Supporting Information

Lymphangion-Chip: a microphysiological system which supports co-culture and bidirectional signaling of lymphatic endothelial and muscle cells

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Supplementary Figure



Figure S1 LMCs orientation exposed to normal shear in monoculture and co-culture conditions. Representative images when LMCs are exposed to normal shear value of 1 dyne/cm² and are either in monoculture or in co-culture with LECs. LMCs alignment shifts significantly toward circumferential direction (perpendicular to flow direction) while co-cultured with LECs.



Figure S2 Fabrication technique of cylindrical Lymphangion-Chip. (A) The device was fabricated by bonding PDMS (made of 3D printed mold) to PDMS-coated glass slide and pretreated to enhance PDMS-collagen adhesion strength. Then, the device was degassed in vacuum to remove the air trapped in the porous polymerized PDMS. (B) The hydrogel-LMCs mixture was perfused into the device by producing vacuum using a syringe connected to the outlet. Then, the devices were rotated 90° (vertical position) so that the microfluidic channel aligned parallel to the direction of gravity. Keeping the device in vertical position, a curved tip filled with LMC medium were added to the inlet while rotating the outlet tip so that both tips share a horizontal plane (equal level) for the cell medium not to pour out of the device. In this case, the less viscous fluid (cell medium) would wash through high viscous fluid

(hydrogel) and form 3D symmetrical lumen. (C) The device was kept for 7 minutes inside the incubator and syringe tips were removed and replaced with cell medium droplet immediately to prevent adhesion of plastic tip to polymerized hydrogel. After 30 minutes, tips filled with fresh medium were inserted into the microfluidic channel. D) After one day, LECs were seeded on top of the hydrogel-LMCs in four steps (each 40 minutes) to form a confluent layer of endothelium.