

Patterning Vasculature

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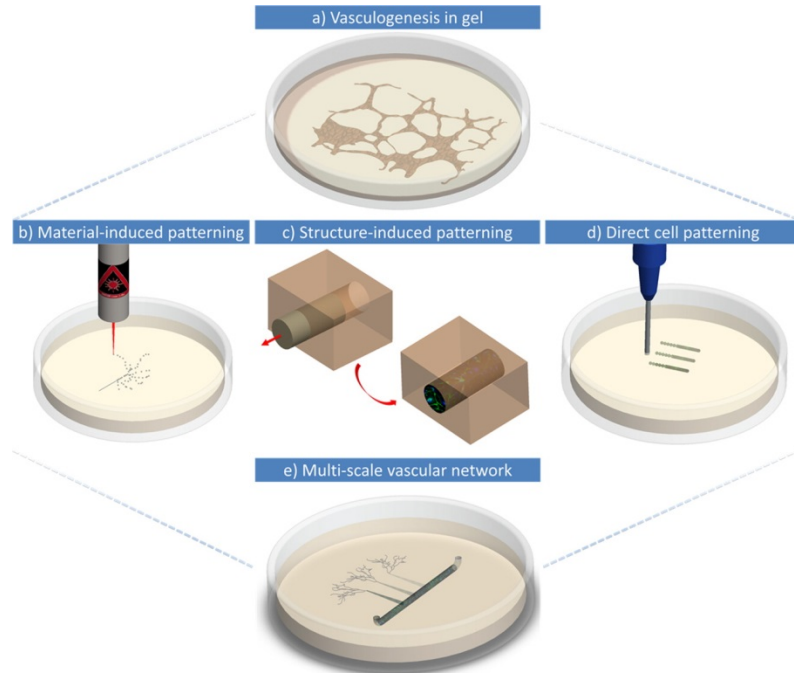
Patterning Vasculature: The Role of Biofabrication to Achieve an Integrated Multicellular Ecosystem

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Traditional Tissue Engineering



- Self-assembly of vessels from randomly seeded endothelial cells within a bulk hydrogel.
- Photo-patterning of physicochemical cues, for instance, cell adhesion peptides (e.g., RGD), growth factors (e.g., VEGF) and protease-sensible cross-links.
- Vascular channel formation through the removal of a channel template within a hydrogel and subsequent endothelialization.
- Bioprinting of cellular spheroids into a biocompatible support (e.g., collagen hydrogel). The spheroids will fuse automatically and self-assemble into vascular structures.
- Combination of biofabrication approaches.

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Vascular Networks

- ▶ Pre-vascularization of constructs is necessary to transplant thick tissues.
 - Diffusion of oxygen and nutrients is limited to 200 μm .
 - Cellular constructs must either undergo neovascularization or be engineered with a vascular network.
- ▶ Scaffold supports are needed that guide cellular self-assembly.
- ▶ In nature vasculogenesis occurs from differentiation of mesodermal progenitor cells into angioblasts. These self-assemble and differentiate into the primary vascular plexus.
 - Angiogenesis can also occur from sprouting endothelial cells (new blood vessels from existing vessels) and splitting of other vessels (intussusception).

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- ▶ Studies have shown that VEGF-releasing hydrogels, hydrogels with covalently bound VEGF, and hydrogels with covalently bound VEGF peptide mimetics are all capable of inducing vascularization in otherwise inert polyethylene glycol (PEG)-based systems.
- ▶ Alternatively, vascular networks will spontaneously form when endothelial cells and appropriate mural cells are cultured together within the hydrogel, with the mural cells providing both angiogenic factors and, crucially, integrating with newly formed vessels to stabilize and further mature nascent vasculature.

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Controlling Vascular Development

- ▶ Material-induced patterning:
 - Selective presentation of physicochemical cues, such as cell adhesion peptides, growth factors, protease-sensible cross-links and/or cross-linking density, to cells within a biomatrix.
- ▶ Structure-induced patterning:
 - Two-step biofabrication processes that consist of the construction of vessel-like architectures through additive or subtractive manufacturing and further lining with endothelial cells.
- ▶ Direct cell patterning:
 - Micromolding or printing techniques to assemble vessels by deposition of vascular specific cells into defined locations

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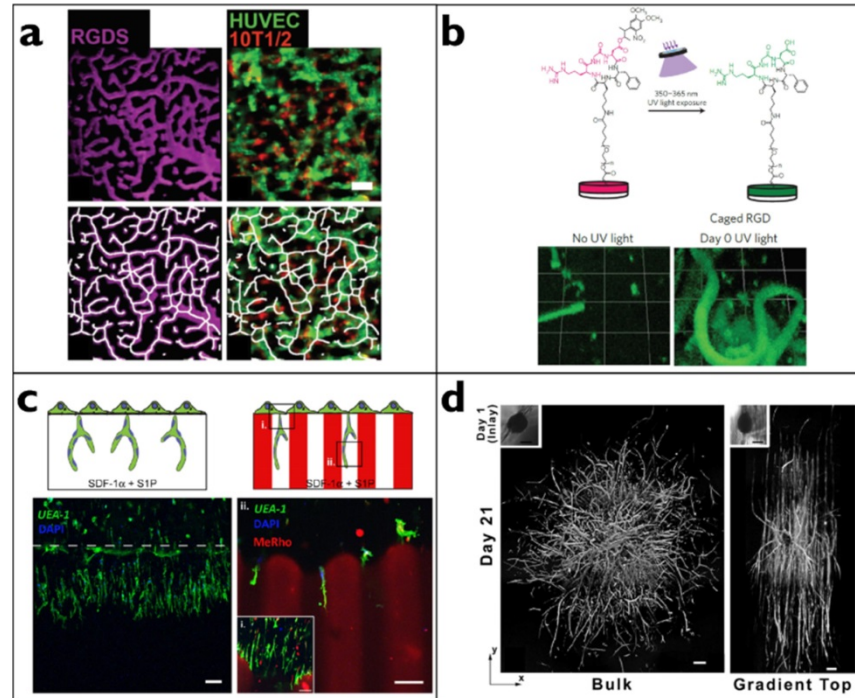
Materials–Based Patterning of Vasculature

- ▶ Growth factors such as VEGF have a direct influence on both vasculogenic and angiogenic processes.
 - Therefore, their presentation to cells in predefined patterns can potentially provide control for region–specific cell differentiation and over sprouting direction.
 - Sprouting angiogenesis anisotropy could also be harnessed with patterning of cell adhesion peptides and MMP–sensible cross–links, which are both required for endothelial cell migration.
 - Spatial control over the degradation of the material can also be utilized to guide vessel formation.

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Materials-Based Patterning of Vasculature

- Photo-patterning of RGD within a degradable PEGDA based hydrogel.
- Photolabile protecting groups, the patterning of RGD sites within a hydrogel can be effected after fabrication of the hydrogel.
- Patterning of MMP cleavable hydrogels with networks that are “permissive” and “nonpermissive” to cellular remodelling.
- complex gradients (d) of MMP cleavable cross-links, RGD concentration, and VEGF concentration have been shown to provide directionality to the growth of vascular networks in hydrogel materials.



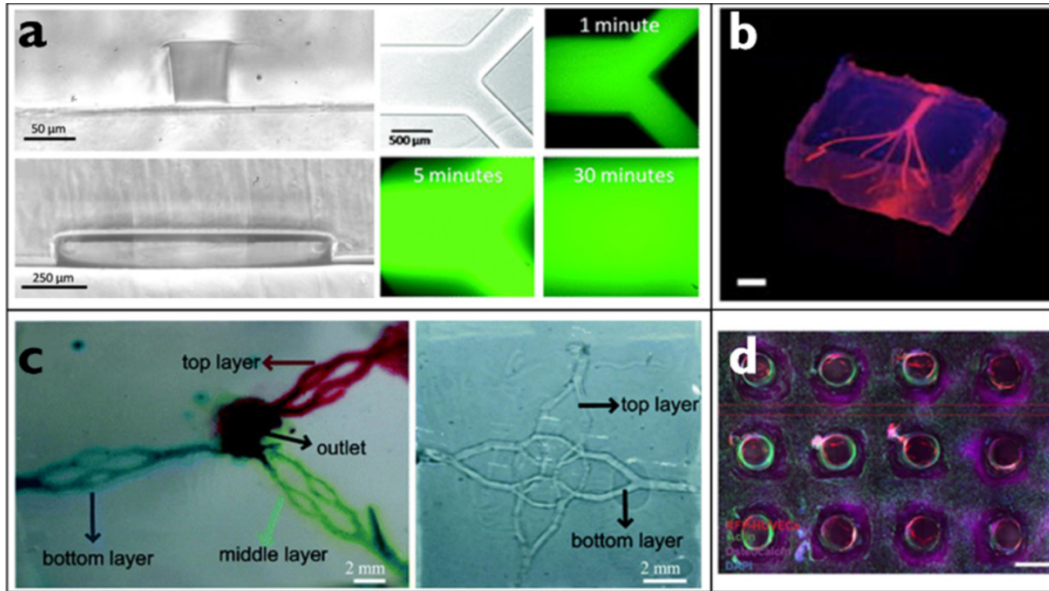
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Structure-Induced Vascularization

- ▶ Integration of cell-laden hydrogels within microfluidic devices for an improved in vivo approximation.
- ▶ Incorporating guiding microchannels within the hydrogel and populating lumens with endothelial cells to increase speed and decrease randomness of vessel formation.
- ▶ Methods include layer by layer casting, removable template molding, and sacrificial molding

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Structure-Induced Vascularization



- Layer-by-layer interface between hydrogel films of agarose with channels.
- Extruded agarose "wires" are used to form microchannel within gelatin (top), shown here with a perfusion of red microbeads in solution.
- A triple layer 3D microfluidic network in gelatin, perfused with different colors (left). An alginate hydrogel formed in a PDMS mold is embedded within gelatin and dissolved using EDTA (right).
- Sacrificial Pluronic F-127/bioprinted hMSCs/fibroblast laden matrix.

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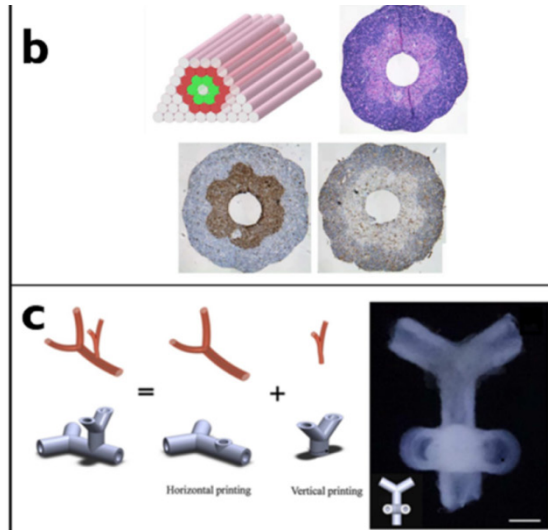
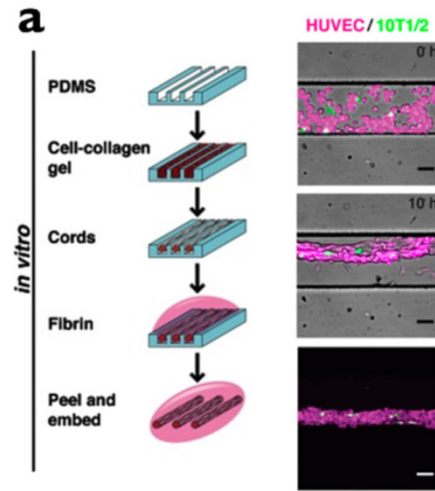
Cell Patterning Strategies

- ▶ Direct cell patterning technologies encompass cell molding, 3D bioprinting, inkjet printing, laser-assisted bioprinting (LAB), and optical guide/tweezers.
- ▶ Faster assembly.
- ▶ Resemble the natural vascular tree with less thrombogenesis and compliance with diffusion limit.
- ▶ Potential for larger anastomosis vessels for implantation.
- ▶ Cell-laden hydrogels are used in tissue micromolding techniques to create geometrically defined and multiscale vessels.
- ▶ Spheroids are multicellular aggregates that can be used as building blocks to construct tissues. Drawbacks include large size, long fusions times and inhomogeneous structures

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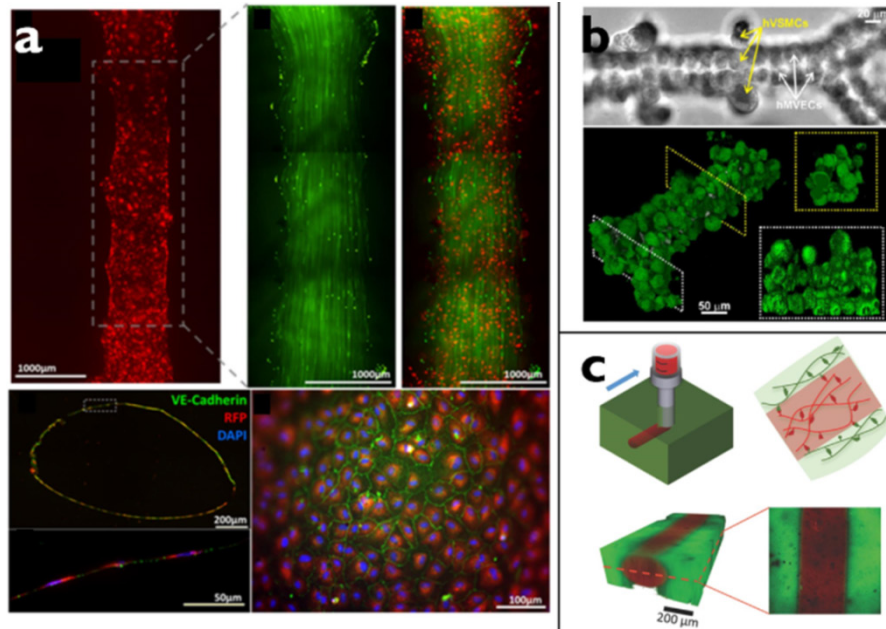
Cell Patterning Strategies

- a) Vascular cords.
- b) 3D printing of concentric multicellular cylinders and enables the formation of a double-layered vascular tube (green represent SMCs and red represents fibroblasts).
- c) Free-from inkjet printing of sodium alginate and fibroblasts into a bath of calcium chloride.



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Combination of Techniques



- Fluorescence imaging of a printed vascular channel fabricated in layer by layer deposition after 1 day of culture on dynamic flow conditions.
- Fabrication of a synthetic capillary via live cell lithography.
- Cell-laden material (guest, red) can be printed into another cell-laden material (host, green) that deforms to accommodate the ink and self-heals to maintain its position

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Summary

- ▶ Pre-vascularization of constructs is necessary to transplant thick tissues.
- ▶ Scaffold supports are needed that guide cellular self-assembly.
- ▶ Approaches include:
 - Material-induced patterning.
 - Structure-induced patterning.
 - Direct cell patterning.