Lab on a Chip

ELECTRONIC SUPPLEMENTARY INFORMATION

Shear and Endothelial Induced Late-stage Calcific Aortic Valve Disease-on-a-chip Develops Calcium Phosphate Mineralizations

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Supplementary Methods

Microchannel fabrication

The flow channel was created using a fabricated wafer mold by photolithography with negative photoresist (HARE SQ-50, KemLab). A 4-inch silicon dioxide wafer (1 µm oxide, University Wafer) was chemically-cleaned in 5:1 acid Piranha solution (5-parts sulfuric acid (H_2SO_4) to 1-part hydrogen peroxide (H_2O_2)) for 25 minutes to remove surface contaminants and dried on 195°C hotplate for 15 minutes. The HARE SQ-50 product produced a 50 µm channel depth following manufacturer's recommended spin settings: 500 RPM at 100 RPM/second for 5 seconds, 2000 RPM at 300 RPM/second for 30 seconds. The pre-soft bake consisted of the following: 65°C for 5 minutes, 95°C for 15 minutes, 65°C for 1 minute, and finally to room temperature. Then, developer solution (SU-8 developer, MicroChem) was used for the edge bead removal, followed by a bake step at 65°C for 2 minutes. The photoresist-coated wafer, Mylar patterned mask (Infinite Graphics Inc.), and quartz slab were placed into the mask aligner (SUSS MicroTec) and exposed to UV for 40 seconds hard contact, followed by the post-exposure bake (65°C for 1 minute, 95°C for 5 minutes, 65°C for 1 minute, and finally room temperature). Finally, the microchannel pattern was chemically-developed (SU-8 developer, MicroChem) in two baths of 4-minutes and 2-minutes, cleaned off with isopropyl alcohol, and dried using nitrogen gas leaving behind a wafer mold for casting.

Device design

PDMS was mixed at 9:1 ratio of base to curing agent, degassed and poured onto the wafer mold to cure for 4 hours at 80ºC for the microchannel and into a custom vertical glass mold to cure for 6 hours at 80°C for the middle layer. PDMS pieces were then prepared and bonded to glass with a corona discharge wand (BD-20AC, Electro Technic Products), utilizing high frequency and high voltage to alter surface properties. The PDMS middle layer was punched with a 6 mm bore (Bore set, Mayhew Pro) for hydrogel placement, was corona-treated for 30 seconds, bonded to a glass slide (3''x2'' microscope slides, ThermoFisher), and placed into 65°C oven for 1 hour to create a permanent bond. The PDMS microchannel with 15-gauge bored (McMaster-Carr) inlet and outlet, was then bonded to the glass-PDMS assembly using corona discharge for 45 seconds with PDMS shadow masks over the microchannel region, placed into 65°C oven for 1 hour, and kept at room temperature overnight to create a permanent bond of the full assembly. Once devices were assembled, they were autoclaved for sterility at 120°C for 40 minutes. Internal reservoirs were then coated with 50 µg/mL poly-d-lysine (Poly-d-lysine Hydrobromide, Sigma-Aldrich) in sterile 1XPBS (10XPBS Ultrapure, VWR) for 5 minutes at 37°C, aspirated, and allowed to dry for 1 hour, followed by 50 µg/mL Cell-TAK™ (Corning) in sterile 18 MΩ water treatment for 20 minutes at 37°C, aspirating, and further allowing it to dry for at least one hour prior to introducing biologics.

Viability assessment

Calcein AM viability images at 14 and 21 days were used to further quantify cell area and circularity in long-term microfluidic cultures compared to static controls. In ImageJ, images were converted to 8-bit, converted to binary using a threshold to distinguish the background from cells, and individual cell areas and perimeters were obtained in the Particle Analysis tool. Circularity was then calculated with the following: $4\pi(area/perimeter^2)$. All conditions contained n≥75 cells and data were presented as Mean±SD, where analyses were conducted in GraphPad Prism 8 (GraphPad). ANOVA with Tukey's post-hoc test was used to compare groups, where p-value<0.05, was considered statistically significant.

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Supplementary Figures

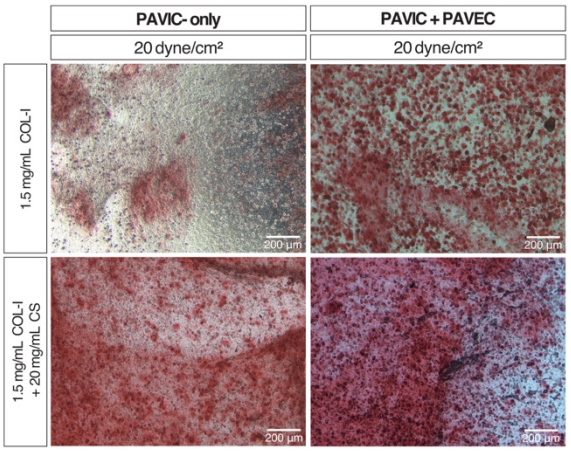

Figure S1. Static and microfluidic Alizarin Red S (ARS) staining after 14 days in culture, porcine aortic valve interstitial cells (PAVIC) only and when co-cultured with porcine aortic valve endothelial cells (PAVEC) at 20 dyne/cm². ARS qualitative images (scale = 200 µm) *(COL-I = collagen-I, PAVIC= Porcine valve interstitial cells, PAVEC = Porcine aortic valve endothelial cells, CS= chondroitin sulfate)*.

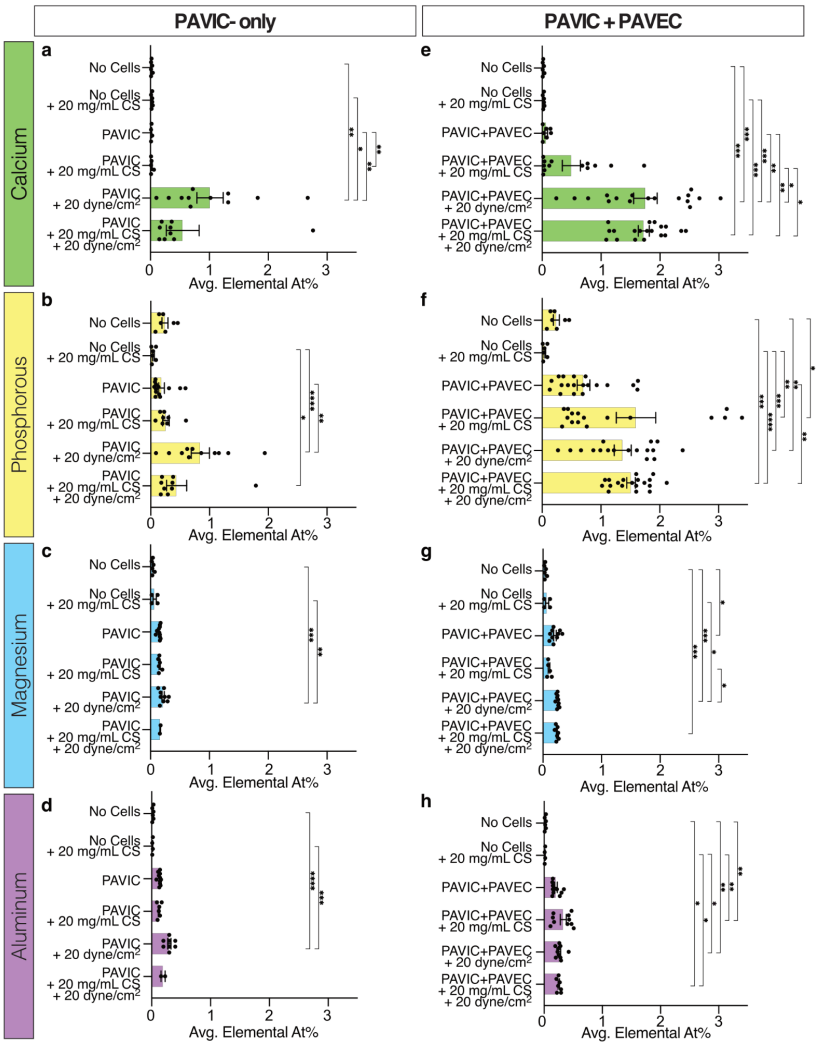

Figure S2. Static and microfluidic energy dispersive x-ray spectroscopy (EDX) analyzes after 14 days in culture, porcine aortic valve interstitial cells (PAVIC) only and when co-cultured with porcine aortic valve endothelial cells (PAVEC). EDX qualitative elemental atomic percentages for PAVIC-only cultures: **a.** calcium, **b.** phosphorous, **c.** magnesium, and **d.** aluminum. PAVIC+PAVEC co-cultures: **e.** calcium, **f.** phosphorous, **g.** magnesium, and **h.** aluminum. Mean±SEM, n≥5 measurements. Statistical significance shown according to Kruskal-Wallis with Dunn's Multiple Comparisons post-hoc test, *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001 *(PAVIC= Porcine valve interstitial cells, PAVEC = Porcine valve endothelial cells, CS= chondroitin sulfate, At% = atomic percent)*.

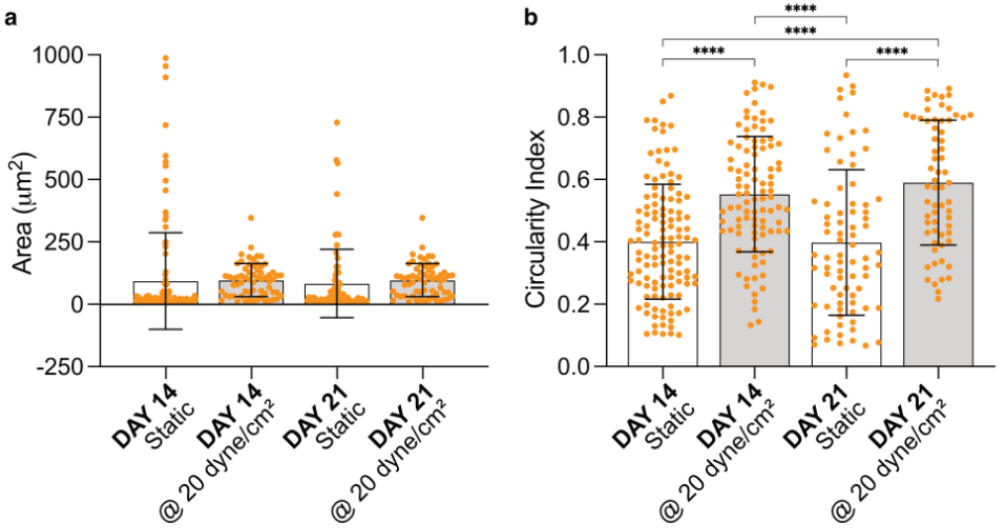

Figure S3. Static and microfluidic long-term culture cell assessment at 14 and 21 days, porcine aortic valve interstitial cells (PAVIC) co-cultured with porcine aortic valve endothelial cells (PAVEC) and 20 mg/mL chondroitin sulfate (CS). Calcein AM viability image assessment of A. Cell area (µm2) and B. Cell circularity (0.0-1.0) for static cultures and at 20 dyne/cm2. Mean±SD, n≥75 measurements. Statistical significance shown according to ANOVA with Tukey's post-hoc test, *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001.

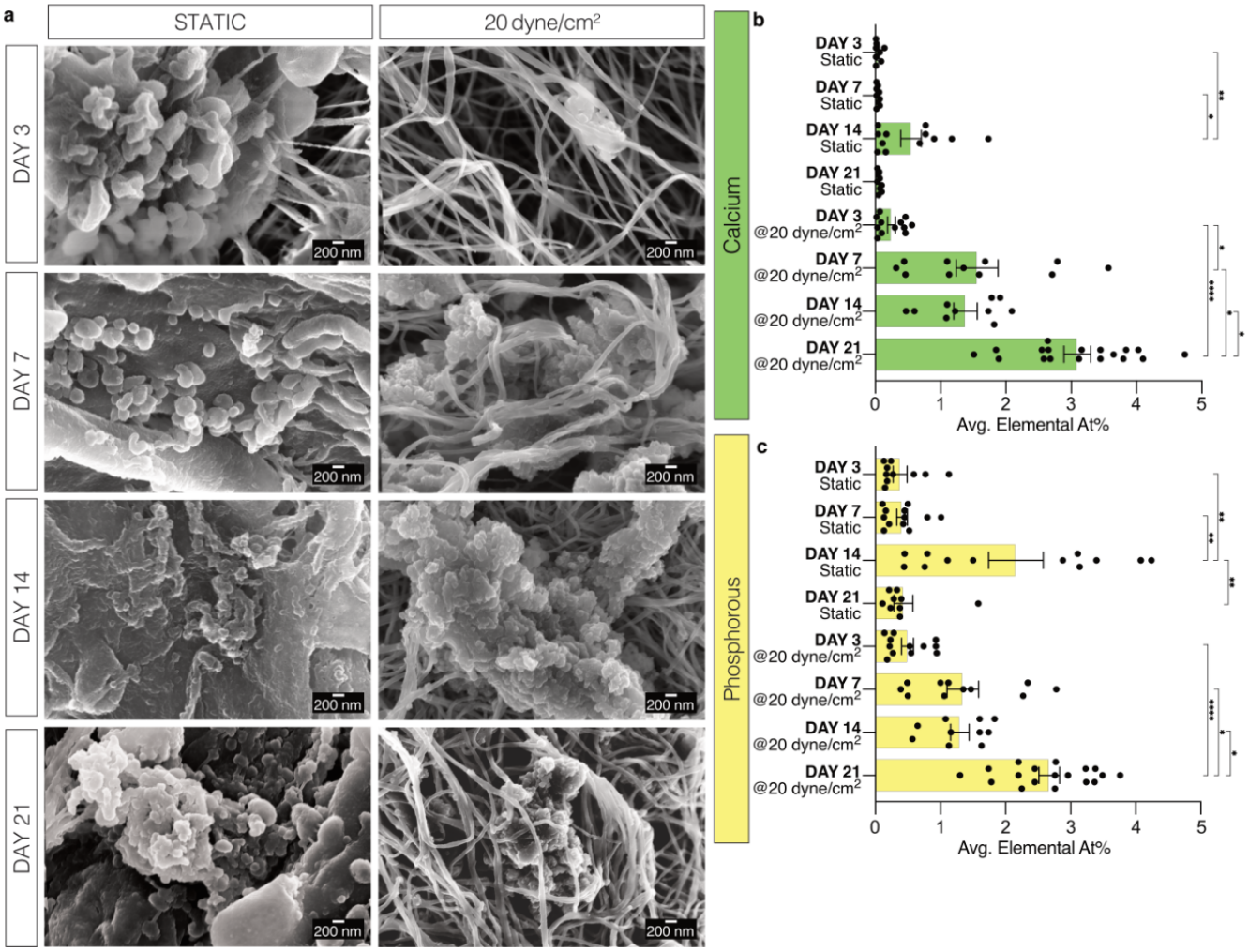

Figure S4. Static and microfluidic calcium phosphate mineralization throughout 21 days, porcine aortic valve interstitial cells (PAVIC) co-cultured with porcine aortic valve endothelial cells (PAVEC) and 20 mg/mL chondroitin sulfate (CS). a. SEM qualitative images throughout 21 days (scale = 200 nm). EDX qualitative elemental atomic percentage throughout 21 days for static and at 20 dyne/cm² of **b.** calcium and of **c.** phosphorous. Mean±SEM, n≥7 measurements. Statistical significance shown according to Kruskal-Wallis with Dunn's Multiple Comparisons post-hoc test, *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001 *(At% = atomic percent)*.