Supporting Information

Cellular Shuttles: Monocytes/Macrophages Exhibit Transendothelial Transport of Nanoparticles under Physiological Flow

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Supporting files and descriptions

Video S1. Uptake of F-SiO_2NP under static conditions. Video acquisition shows J774A.1 cells arresting onto recombinant ICAM-1 treated surface (t = -5 min, Figure 3Cii) at a τ_w of 0.1 dyn/cm^2. After 4 min, flow was increased to 1.5 dyn/cm^2 to remove non-adherent J774A.1 cells and NPs were introduced to the flow system. Following 1 min (t=0, Figure 3C iv), we stopped flow (τ_w = 0.0 dyn/cm^2) and observed particle uptake via fluorescence intensity increase (red, Cy3). Scale bar represents 100 μm and time is displayed for the transmitted light phase contrast (TL Phase) and Cy3 channels [min:sec.ms].

Video S2. Uptake of F-SiO_2NP under low flow conditions (0.1 dyn/cm^2). Video acquisition shows J774A.1 arresting onto a recombinant ICAM-1 treated surface (t = -5 min) at a τ_w of 0.1 dyn/cm^2. After 4 min, we increased flow to 1.5 dyn/cm^2 and introduced F-SiO_2NP to the flow system. Following 1 min (t=0, Figure 3C iv), we reduced flow (τ_w = 0.1 dyn/cm^2) and observed particle uptake via fluorescence (red). Scale bar represents 100 μm and time is displayed for the transmitted light phase contrast (TL Phase) and Cy3 channels [min:sec.ms].

Video S3. Uptake of F-SiO_2NP under physiological flow conditions (1.5 dyn/cm^2). Video acquisition shows J774A.1 arresting onto an recombinant ICAM-1 treated surface (t = -5 min) at a τ_w of 0.1 dyn/cm^2. After 4 min, we increased flow to 1.5 dyn/cm^2 and introduced F-SiO_2NP to the flow system. After 1 min (t=0, Figure 3C iv), we maintained flow (τ_w = 1.5 dyn/cm^2) and observed particle uptake via fluorescence (red). Scale bar represents 100 μm and time is displayed for the transmitted light phase contrast (TL Phase) and Cy3 channels [min:sec.ms].

Video S4. Magnified image of J774A.1 cells interacting with the TNF-α stimulated pMLuECs. Left panel shows transmitted light phase contrast images of J774A.1 polarized and actively crawling along the pMLuEC. The center Cy3 channel shows red fluorescence from the F-SiO_2NP uptake. Clearly apparent within 6 minutes is the localization of F-SiO_2NP within the J774A.1 uropod. Upon slowing of the crawling activity (20:36 min) the particles then become more homogeneously distributed throughout the cell and then resume distribution to the uropod when crawling reactivates. Scale bar represents 20 μm and time is displayed [min:sec.ms].

Video S5. Uptake of F-SiO_2NP by J774A.1 cells adherent to the pMLuEC layer under static conditions. Video acquisition shows J774A.1 arresting onto an pMLuEC (t = -5 min) at a τ_w of 0.1 dyn/cm^2. After 5 min, flow was switched to F-SiO_2NP at 1.5 dyn/cm^2 to introduce NP to the flow system. Following 1 min, the flow was stopped (τ_w = 0.0 dyn/cm^2) and particle uptake was observed via fluorescence (red). pMLuEC were also transfected with LifeAct-GFP to show actin filaments. Scale bar represents 100 μm and time is displayed for the transmitted light phase contrast (TL Phase), Cy3, and enhanced green fluorescent protein (EGFP) channels [min:sec.ms].

Video S6. Uptake of F-SiO_2NP by J774A.1 attached to the pMLuEC layer under static conditions. Video acquisition shows J774A.1 arresting onto an pMLuEC (t = -5 min) at a τ_w of 0.1 dyn/cm^2. After 5 min, flow was switched to F-SiO_2NP at 1.5 dyn/cm^2 to introduce NP to the flow system. Following 1 min, the flow was continued (τ_w = 1.5 dyn/cm^2) and particle uptake was observed via fluorescence (red). pMLuEC were also transfected with LifeAct-GFP to show actin filaments. Scale bar represents 100 μm and time is displayed for the transmitted light phase contrast (TL Phase), Cy3, and enhanced green fluorescent protein (EGFP) channels [min:sec.ms].

Video S7. Diapedesis of J774A.1 through the pMLuEC layer. Magnified image of J774A.1 actively crawling on the pMLuEC layer and uptaking F-SiO_2NP (red) under flow (1.5 dyn/cm^2). Magenta arrows indicate instances of J774A.1 migrating across the endothelial layer. Actin filament of pMLuEC were stained with LifeAct-GFP (green), and J774A.1 are visible in the transmitted light phase images. Time for transmitted light phase (TL Phase), fluorescence from F-SiO_2NP (Cy3), and green fluorescent from LifeAct-GFP (EGFP) is shown in [min:sec.ms].

Supplementary methods and figures

Fixed cell confocal laser scanning microscopy

Following video acquisition, cells were fixed in a 4 vol% solution of formaldehyde in PBS. μ-Dish bottoms were then extracted and cells were permeabilized with 0.1 vol% Triton X-100 and stained with Alexa Fluor 488 phalloidin (for filamentous actin) and DAPI (for cell nuclei). μ-Dish bottoms were then mounted in Glycergel aqueous mounting medium (Dako Schweiz GmbH; Baar, Switzerland). Confocal laser scanning microscopy images were then acquired on a 710 meta laser scanning microscope (Carl Zeiss AG; Jena, Germany) outfitted with a Plan-Apochromat 63× oil immersion objective.

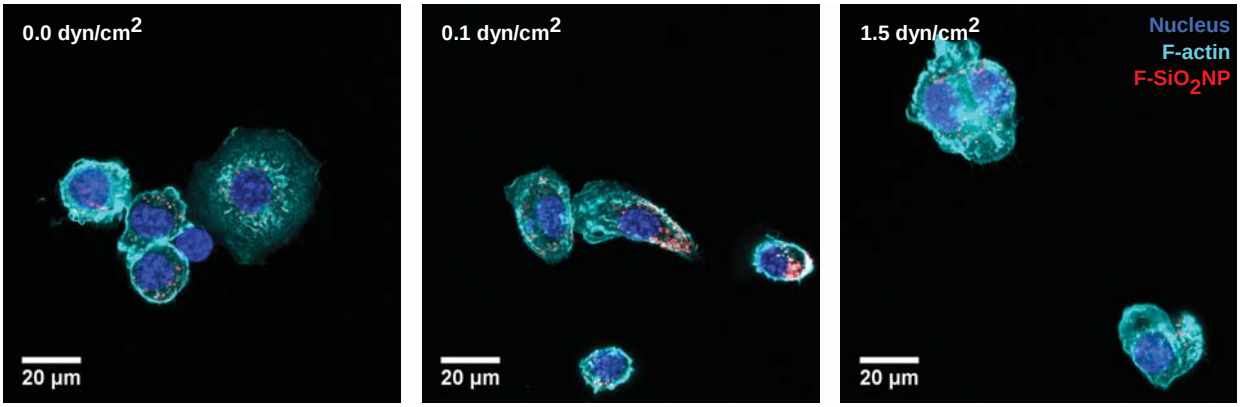

Figure S1. Laser scanning confocal microscopy images confirming the internalization of NP within cells stained for actin and nuclei. Images were acquired as three-dimensional confocal stacks and processed as a maximum intensity projection. Scale bars represent 20 μm.

Endothelial cell activation and immunofluorescence staining

For immunofluorescence staining, pMLuECs were cultured on laminin-coated wells of a 96-well plate at 5×10^6 cells/well. To induce an inflammatory phenotype, pMLuECs were stimulated with 10 ng/ml TNF-α for 16 hours. All subsequent steps were carried out at room temperature. pMLuECs were washed with PBS and fixed with 1% paraformaldehyde in PBS for 30 minutes. Following this, pMLuECs were blocked for 15 minutes with blocking solution (Rockland Immunochemicals Inc, Bioconcept, Allschwil, Switzerland). Fixed cells were incubated with rat anti-mouse hybridoma supernatants containing monoclonal antibodies to ICAM-1 (25ZC7) or VCAM-1 (9DB3) diluted 1:1 in blocking solution for 45 minutes. Then, pMLuECs were washed with PBS, stained with 10 μg/ml goat-anti rat Cy3 labeled secondary antibody (Jackson ImmunoResearch) in blocking solution together with DAPI (1 μg/ml) for 30 minutes, washed again and mounted within the 96 well plate with mounting medium (ibidi® GmbH, Vitaris, Baar, Switzerland). Images were acquiredat 40× magnification with an inverted fluorescence

microscope (AxioObserver.Z1, Carl Zeiss Vision Swiss AG, Feldbach, Switzerland) using a monochrome CCD camera (AxioCam. MRmRev, Carl Zeiss Vision Swiss AG, Feldbach, Switzerland) and processed with ZEN Blue software (Carl Zeiss Vision Swiss AG, Feldbach, Switzerland).

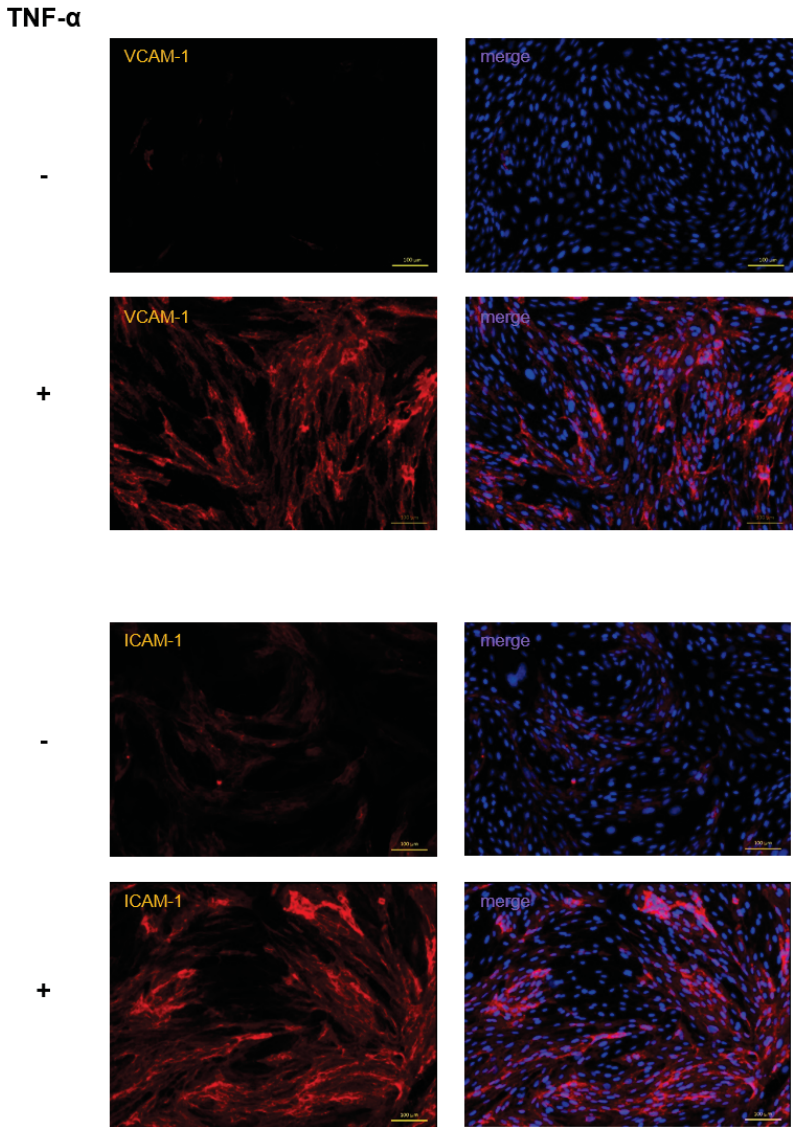

Figure S2. Immunofluorescence analysis of pMLuECs shows the upregulation of VCAM-1 and ICAM-1 upon TNF-α stimulation. Stimulated (+) and unstimulated (-) pMLuECs were stained for ICAM-1 and VCAM-1 (left column, red). Merged images with DAPI are shown on the right side. Scale bar indicate 100 µm.

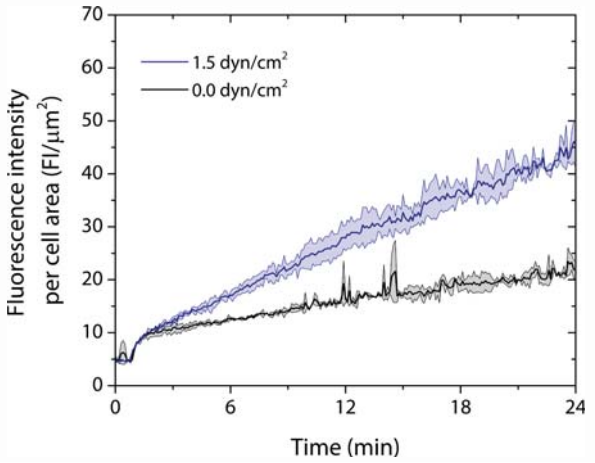

Figure S3. Preliminary video analysis of F-SiO_2NP uptake by J774A.1 that are arrested onto a pMLEC layer. Solid lines represent average values $\pm$ standard deviation (n = 2).

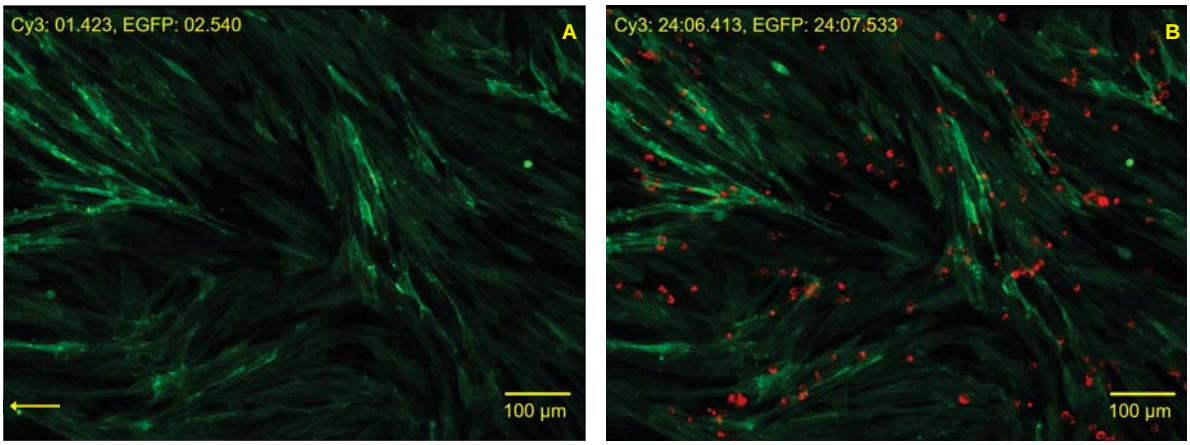

Figure S4. While J774A.1 monocytes/macrophages readily took up NP, there was no observable uptake of NP by pMLuEC. The enhanced green fluorescent protein channel (EGFP, green) which shows the F-actin filaments of LifeAct-GFP-transfected pMLuEC and the Cy3 channel (Cy3, red) show the localization of pMLuEC and NP. At (A) t = 0:00 there is no presence of NPs as they are just being introduced into the flow channel. At t = 24 min there is distinct red coloration of NP uptaken within J774A.1 cells but no red is localized within the pMLuEC. This indicates there is little to no uptake of NP by the pMLuEC. Arrow (in panel A) represents the direction of flow and scale bars are 100 µm. Acquisition time for Cy3 and EGFP channels are displayed at the top [min:sec.ms].