MiR-146a Encapsulated Liposomes Reduce Vascular Inflammatory Responses

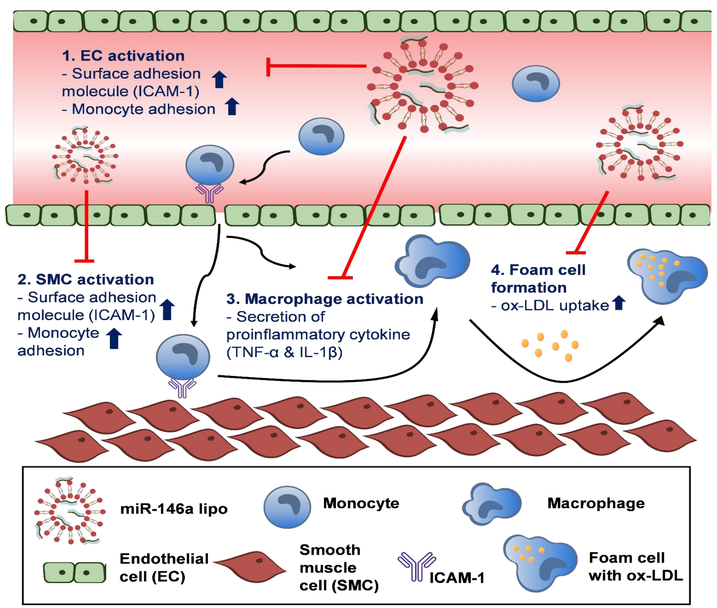
Claire Jun, [a,b] Donald Ho,[c,d] Tyler O. Lynd,[d] and Patrick T.J. Hwang [b,d]

[a] School of Arts and Sciences, University of Pennsylvania; [b] Endomimetics, LLC; [c] Department of Pediatric Dentistry;
[d] Department of Biomedical Engineering, University of Alabama at Birmingham

ABSTRACT

- Vascular insults can create an inflammatory cascade involving endothelial cell, smooth muscle cell, and macrophage activation which can eventually lead to vascular disease such as atherosclerosis.
- Several studies have identified microRNA 146a's (miR-146a) anti-inflammatory potential based on its role in regulating the nuclear factor kappa beta (NF-κβ) pathway.
- We introduced exogenous miR-146a encapsulated by liposomes to lipopolysaccharide (LPS) stimulated vascular cells and macrophages to reduce inflammatory responses.
- We demonstrated that miR-146a encapsulated liposomes reduced vascular inflammation responses in human aortic endothelial cells (HAECs) and human aortic smooth muscle cells (AoSMCs) through inhibition of ICAM-1 expression and decreased monocyte adhesion.
- In macrophages, miR-146a liposome treatment demonstrated decreased production of proinflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), as well as reduced oxidized low-density lipoprotein (ox-LDL) uptake.
- miR-146a encapsulated liposomes may be promising for reducing vascular inflammation by targeting its multiple associated factors.

BACKGROUND



RESULTS

1. Characterization of empty and miRNA loaded liposomes

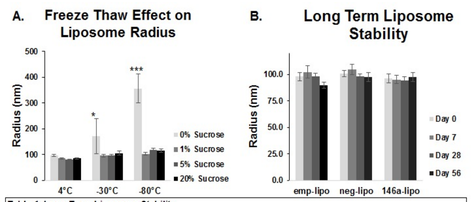
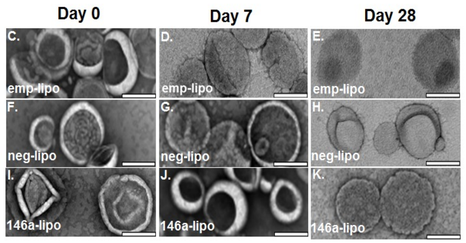


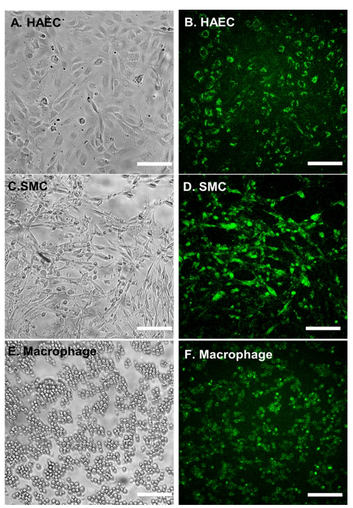
Table 1. Long Term Liposome Stability

	emp-lipo		neg-lipo		146a-lipo	
Time points	Radius (nm)	PDI	Radius (nm)	PDI	Radius (nm)	PDI
Day 0	98.0 ± 3.9	0.19 ± 0.08	100.7 ± 3.0	0.22 ± 0.07	96.4 ± 4.2	0.06 ± 0.02
Day 7	102.5 ± 5.8	0.07 ± 0.04	104.9 ± 4.7	0.16 ± 0.06	95.2 ± 3.8	0.16 ± 0.06
Day 28	98.5 ± 2.9	0.17 ± 0.07	98.1 ± 2.6	0.22 ± 0.10	94.3 ± 3.5	0.23 ± 0.08
Day 56	90.0 ± 3.0	0.26 ± 0.1	97.8 ± 4.1	0.28 ± 0.09	97.6 ± 4.1	0.2 ± 0.08



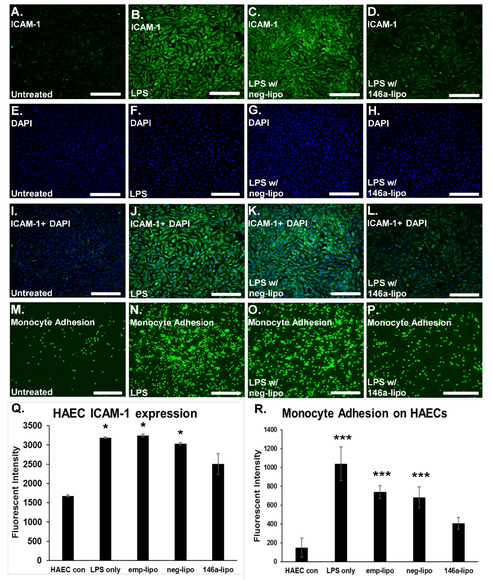
A.) DLS analysis of liposomes after freeze-thaw. Liposomes were stored at 4, -30, -80°C with 0, 1, 5, or 20% sucrose as a cryoprotectant. *p < 0.05 vs. liposome with 1% sucrose at -30°C and ***p < 0.001 vs. liposome with 1% sucrose at -30°C. **B.**) DLS analysis of long-term liposome stability at 0, 7, 28, 56 days (Table 1). **C-K.**) Representative TEM images of unloaded/loaded liposomes at 0, 7, 28 days. Scale bar is 200 nm.

2. Liposome-miRNA transfection



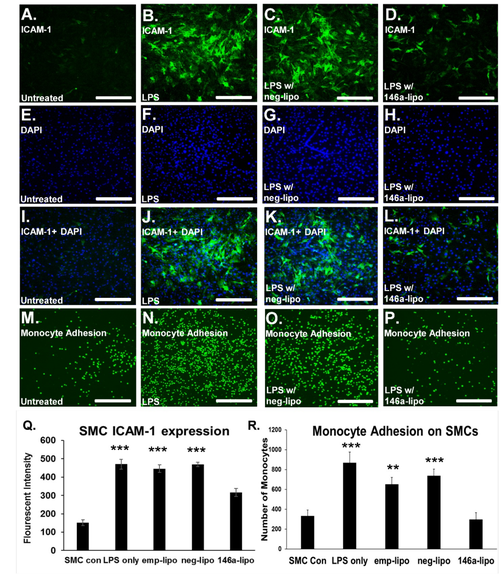
A. and **B**.) Bright field and fluorescent images of HAECs transfected with fluorescent tagged miR-146a loaded liposomes. **C**. and **D**.) Bright field and fluorescent images of SMCs transfected with fluorescent tagged miR-146a loaded liposomes. **E**. and **F**.) Bright field and fluorescent images of macrophages (differentiated U937 cells) transfected with fluorescent tagged miR-146a loaded liposomes. Scale bar is 50 μm with 20x magnification.

3. MiR-146a effects on HAEC activation



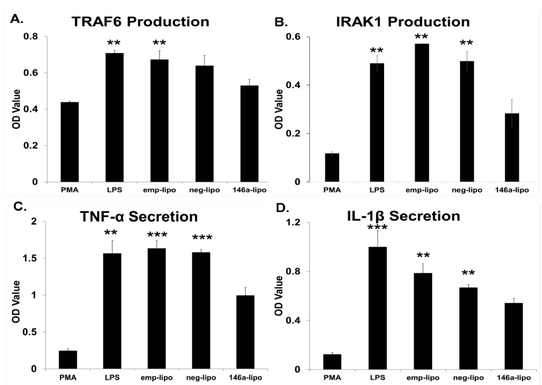
A.-D.) Fluorescent images of immunocytochemistry stained ICAM-1 on untreated, LPS only, neg-lipo, and 146a-lipo HAECs. **E.-H.**) Fluorescent images of DAPI stained HAECS. Same cells as previous set. **I.-L.**) Overlayed images of ICAM-1 and DAPI stained untreated/ treated HAECs. **M.-P.**) Monocyte adhesion assay. Fluorescent images of Calcein-AM stained monocytes adhering to untreated, LPS only, neg-lipo, and 146a-lipo treated HAECs. Scale bar is 100 μm with 10x magnification. **Q.**) ICC analysis of ICAM-1 expression on HAECs after no treatment (HAEC con) and treatment with LPS only, emp-lipo, neg-lipo, and 146a-lipo. *p < 0.05 vs. 146a-lipo. **R.**) Monocyte adhesion analysis on HAECs following no treatment (HAEC con) and treatment with LPS only, emp-lipo, neg-lipo, and 146a-lipo. ***p < 0.001 vs. 146a-lipo.

4. MiR-146a effects on SMC activation



A.-D.) Fluorescent images of immunocytochemistry stained ICAM-1 on untreated, LPS only, neg-lipo, and 146a-lipo SMCs. **E.-H.**) Fluorescent images of DAPI stained SMCs. Same cells as previous set. **I.-L.**) Overlayed images of ICAM-1 and DAPI stained untreated/ treated SMCs. **M.-P.**) Monocyte adhesion assay. Fluorescent images of Calcein-AM stained monocytes adhering to untreated, LPS only, neg-lipo, and 146a-lipo treated SMCs. Scale bar is 100 μm with 10x magnification. **Q.**) ICC analysis of ICAM-1 expression on SMCs after no treatment (SMC con) and treatment with LPS only, emp-lipo, neg-lipo, and 146a-lipo. ***p < 0.001 vs. 146a-lipo. **R.**) Monocyte adhesion analysis on SMCs following no treatment (SMC con) and treatment with LPS only, emp-lipo, neg-lipo, and 146a-lipo. **p < 0.01 vs. 146a-lipo and ***p < 0.001 vs. 146a-lipo.

5. MiR-146a effects on macrophage proinflammatory cytokine release



A.) Analysis of TRAF6 production in untreated differentiated U937 cells (PMA) and LPS, emp-lipo, neg-lipo, and 146a-lipo treated differentiated U937 cells. **p < 0.01 vs. 146a-lipo. **B.**) Analysis of IRAK1 production in untreated differentiated U937 cells (PMA) and LPS, emp-lipo, neg-lipo, and 146a-lipo treated differentiated U937 cells. **p < 0.01 vs. 146a-lipo. **C.**) Analysis of TNF-α secretion in untreated differentiated U937 cells (PMA) and LPS, emp-lipo, neg-lipo, and 146a-lipo treated differentiated U937 cells. **p < 0.01 vs. 146a-lipo and ***p < 0.001 vs. 146a-lipo. **D.**) Analysis of IL-1β secretion in untreated differentiated U937 cells (PMA) and LPS, emp-lipo, neg-lipo, and 146a-lipo treated differentiated U937 cells. **p < 0.01 vs. 146a-lipo and ***p < 0.001 vs. 146a-lipo.

CONCLUSION

- The liposomes maintained their size, shape, and uniformity after freeze-thaw process. Long term stability studies indicated the liposomes had a shelf life of at least one month. Additionally, the liposomes demonstrated efficient encapsulation of microRNA.
- miR-146a encapsulated in liposomes (146a-lipo) successfully demonstrated our 146a-lipo's effect on reducing endothelial cell (HAEC) and smooth muscle cell (SMC) activation, and macrophage proinflammatory cytokine release.
- The 146a-lipo reduced inflammatory activation of HAECs and SMCs, as shown by the reduction of ICAM-1 expression and monocyte adhesion.
- In LPS induced macrophages, 146a-lipo mitigated pro-inflammatory cytokine release by targeting regulators of the NF-κβ pathway.
- As our results indicate, miR-146a liposomes have the potential to reduce multiple factors associated with vascular inflammation.