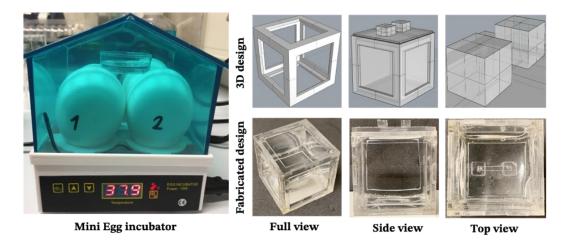
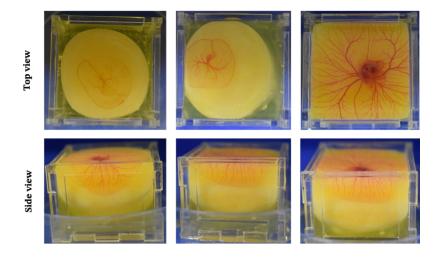
Electronic Supplementary Material (ESI) for Lab on a Chip. This journal is © The Royal Society of Chemistry 2024

## **Supplementary Figures**

**\$1.** Egg incubator and engineered eggshell design

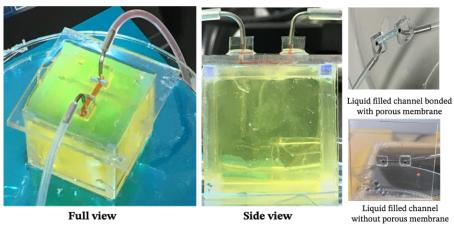


**S2.** Fertilized egg showing CAM vasculature cultured within engineered eggshell



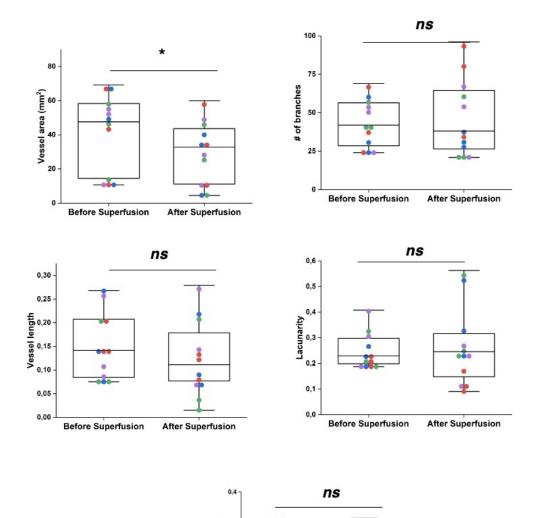
**S3.** Proof-of-concept test: Impact of superfusion on porous membrane

## Superfusion on porous membrane integrated with flow channel



S4. Statistical test

Statistical significance was analyzed using OriginPro V.9.85 performing Wilcoxon signed Rank Test (non-parametric statistical test) due to limited sample numbers. Differences were considered statistically significant with P<0.05. Data shown here are from 3 independent experiment samples, with at least four region of interest (ROIs) per sample.



nm) vasei diameter (ng. 0,2		*	
0,0	Before Superfusion	After Superfusion	
	n. valua		

p- value					
Morphology parameter	S1 (BS vs AS)	S2 (BS vs AS)	S3 (BS vs AS)	All samples (BS vs AS)	
Vessel area	0,20124	0,10035	0,10035	0,00326	
Vessel diameter	0,58388	0,36131	0,85513	0,50474	
Branch number	0,36131	0,85513	0,20124	0,84452	
Lacunarity	0,85513	0,85513	0,85513	0,84452	
Vessel length	0,58388	0,58388	0,20124	0,06526	

## **Supplementary Videos**

**Supplementary video 1a**: Red blood cells flowing through multi-sized venous vessels of vascularized CAM tissue.

**Supplementary video 1b**: Red blood cells flowing through multi-sized micro capillaries of vascularized CAM tissue.

Supplementary video 2a: Red blood cells flowing through large artery of vascularized CAM tissue.

Supplementary video 2b: Red blood cells flowing through artery-veins of vascularized CAM tissue.

**Supplementary video 2c**: Red blood cells flowing through artery, microcapillaries and veins of vascularized CAM.

**Supplementary video 3a:** Chick embryo containing hierarchical vascular networks cultured within engineered eggshell system – chick embryo moving within engineered eggshell system.

**Supplementary video 3b:** Chick embryo containing hierarchical vascular networks cultured within engineered eggshell system – integrated microchannel perfusion on CAM vasculature.

**Supplementary video 4a:** External flow application within microchannel integrated on CAM within engineered eggshell system – introducing external fluids via an integrated microchannel.

**Supplementary video 4b:** External flow application within microchannel integrated on CAM within engineered eggshell system – red blood cells moving within capillaries by external fluid perfusion.

**Supplementary video 5:** Proof-of-concept testing of integrated porous membrane to microfluidic channels within engineered eggshell system.