# Fabrication of a modular tissue construct in a microfluidic chip

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# Supplemental information

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#### **Cell culture**

We cultured NIH 3T3 fibroblast cells (American Type Culture Collection, Rockville, MD) in Dulbecco's Modified Eagle Medium with *L*-glutamine (Invitrogen, Carlsbad, CA), 10% fetal calf serum (Sigma-Aldrich), and 2% penicillin and streptomycin (Invitrogen) in 25 cm tissue culture flasks at 37 °C in a humidified atmosphere of 5% CO, 95% air. Each week, we fed the cells with fresh medium and sub-cultured the cells (1:20 splitting ratio).

#### **Confocal microscopy**

Modules were fixed with 3% formaldehyde (20 minutes, room temperature) and stained with 10  $\mu$ M carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene) for 30 minutes at 37 °C, and with rhodamine-labeled phalloidin (Molecular Probes) and 4',6-diamidino-2-phenylindole (DAPI; Invitrogen) according to the provided instructions. Samples were mounted with Gel/Mount (BioMeda) between slides coated with poly-*L*-lysine (Fisher) and a coverslip; Kim-Wipes were used to remove excess liquid.

We used a Zeiss LSM 510 system with three diode lasers (488 nm, 548 nm, 633 nm) and a two-photon laser (Coherent Chameleon) to collect confocal images. We imaged the modules with oil-immersion lenses (25x, 40x, 63x).

#### Mutli-color labeling of modules

Modules were stained with using the Vybrant cell staining kit (Molecular probes) according to the manufacturer's instructions.

#### Organization of glass and agarose objects in a microfluidic channel

To allow studies with multiple cell types, we used a microchannel to template the assembly of modules into an ordered structure (we used methods similar to those of previous work, in which glass beads were ordered in microchannels1). To explore the differences between the packing of rigid spheres (as has been described previously) and that of soft gel cylinders, we first examined the packing of rigid objects (260-µm diameter glass beads) in microchannels of various widths; then we repeated those packing experiments with a stiff gel (agarose), and then with our cell-containing gel modules. Figure 3A-C shows light microscopic images of glass beads assembled in microfluidic channels of different widths, and Figure 3D-F shows similar images of soft cylindrical agarose modules within a channel. When objects of any material (glass beads, agarose cylinders, or gel-containing collagen modules) packed in channels much wider than the templated objects, random packing of the components occurred. In channels approximately twice the width of the objects, the glass beads packed into well organized structures, and soft cylindrical modules of both agarose and collagen became noticeably more organize. Soft components did not become as ordered as the rigid glass beads, however.

Channels with widths comparable to the dimensions of the objects being packed resulted in the greatest degree of organization. The glass beads formed a single line, as expected. Since two orientations were possible for the cylindrical agarose modules, the narrow channel templated their

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packing into a single line, but their orientations varied. Decreasing the height of the microchannel

forced the agarose modules to lie along their length in one orientation.

## References

1 H. Wu, V. R. Thalladi, S. Whitesides, and G. M. Whitesides, J. Am. Chem. Soc., 2002, 124,

14495-14502.

Supplemental figures

Fig. S1 Schematic of modular tissue engineering.

