Supplementary Information (SI) for Nanoscale Horizons.

Supporting information

Endothelial-Targeting miR-145 Micelles Restore Barrier Function and Exhibit Atheroprotective Effects

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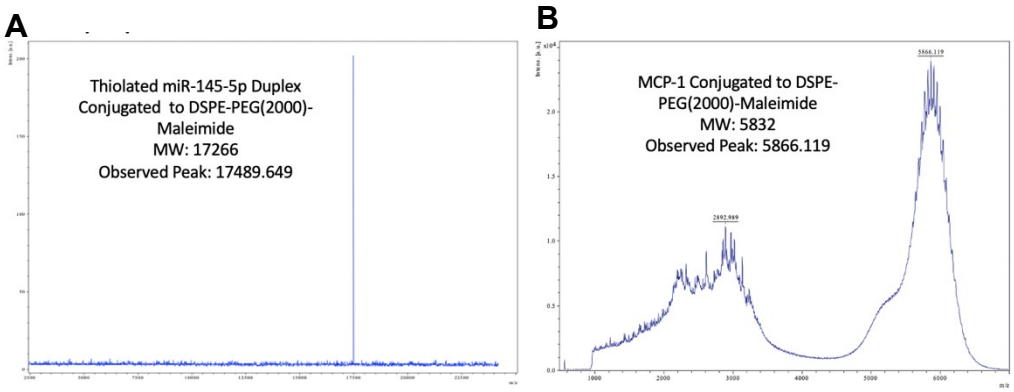

Figure S1. MALDI characterization of DSPE-PEG(2000)-miR145 and DSPE-PEG(2000)-MCP-1: A) miR-145-SH conjugation to DSPE-PEG(2000)-Maleimide was confirmed thorough the presence of peak at 17489.649 m/z (expected m/z: 17266 g/mol). **B)** MCP-1 peptide conjugation to DSPE-PEG(2000)-Maleimide was confirmed through the presence of peak at 5866.119 m/z (expected m/z: 5832 g/mol).

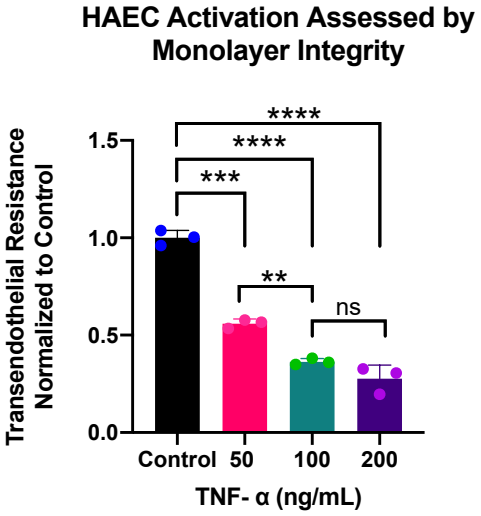

Figure S2. TEER measurement confirmed the activation of endothelial cells by TNF-α: HAEC monolayer integrity was reduced from healthy (1.00 ± 0.04) to 0.56 ± 0.02 ($p<0.001$) when treated with treated with 50 ng/mL TNF-α. When treated with 100 ng/mL, integrity was further reduced to 0.37 ± 0.02 compared to 50 ng/mL and healthy ($p<0.01$, $p<0.0001$). Non-significant changes were observed when increasing from 100ng/mL to 200ng/mL (0.37 ± 0.02 vs. 0.28 ± 0.07, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, $n\geq3$).

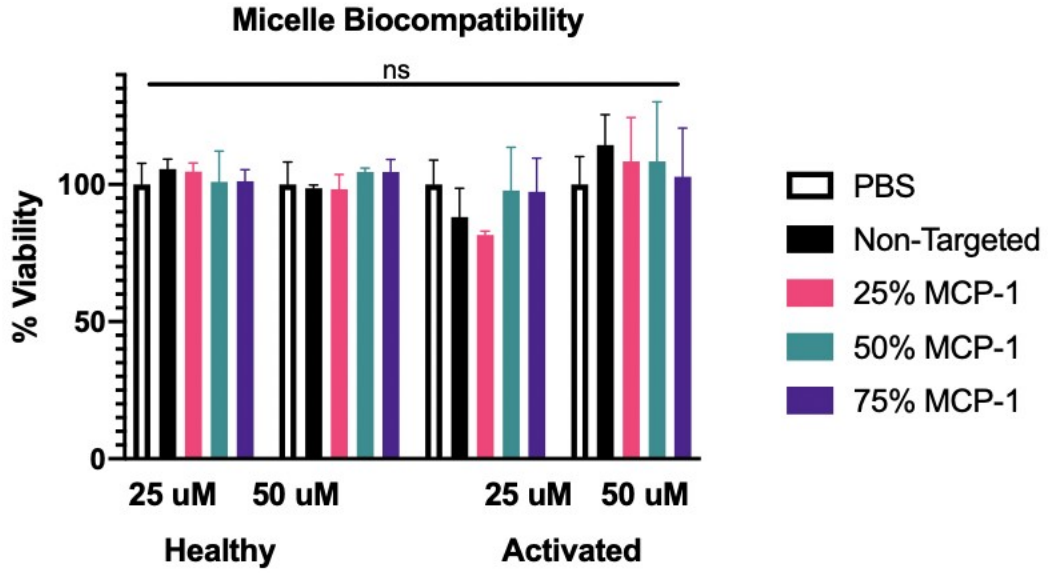

Figure S3. Biocompatibility of MCP-1 micelles: Micelle biocompatibility was assessed by MTS on healthy and activated cells and 0-75% molar MCP-1 micelles up to 50 μM showed no significant changes in percent viability compared to PBS-treated controls ($n \geq 5$).

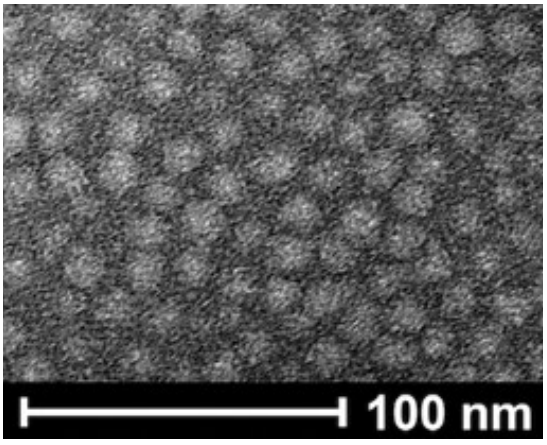

Figure S4. TEM of miR-145 micelles: miR-145 micelles were negatively stained with uranyl acetate and visualized with TEM which shows a spherical, monodisperse micelle population.

Micelle	Dispersant	Time (min)	Size (nm)	Polydispersity	Zeta (mV)
miR-145	PBS	0	18.70 ± 0.06	0.04 ± 0.015	1.15 ± 1.89
miR-145	PBS	30	17.30 ± 0.65	0.05 ± 0.006	-4.14 ± 1.41
miR-145	PBS	240	19.80 ± 0.48	0.05 ± 0.002	-2.99 ± 0.39
miR-145	Media	0	22.10 ± 0.15	0.05 ± 0.005	-3.54 ± 1.92
miR-145	Media	30	18.60 ± 1.58	0.10 ± 0.007	-3.85 ± 2.25
miR-145	Media	240	19.00 ± 2.55	0.05 ± 0.001	-5.19 ± 1.05
MCP-1	PBS	0	15.90 ± 0.31	0.05 ± 0.006	-1.22 ± 1.12
MCP-1	PBS	30	23.80 ± 1.21	0.12 ± 0.016	5.49 ± 3.042
MCP-1	PBS	240	24.00 ± 2.03	0.06 ± 0.031	-8.46 ± 4.31
MCP-1	Media	0	22.60 ± 1.15	0.11 ± 0.072	0.66 ± 3.42
MCP-1	Media	30	20.21 ± 0.85	0.07 ± 0.04	-4.22 ± 2.03
MCP-1	Media	240	20.84 ± 1.56	0.07 ± 0.02	-6.35 ± 1.09

Figure S5. Stability of miR-145 and MCP-1 micelles in media conditions: miR-145 and 50% molar MCP-1 micelles were incubated in PBS or media at 37°C for up to 4 hours (240 minutes) and their size, polydispersity, and zeta potential were measured through dynamic light scattering and Zetasizer. No significant changes in size, polydispersity, or chrage were found throughout the incubation (n≥3).

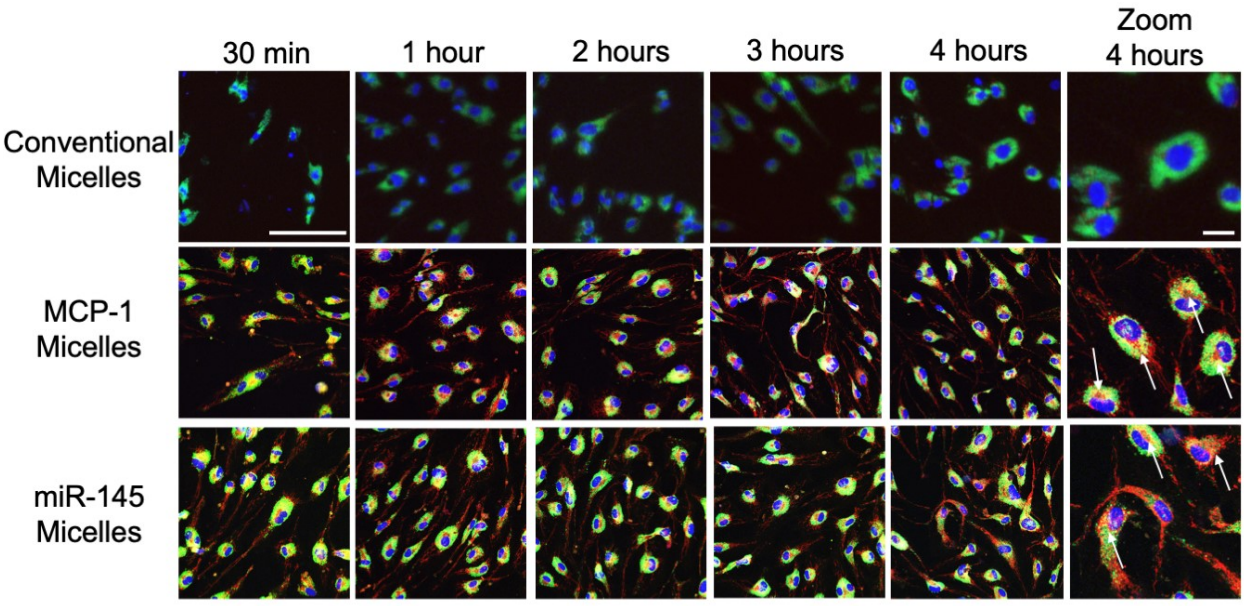

Figure S6. Micelle binding and internalization over time: Activated HAEC were incubated with 50% molar MCP-1 micelles and miR-145 micelles for up to 4 hours and visualized via confocal microscopy and white arrows indicate endosomal escape (green = lysotracker, blue = cell nuclei, red=micelles, top left scale bar = 100μm, top right scale bar =10 μm, n≥6).