Supplementary Information for

An intimal-lumen model in a microfluidic device: Potential platform for atherosclerosis-related studies

Method:

S1. Reynold number calculation:

The formula for the Reynold number is as below:

$Re=\rho v D_h/\mu$

Where:

ρ = fluid density =1000 kg/m^3

v = fluid velocity

D_h = hydraulic diameter of the channel

μ = dynamic viscosity of the fluid = 0.964×10−3 Pa.s

For a rectangular channel, the hydraulic diameter is given by:

$$D_h=\frac{2\,w\,h}{w+h}$$

Where, w=1 mm=0.1 cm, and h=100 μm=0.01 cm

So, D_h= 0.1818 cm

To calculate the velocity, we use the flow rate Q and the cross-sectional area A:

$v=Q/A$

Where, Q=100μL/min = 1.667×10−3cm^3/s, and A=w·h=0.1cm×0.01cm=0.001cm^2

Now, calculate the velocity:

$v=1.667\times10^{-3}/0.001=1.667$ cm/s

Now we can plug the values into the Reynolds number formula:

$Re=\rho v D_h/\mu$

Substituting the values:

$Re=1\times1.667\times0.01818/0.964\times10^{-2}=0.0303/0.00964$

$Re \approx 3.144$

S2. Measurement of oxidized low-density lipoprotein (ox-LDL)

Low-density lipoprotein (LDL) concentration was measured using the Micro BCA™ Protein Assay Kit (ThermoFisher Scientific, 23235), and oxidized low-density lipoprotein (ox-LDL) was quantified using a Lipid Peroxidation (MDA) Assay Kit (ab118970), following the manufacturer's protocol. Briefly, 20 µL of LDL stock (6.53 mg/ml) were gently mixed with 500 µL of 42 mM H_2SO_4, and 125 µL of phosphotungstic acid solution was added to precipitate lipids while minimizing protein contamination. After vortexing, the mixture was incubated at room temperature for 5 minutes and centrifuged at 13,000 × g for 3 minutes to collect the lipid pellet. The pellet was resuspended on ice with 100 µL of double-distilled water (ddH_2O) containing 2 µL of 100× butylated hydroxytoluene (BHT), adjusting the final volume to 200 µL with ddH_2O. To the resuspended lipid samples and malondialdehyde (MDA) standards, 600 µL of 2-thiobarbituric acid (TBA) solution (33.3 mg/mL) was added to 200 µL of both the standard (malondialdehyde – MDA) and samples. The mixture was incubated at 95°C for 60 minutes, then cooled to room temperature in an ice bath for 10 minutes. Subsequently, 200 µL of the reaction mixture, containing the MDA-TBA adduct, was transferred to a 96-well microplate for analysis. A standard curve was prepared using MDA concentrations of 0, 2, 4, 6, and 8 µM. Absorbance was measured immediately using a microplate reader (BMG LABTECH FLUOstar Omega) at 532 nm.

Figures:

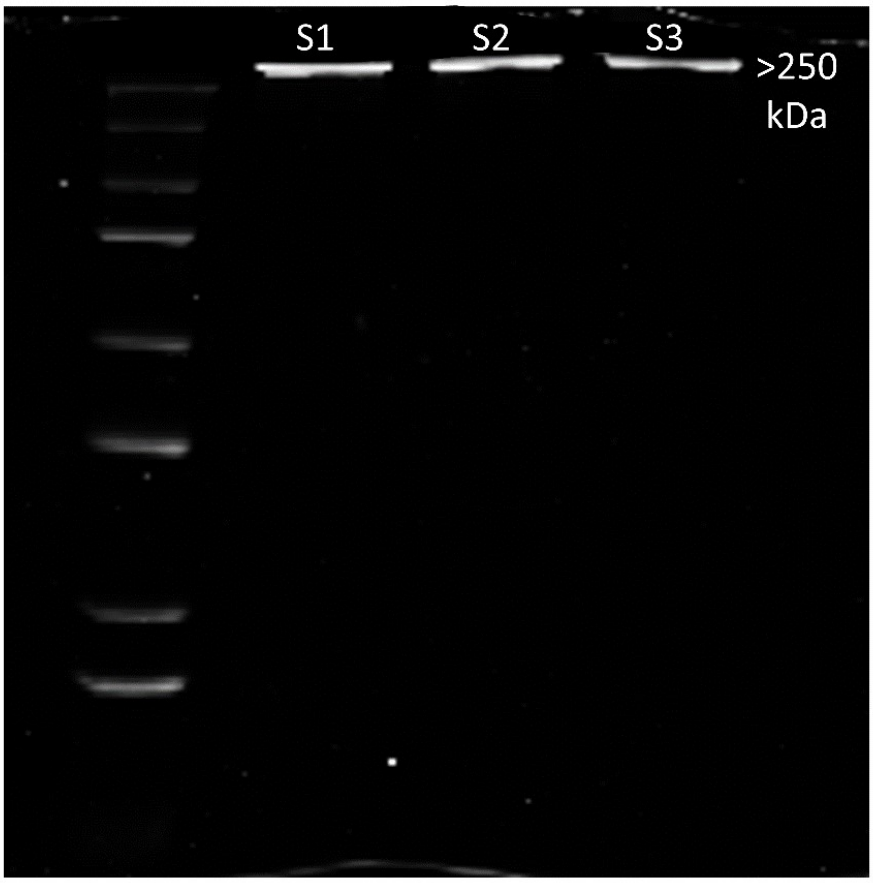

Figure S1: Confirming the low-density lipoprotein (LDL) isolation from whole blood by SDS-PAGE electrophoresis. The band achieved above 250 kDa proved successful LDL isolation. Only one band detected in the sample proved the purity. The three separate bands represented three separate isolates.

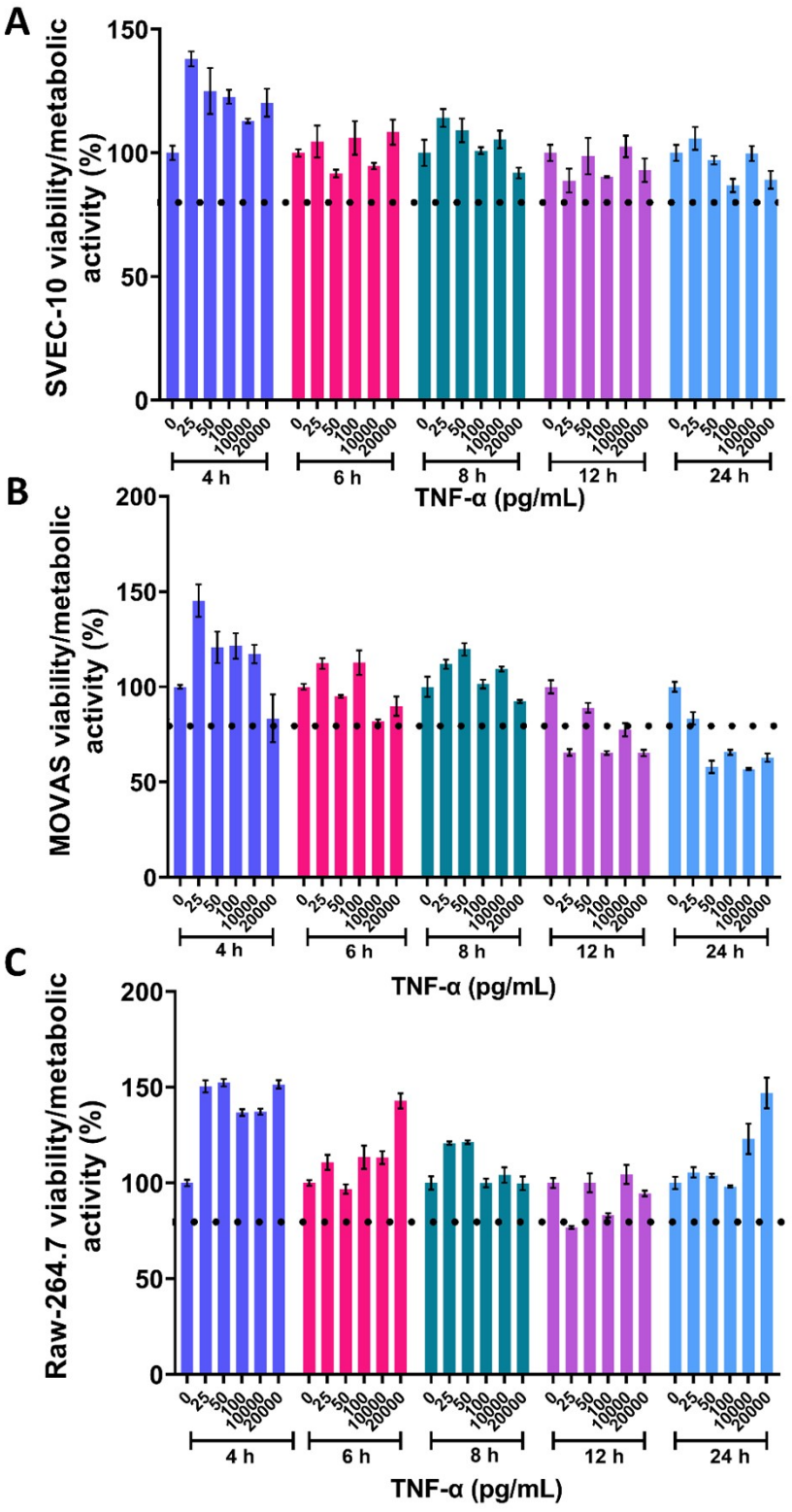

Figure S2: Cell viability/metabolic activity profiles in different TNF-α concentrations and time points. The viability/metabolic activity of **(A)** SVEC-10, **(B)** MOVAS, and **(C)** RAW 264.7. Both SVEC-10 and RAW 264.7 cells maintained good cell viability (above 80%) in all concentrations up to 24 h, while a reduction in viability/metabolic activity was observed in MOVAS after 8 h of treatment.

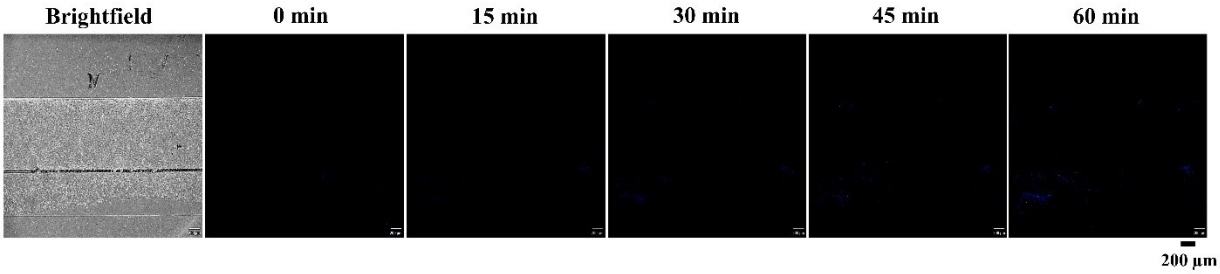

Figure S3: Monocyte adhesion on endothelial monolayer. No monocyte adhesion was observed on the endothelial monolayer in the absence of LDL and TNF-α stimulation.

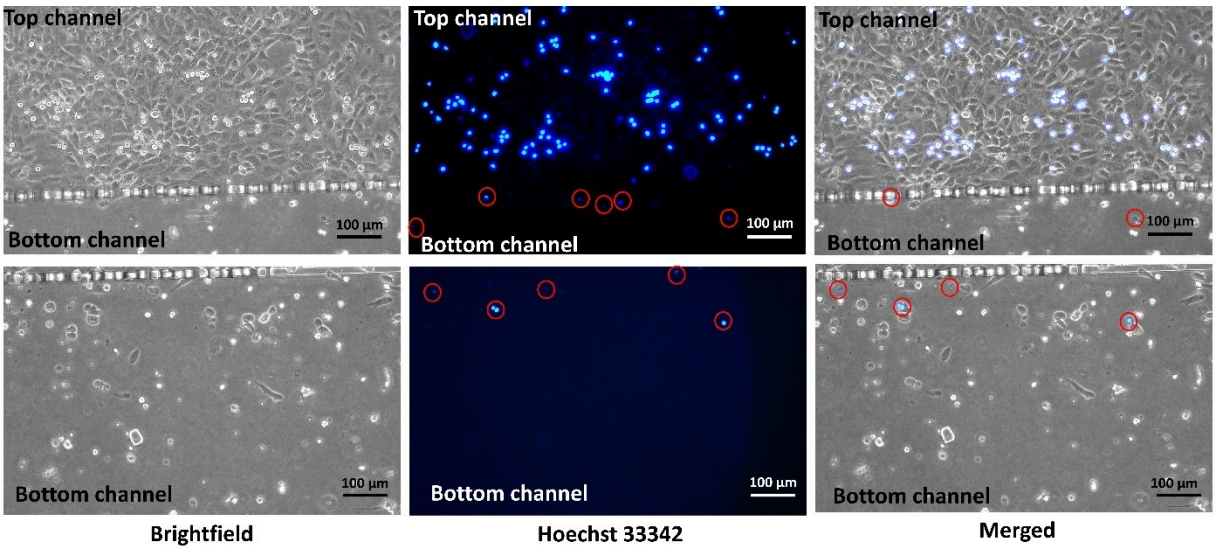

Figure S4: Subendothelial transmigration of monocyte during cell infusion through the top channel. TNF-α stimulation caused endothelial dysfunction and initiated subendothelial transmigration within 15 min of infusion. The blue colour from the nucleus staining indicated the attached monocytes in the top channel and the transmigrated monocytes in the bottom. The red circle showed the transmigrated monocytes in the fluorescence and merged images.