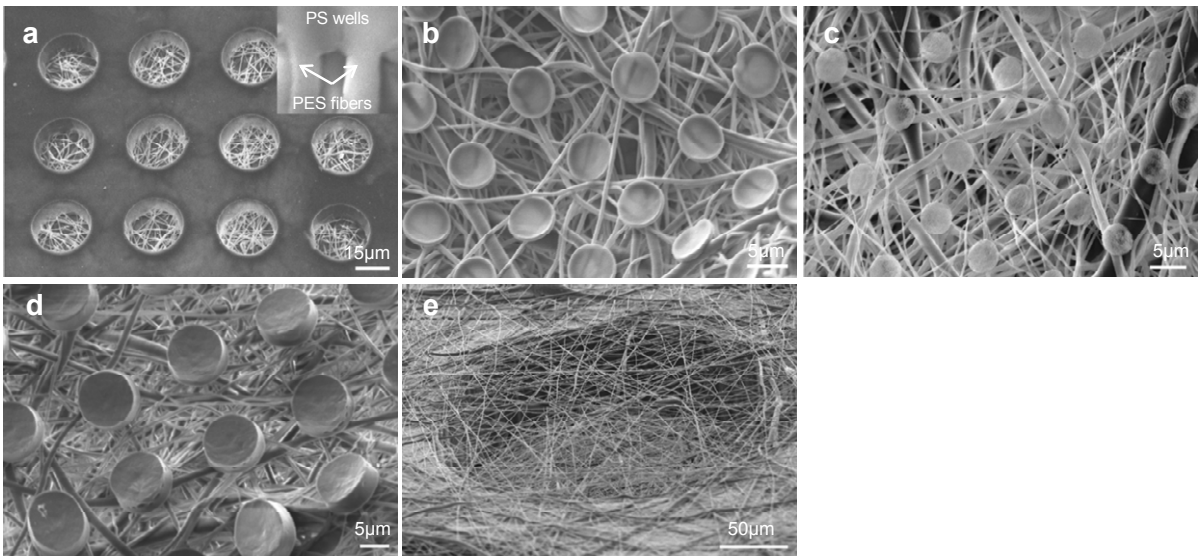

Supplementary Figure 1 (a) Temperature-assisted device assembly and (b-e) fabrication of micro/nano devices with more complex microstructures. (a) Micro/nano device (20 μm PS wells/electrospun PES fibers) assembled using temperature-assisted bonding. The inset shows a higher magnification micrograph (20,000X) of the well-fiber interface illustrating a smooth fusion between the two polymers. **(b-d)** Arrays of discrete cylindrical microstructures (PCL, PLGA, and PVDF respectively) interfaced with mats of electrospun PCL fibers. **(e)** PCL microwell (300 μm) array sandwiched between electrospun PCL fibers.

Supplementary Figure 1 Notes

Temperature-assisted device fabrication: we demonstrated that temperature-assisted bonding could also be employed in the assembly of micro/nano devices. This could be particularly important in cases where the presence of residual solvent in the freshly deposited fibers is not sufficient to achieve a strong bond between the microstructures and the fibers. For demonstration purposes, a PS microwell array was first placed on a sheet of electrospun PES fibers and a pressure of 5-10 psi was applied at 60-100°C for 10-20 sec. This temperature softened the surface of the microwell array and provided strong bonding between the wells and the fibers. This approach can be used when the physical and chemical integrity of the individual parts is not affected by the temperature used.

Fabrication of micro/nano devices with more complex microstructures: cylindrical microstructures, made from different polymers (PCL, polylactic-co-glycolic acid-PLGA-, polyvinylidene fluoride-PVDF-), were patterned on a sacrificial polyvinyl alcohol (PVA) layer following a procedure previously developed by J. Guan, *et al*.[1] The fibers were then spun on the microstructures, and the PVA layer was dissolved in deionized water. The microstructures remained attached to the electrospun fibers. The sandwiched microwell configuration was achieved by sequentially electrospinning fibers on both sides of the array (10 min on one side, and 30 sec on the other). Micro/nano devices with more complex structures could be implemented in a number of applications, including the development of biomimetic tissue engineering scaffolds capable of more closely resembling structural and chemical cues presented by the extracellular matrix (ECM) *in vivo*, which typically possesses features of different length

scales, morphologies and compositions.[2,3] The sandwiched microwell configuration could potentially be used to improve hepatocyte functions even further by both inducing/controlling cluster formation and presenting appropriate polarity cues.[4]

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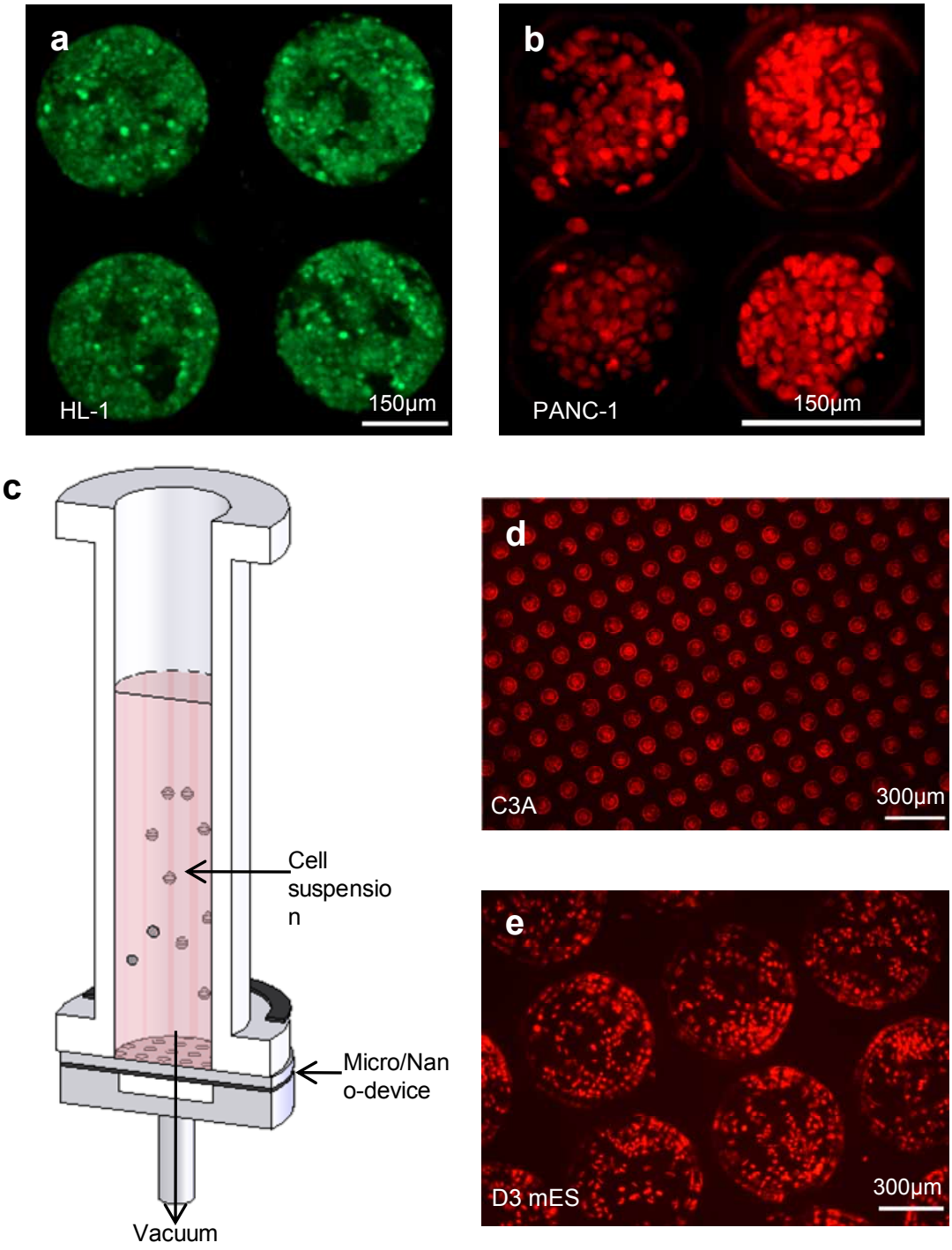


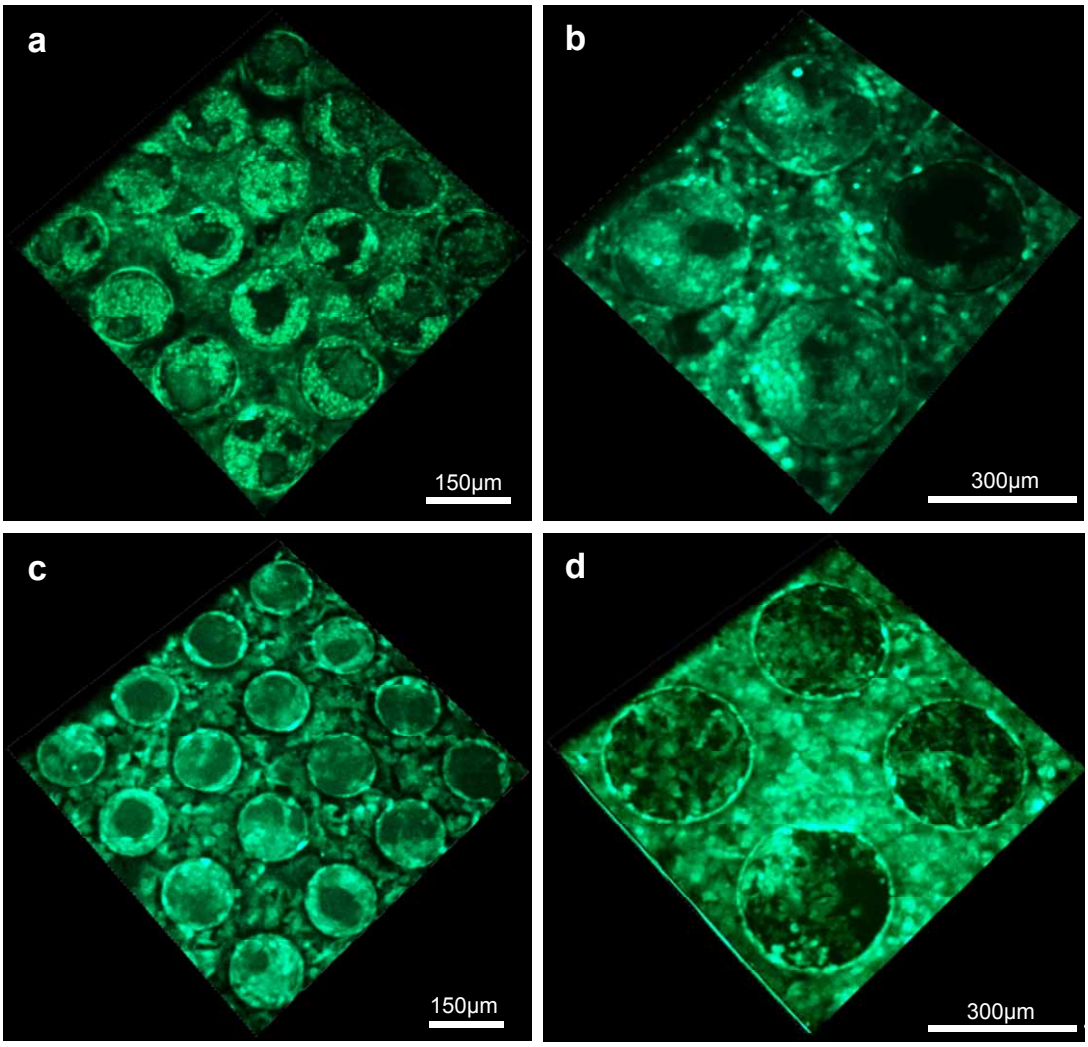
Supplementary Figure 2 Preferential seeding and guided cluster formation with other cells of interest. (a, b) Fluorescence microscopy images of HL-1 mouse cardiomyocytes and PANC-1 human ductal epithelial-like cells passively seeded (i.e. cell settling) on the micro/nano device (PS wells/PCL fibers). The cells were cultured for 2 and 4 days, respectively. Live HL-1 cells were stained green with calcein, and the nuclei of post-fixed PANC-1 cells were stained red with

PI-RNase. **(c)** Schematic illustration of the active vacuum-assisted cell seeding procedure. **(d, e)** Fluorescence images of C3A human hepatoblastoma cells and D3 mouse Embryonic Stem (mES) cells preferentially placed inside the wells of the micro/nano (PCL wells/PCL fibers) device using vacuum-assisted seeding. These cells were cultured for 2 h after seeding, and the nuclei were stained red with PI-RNase after fixation.

Supplementary Figure 2 Notes

Cell maintenance: D3 mES cells (ATCC) were maintained on 0.1% gelatin (Sigma)-coated tissue culture flasks in high-glucose Dulbecco's modified Eagle's medium (DMEM, ATCC) supplemented with 15% fetal bovine serum (FBS) (Invitrogen), 2mM L-glutamine (Invitrogen), 0.1 mM nonessential amino acids (Invitrogen), 1 mM sodium pyruvate, penicillin (50IU/mL), and streptomycin (50mg/mL) (Invitrogen). Leukemia inhibitory factor (LIF, 1000units/mL; Chemicon, Temecula, CA) was added to keep mES cells in the undifferentiated state. Mouse cardiomyocytes (HL-1) were cultured on gelatin/fibronectin (1mg of fibronectin in 80 mL of 0.02% gelatin)-coated flasks in Claycomb medium (Sigma) supplemented with 10% FBS (Sigma), 2mM L-glutamine (Invitrogen), penicillin–streptomycin (100U/mL–100*m*g/mL; Invitrogen), and 0.1mM norepinephrine (Sigma). Human ductal epithelial-like cells (PANC-1) were maintained in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin (100 U/mL–100*m*g/mL; Invitrogen). All cell lines were incubated in a humid atmosphere at 37°C and 5% CO_2.

Vacuum-assisted cell seeding: Cells were selectively placed in the microwells using a microanalysis vacuum filtration setup (Fisher Scientific). The platform was clamped between a glass funnel and the fritted glass base. A cell suspension in culture medium with the desired cell concentration was placed in the funnel, and vacuum (≥20KPa) was applied to pull the medium through the electrospun fibers of the platform and trap the cells in the microwells. The platforms were then removed from the filtration apparatus and incubated under normal culture conditions. In some cases the cells can get pulled a few micrometers into the fibrous scaffold. This depends on the vacuum pressure, size of the cells, and pore size of the fiber mat. Preliminary experiments (data not shown) have revealed that this could interfere with clustering in some cases, as the cells seem to increase interactions with the fibers, and decrease cell-cell contacts. Optimum vacuum-assisted cell seeding parameters for achieving cluster formation are currently being studied and will be addressed in a future publication.



Supplementary Figure 3 C3A cells cultured on conventional microwells. (a, b) 4 day culture. **(c, d)** 8 day culture.

Supplementary Figure 3 Notes

C3A cells cultured on conventional microwell arrays (i.e. no fibrous bottom) showed a spatially disorganized growth pattern. The cells were not successfully confined within the wells. Instead, they populated the entire surface of the array (well bottoms and walls, and the outside of the wells), forming monolayers in some regions, and small aggregates in others. We believe this is a result of the disorganized cell distribution obtained after the PBS rinsing step during cell seeding (already illustrated in figure 2C). This clearly shows an advantage of our design, where topographical cues from the fibrous well bottom presumably allow for more adequate cell positioning within the wells, which results in microtissues that adjust better to the geometrical parameters of the microwell array.

Supplementary Table 1 Fabrication parameters for the microwell arrays.

Type of microstructure		Polymer solution		Spin speed (rpm)
Geometry	**Microwell Dimensions**	**Polymer**	**Concentration (w/w)**	
Circular wells	20 µm diameter, 40 µm spacing (center-center), 10 µm thickness	PS	10%	2000
		PCL		
	150 µm diameter, 180 µm spacing (center-center), 100 µm thickness	PS	20%	2000
		PCL	25%	
	300 µm diameter, 360 µm spacing (center-center), 100 µm thickness	PS	20%	2500
		PCL	25%	
Triangular wells	Equilateral triangles (300 µm side) with 100 µm wide spacing lines, and 100 µm thick	PCL	25%	1500
Square wells	30 µm side, 50 µm spacing (center-center), and ~8 µm thick	PCL	10%	2000