Engineering biomimetic microvascular meshes for subcutaneous islet transplantation

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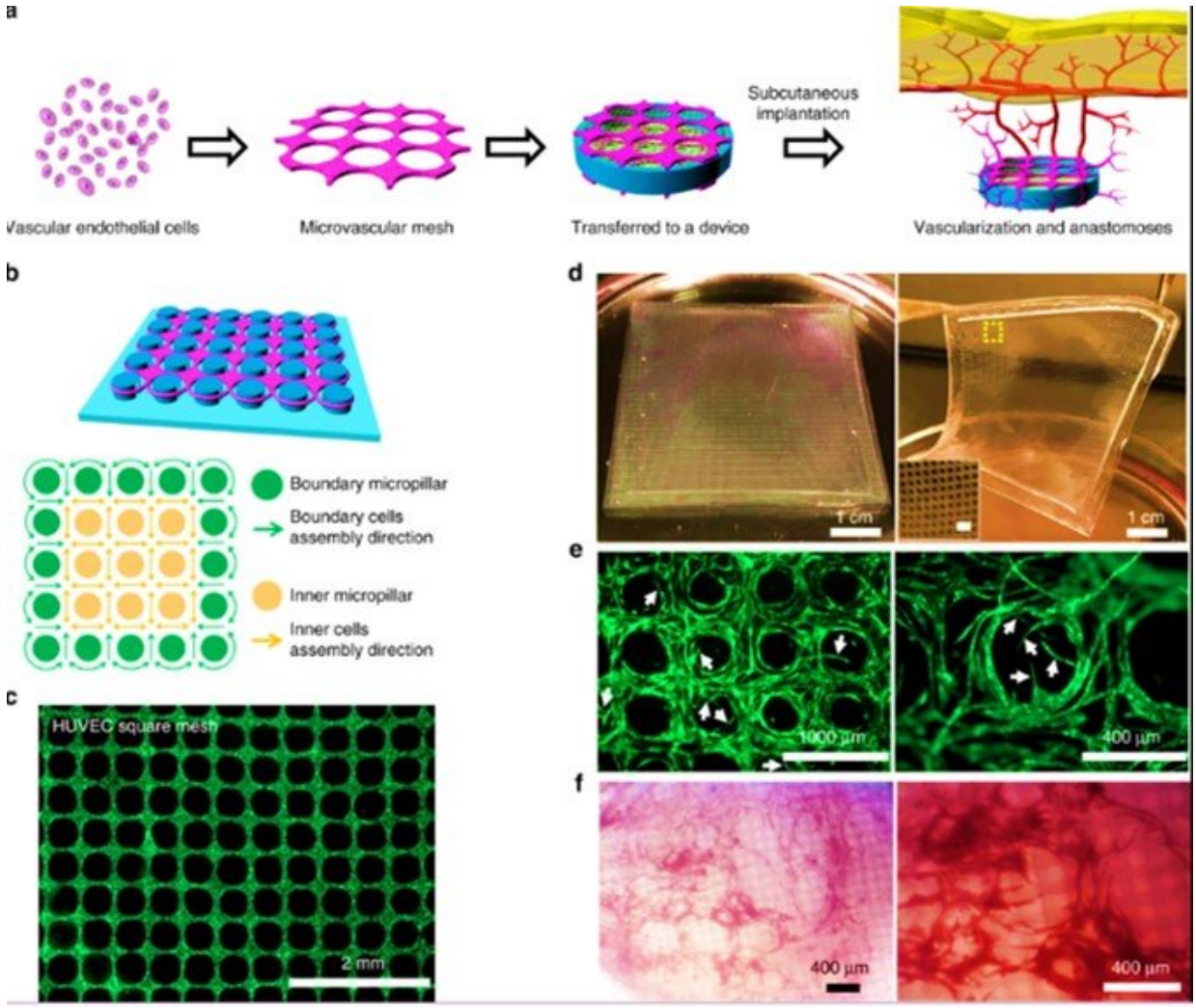


Fabrication and function of transferrable microvascular meshes via anchored self-assembly. (a) Schematic illustration of organization of vascular endothelial cells

into a microvascular mesh, which is transferred and attached to a cellular device. In a poorly vascularized subcutaneous space, microvascular mesh can enhance vascularization and anastomoses with host vasculature to provide oxygen/nutrients to donor cells. (b) Design of micropillar arrangement (blue) and ASA-enabled cell organization (purple): the key to the ASA is that inner micropillars (yellow) provide a geometric template for cell self-assembly to form square mesh and boundary micropillars (green) serve as anchoring points for cell attachment to prevent assembled mesh structure from shrinking. (c) A fluorescent image of HUVECs expressing GFP in fibrin matrix organized into a square mesh (1 × 1 cm) on a micropillar substrate via ASA after 2 days of culture. (d) After 2 days of culture, a HUVEC square mesh (5 × 5 cm) is lifted from the micropillar substrate for transfer. The insert is a magnified image of HUVEC mesh and scale bar is 1 mm. (e) HUVEC microvascular mesh generates angiogenic sprouts (white arrows) when embedded in fibrin matrix during 2 weeks of culture in vitro. (f) Microscopic images of retrieved HUVEC microvascular mesh device show a high degree of vascularization after 2 weeks of subcutaneous implantation in SCID-Beige mice. Credit: Nature Communications, doi: 10.1038/s41467-019-12373-5

To successfully engineer cell or tissue implants, bioengineers must facilitate their metabolic requirements through vascular regeneration. However, it is challenging to develop a broad strategy for stable and functional vascularization. In a recent report on *Nature Communications*, Wei Song and colleagues in the interdisciplinary departments of Biological and Environmental Engineering, Medicine, Mechanical and Aerospace Engineering, Clinical Sciences and Bioengineering in the U.S. described highly organized, biomimetic and resilient microvascular meshes. The team engineered them using controllable, anchored self-assembly methods to form microvascular meshes that are almost defect-free and transferrable to diverse substrates, for transplantation.

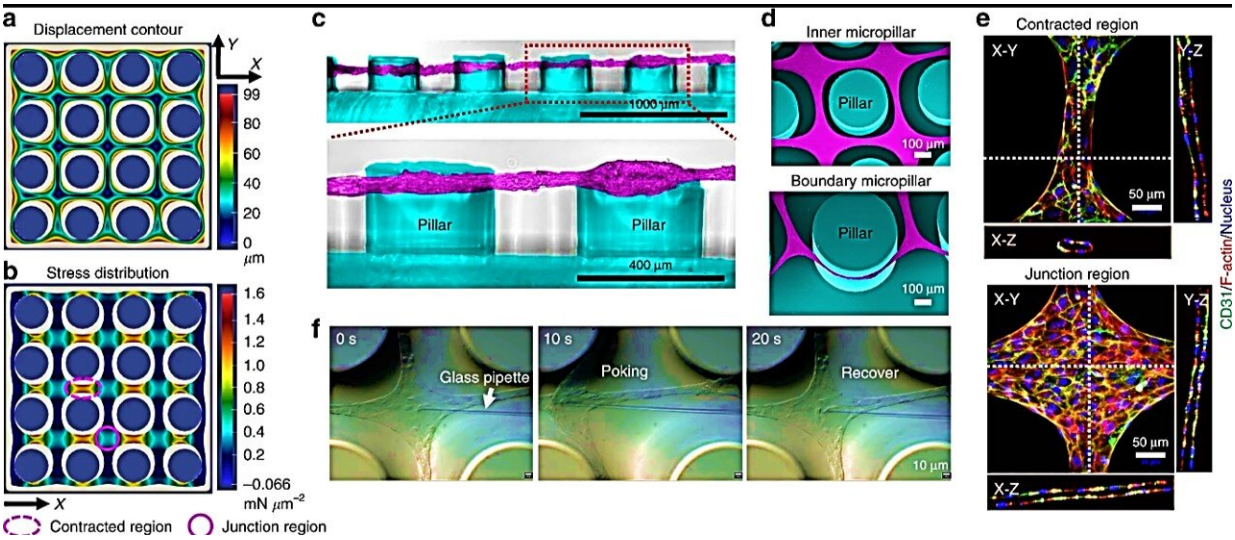
The scientists promoted the formation of functional blood vessels with a density as high as ~200 vessels per mm^{-2} within the subcutaneous space

of SCID-Beige mice. They demonstrated the possibility of engineering microvascular meshes using human induced pluripotent stem-cell (iPSCs) derived endothelial cells (ECs). The technique opens a way to engineer patient-specific type 1 diabetes treatment by combining microvascular meshes for subcutaneous transplantation of rat islets in SCID-beige mice to achieve correction of chemically induced diabetes for 3 months.

Vasculature is an essential component of any organ or tissue, and vascular regeneration is critical to successfully bioengineer implants. For instance, during cell replacement therapy for type 1 diabetes (T1D), transplanted insulin producing cells rely on the vasculature to function and survive. Bioengineers often use vascular endothelial cells such as human umbilical vein endothelial cells (HUVECs) to spontaneously assemble into tubular structures within the extracellular matrix (ECM). But the resulting structures can be random, uncontrollable and less efficient for microvascular regeneration. Scientists have recently developed three-dimensional (3-D) printing techniques to engineer controlled cellular constructs with embedded vessels. However, it remains challenging to 3-D print resilient and transferrable, high-resolution, microvasculature.

Bioengineering the microvascular meshes

In the present work, Song et al. engineered a microcapillar-based, anchored self-assembly (ASA) strategy to create controllable, transferrable and scalable microvascular meshes for T1D cell treatment therapies. During ASA, the researchers implemented the micropillars to guide self-assembly of endothelial cells within a fibrin matrix. The pillars served as anchoring points to prevent structural shrinkage during cellular maturation for controllable and resilient microvascular meshes.



Simulation and characterization of the ASA-enabled microvascular meshes. (a, b) The contraction simulation shows an in-plane displacement contour plot of organized cellular mesh structure (a) and the normal stress distribution in the X (Cauchy stress component 11) direction (b) on a 4 × 4 micropillar substrate. The initial shape of cells and fibrin matrix is displayed in light gray. The micropillar diameter is 400 μm and micropillar-to-micropillar interval is 200 μm. The contracted region is marked as dotted purple ellipse and the junction region is purple circle. The displacement unit is μm and the unit of stress is mN μm−2. (c) Cross-sectional images showing a HUVEC mesh suspended between micropillars. The micropillars are pseudo-colored as blue and HUVECs are pseudo-colored as purple. (d) SEM images of a HUVEC mesh (purple) at the inner and boundary regions on the micropillar substrate (blue). (e) Confocal images of a HUVEC mesh at the contracted and junction regions on the micropillar substrate showing the tubular structures. Human CD31 antibody is green, F-actin is red, and nucleus is blue. (f) Screenshots of a glass pipette poking a HUVEC mesh showing high resilience of the mesh. Credit: Nature Communications, doi: 10.1038/s41467-019-12373-5

The research team tuned the dimension and arrangement of micropillars to control the geometry of the microvascular mesh. On transplantation,

the microvascular meshes promoted the formation of new blood vessels (neovascularization) and vascular anastomoses (connection between two vessels). They developed the meshes using both HUVECs and human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) suited for patient-specific microvasculature development. Using both types of microvascular meshes, the research team significantly improved vascularization, and corrected diabetes in chemically induced, i.e., streptozotocin (STZ)-based SCID-beige mice for three months. The work provides proof of concept for the use of microvascular meshes in cell replacement therapies for T1D and other diseases.

To achieve rapid and functional vascularization around a cellular device, the research team attached a preformed vascular structure with sufficient density and resolution to the device. After transplantation, the structure induced angiogenesis and promoted anastomoses (connection between two vessels) with the host vasculature. During the process, the researchers observed HUVECs together with a fibrin matrix to self-assemble into an almost defect-free square mesh after 2 days of cell culture on a micropillar substrate. They adjusted the size and arrangement of micropillars to precisely control the mesh geometry and dimension. The cells remained viable and stable on micropillar substrates for at least 4 weeks and rapidly generated sprouts on transfer to embed within a fibrin matrix. Due to constant remodeling, the mesh network did not preserve their original shape.

Simulating and experimentally testing the microvascular meshes

A large piece of HUVEC mesh (5×5 cm) is being lifted up from micropillar substrate and microvascular mesh can be easily transferred to other devices or substrates. Credit: Nature Communications, doi: 10.1038/s41467-019-12373-5

To understand the ASA process, the researchers conducted finite element analysis simulation of the cell assembly process and further tested the microvascular mesh. The simulation considered the contractility of cells on the fibrin matrix to generate an in-plane displacement contour plot of organized mesh structure and stress/strain distribution on the micropillar substrate. The experimental results were consistent with the simulation to validate that microvascular meshes were tightened and suspended between micropillars instead of settling at

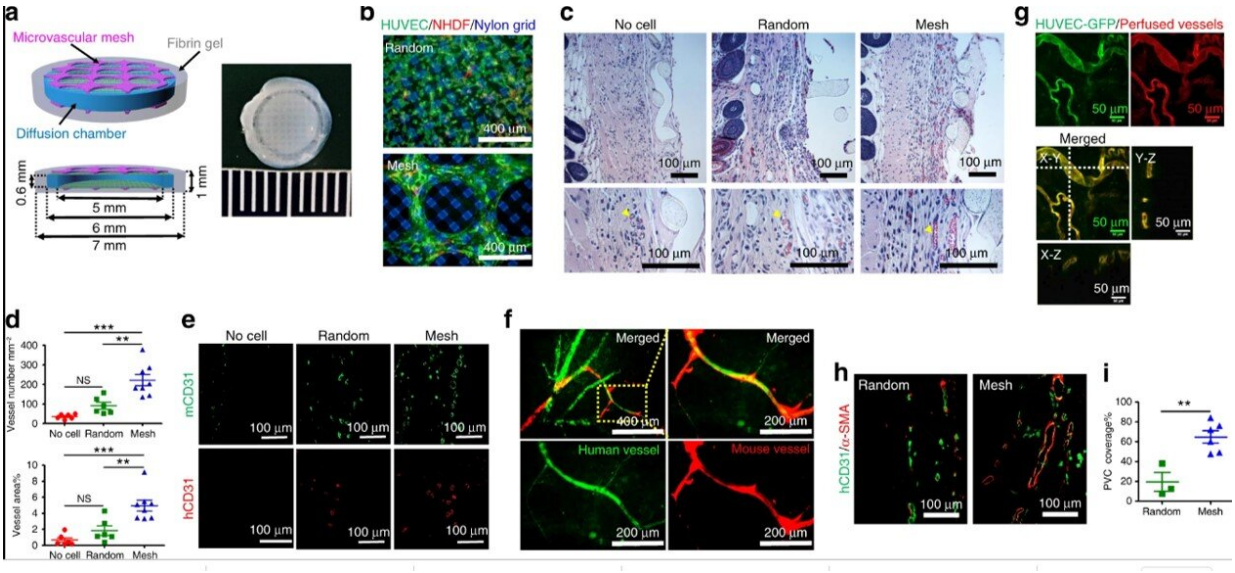
the bottom of the culture setup. The work supported the hypotheses that stable cell constructs formed by relying on the micropillars. The meshes were also elastic, although mechanically robust and resilient to even withstand poking with a glass pipette. The scientists used these remarkable properties to manipulate and transfer the mesh to diverse substrates without affecting its integrity.

Improved vascularization of micro meshes in SCID-Beige mice.

The research team investigated how microvascular meshes enhanced vascularization by comparing normal HUVEC meshes with normal human dermal fibroblast (NHDFs) added constructs. The combination supported and enhanced vessel formation. The scientists transplanted the constructs within the subcutaneous space of SCID-Beige mice. The subcutaneous space is a poorly vascularized area offering many advantages for cell replacement therapy, including easy access, minimal invasiveness and high transplant capacity. After two weeks, the scientists retrieved the devices and compared vascularization using histology with hematoxylin/eosin (H&E) staining to quantify the blood vessels surrounding the chambers. They revealed a highly vascularized mesh device compared to the control mesh without cells. Immunohistochemical staining further revealed the newly formed vessels to be chimeric in nature, indicating anastomoses and vascular remodeling during vascularization.

To further confirm anastomoses, Song et al. perfused mice with mesh devices via tail injections using two lectin dyes (labeled with green fluorescence protein dye—GFP and a red dye). The overlap of the two different lectin strains confirmed the formation of blood-perfused human vasculatures that anastomosed with the mouse vascular meshes after implanting the HUVEC-GFP/NHDF within the mouse vascular

system at different time frames. The results substantiated the ASA-enabled process to transfer microvascular meshes to vascularize cell delivery devices.



Enhancement of vascularization and anastomoses in subcutaneous space of SCID-Beige mice. (a) Schematics and a digital photo of a Mesh device, which is a diffusion chamber with HUVEC meshes (~25 μm thick, purple) in the fibrin gel (gray) on the top and bottom. The diffusion chamber is a cylindrical cell container with a PDMS ring (blue) as the wall and two nylon grids (green) as the top and bottom. The dimensions of each component are labeled in the schematic. (b) Fluorescent images of randomly mixed cells (top) and microvascular mesh (bottom) placed on diffusion chambers after 2 days of culture in EGM-2 medium. HUVEC:NHDF = 9:1, Human CD31 antibody is green to show HUVEC, α-smooth muscle actin (α-SMA) antibody is red to show NHDF, and nylon grid is blue. (c) Cross-sectional hematoxylin/eosin staining images of retrieved devices after 14 days of implantation. Yellow arrowheads point to blood vessels with erythrocytes inside. (d) Density and area percentage of blood vessels at the interface between the device and panniculus carnosus muscle. n = 6 in the No cell and Random, and n = 8 in Mesh groups. Data are mean ± SEM; **P