

Engineered tissues with perfusable vascular networks created by sacrificial templating of laser sintered carbohydrates

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Introduction: Engineered tissues densely populated with cells rapidly develop a necrotic core in the absence of convective transport of oxygen and nutrients through vascular networks. Several additive manufacturing strategies have emerged for fabricating vessel-like channels, all of which involve the deposition of a sacrificial material whose removal from a surrounding matrix yields an open lumen.¹⁻³ These techniques are limited, either by compatibility with a narrow range of materials,^{2,3} or by poor reproducibility and control over 3D architectures.¹ Here, we introduce laser sintered carbohydrate materials and their use as biocompatible templates for vascular networks. Sintered carbohydrates are ideal sacrificial materials because they are rigid, self-supporting, water-soluble, and biocompatible. 3D vessel networks can be created by encasing sintered carbohydrate templates in diverse hard and soft ECM materials, then dissolving away the templates. This approach provides 3D geometric control, speed, reproducibility, and flexibility with respect to materials.

Methods: Laser sintering of carbohydrates was performed on a custom open-source laser sintering system. A hydrophobic coating was applied before sintered templates were encapsulated in a bulk matrix. Once the bulk matrix was stable, the sintered template was removed by incubation in PBS and the bulk material was analyzed by light or epifluorescent microscopy or by x-ray microcomputed tomography (μ CT).

Results and Discussion: Based on our experience using carbohydrates as sacrificial vascular templates¹, we hypothesized that sintered carbohydrates would provide improved control over the architecture of 3D vascular templates. Indeed, we demonstrated that carbohydrate starting materials can be sintered into 3D filament networks (Figure 1). Sintered filaments were self-supporting, water-soluble, and cylindrical and smooth after post-processing. Laser sintered carbohydrate filament networks were amenable to sacrificial templating in a variety of bulk matrices to yield open, perfusable channels (Figure 2). Specifically, 3D perfusable networks were formed in cell-laden fibrin, polycaprolactone foams, and PDMS. Thus, this approach is expected to be useful for patterning vascular networks in diverse soft tissues, bone, and microfluidic devices.

Conclusions: Sacrificial templating using laser sintered isomalt yields 3D perfusable vascular networks in a diverse array of ECM materials. This novel approach has the potential to meet a major need in the field for reproducible perfusable vasculature within engineered tissue. We expect this technique to be useful for sustaining high cell densities inside large-scale engineered tissues. Independent control of vascular architecture, ECM material, and cell populations also makes this an ideal experimental platform to study angiogenesis, vasculogenesis, interaction between vessels and parenchymal cells, and the effects of flow-perfusion on these phenomena.

1. Miller, J. S. *et al.* Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat. Mater.* **11**, 768–774 (2012).
2. Kolesky, D. B. *et al.* 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. *Adv. Mater.* **26**, 3124–3130 (2014).
3. Bertassoni, L. E. *et al.* Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. *Lab Chip* **14**, 2202–11 (2014).

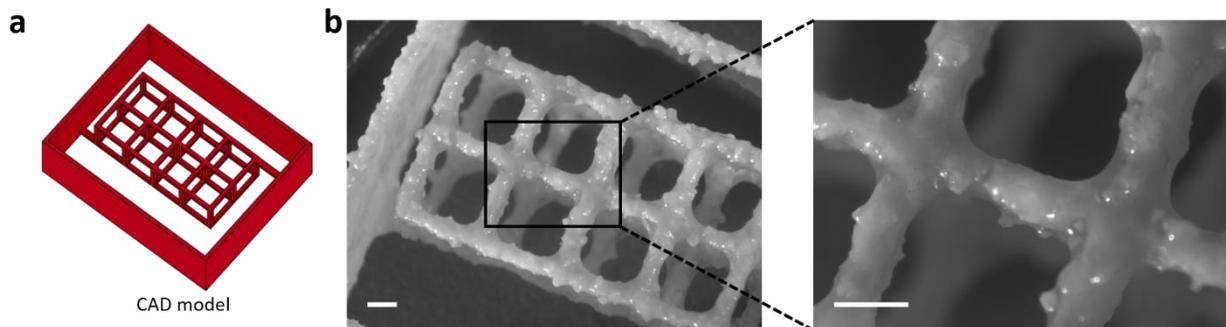


Figure 1. a) A 3D square filament lattice was designed and b) laser sintered from powdered carbohydrates (scale bars = 1mm). Sintered filaments were efficiently fabricated (9 in ~90 mins) with high reproducibility and fidelity on a custom laser sintering platform (github.com/MillerLabFTW/OpenSLS).

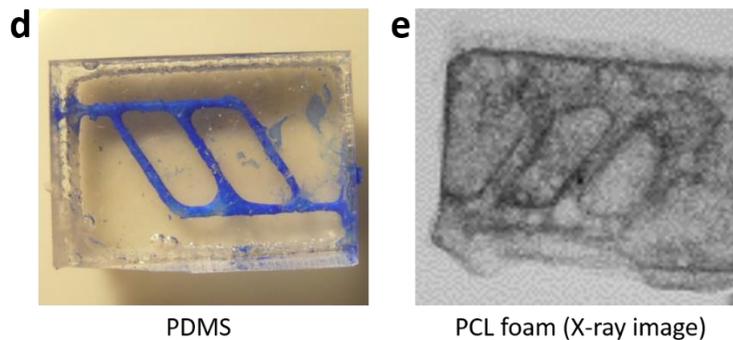
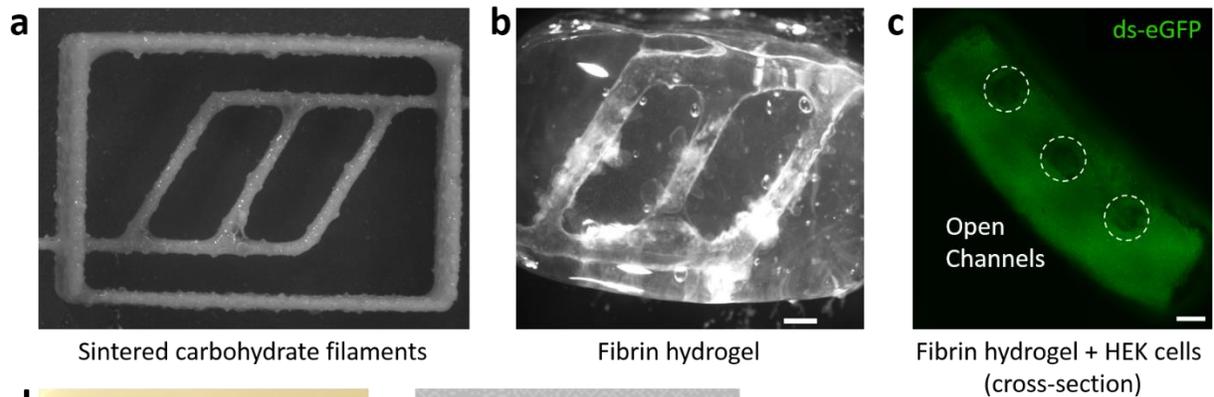


Figure 2. a) A laser sintered carbohydrate ladder network is used as a sacrificial template for open, perfusable channels in diverse materials. Encapsulation in and subsequent dissolution out of b) fibrin hydrogels, c) cell-laden fibrin hydrogels, d) PDMS, and e) PCL foams highlights the flexibility of this approach (scale bars= 1mm).