactic acid (PLA) scaffolds. All scaffolds have 500 μ m pore diameter. Images highlight sectional views of the complex structures that were sliced and sectioned post-printing and show intrascaffold assembly structural transitions at four-layer intervals. Areas with varying color intensities as shown in the vascular structure (c) in the bottom- and middle sections refer to material deposition at varying depth within the respective four layers. Scale 2 mm.