PREPARATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF SPECIALIZED PRO-RESOLVING LIPID MEDIATORS (SPMS)-LOADED NANOEMULSIONS AS NANOCARRIERS FOR INFLAMMATION RESOLUTION

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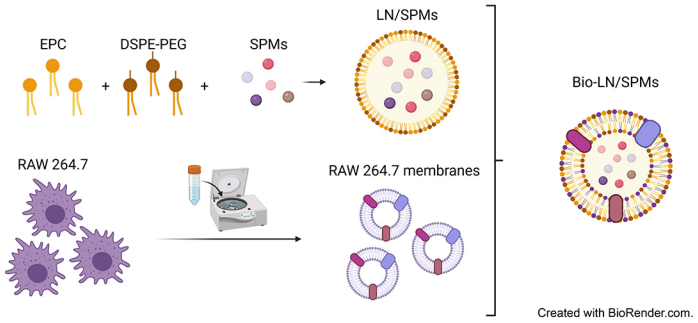
INTRODUCTION

The pathology underlying cardiovascular disease (CVD) is atherosclerosis (AS), a condition characterized by dyslipidemia and a chronic inflammatory process in the arteries. Compelling evidence suggests that chronic inflammation observed within AS lesions is a consequence of failed resolution. The inflammation resolution is actively controlled by cellular effectors and by an anti-inflammatory network consisting of endogenous proteins (such as prostaglandin E2, annexin A1) and specialized pro-resolving lipid mediators (SPMs) (i.e. lipoxins, resolvins, protectins and maresins) [1,2]. Therefore, we envisioned a biomimetic nanocarrier system comprised of SPMs-loaded lipid nanoemulsions (LN) that could effectively accumulate at the inflamed site via their coating with macrophage membranes, thereby reducing the inflammatory process.

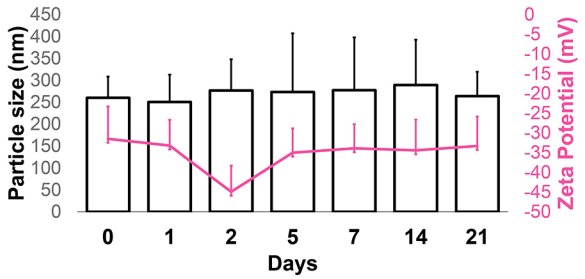
MATERIALS AND METHODS

The LNs were prepared using the ultrasonication method by mixing the organic phase-containing a cocktail of five SPMs and an aqueous phase containing glycerine as a surfactant. High-performance liquid chromatography (HPLC) analysis, dynamic light scattering (DLS), and electrophoretic light scattering (ELS) were used to characterize LN/SPMs over 21-days. Cell membranes were isolated from RAW264.7 macrophages by centrifugation and were used to obtain biomimetic LN/SPMs (Bio-LN/SPMs). The presence of macrophage membrane proteins on the surface of LN was assessed by Western Blotting.



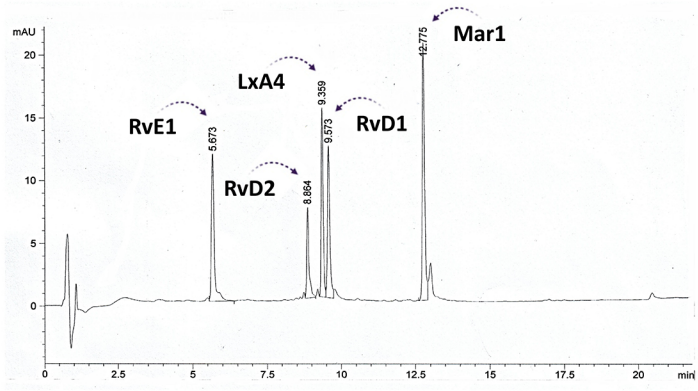
RESULTS

1 Size and Zeta-potential of LN/SPMs



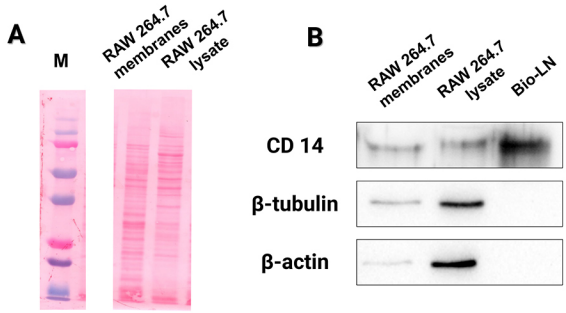
Days	Hydrodynamic diameter (nm)	Zeta-potential (mV)
0	260.5 ± 48.28	-31.5 ± 11.2
1	250.5 ± 62.61	-33.2 ± 6.50
2	277.0 ± 71.16	-44.9 ± 6.63
5	273.6 ± 133.7	-35.0 ± 6.15
7	277.7 ± 180.3	-33.9 ± 6.10
14	289.5 ± 103.3	-34.4 ± 7.77
21	264.2 ± 55.36	-33.3 ± 7.46

2 HPLC analysis of SPMs encapsulation



Days	SPMs (ng/ml)				
	RvE1	RvD2	LXA4	RvD1	Mar1
0	448.75	500.9	316.45	560.82	645.52
2	228.65	322.68	136.32	259.34	361.80
7	203.35	116.27	122.71	244.40	355.45
14	176.49	117.23	114.50	240.00	350.93
21	154.45	113.00	94.85	217.50	361.28

3 Macrophage membrane isolation and protein detection in Bio-LN



Protein profiles of isolated RAW 264.7 membranes and whole lysates (A), as well as macrophage membrane protein CD14 expression in Bio-LN via Western Blotting technique (B).

CONCLUSIONS

The nanocarrier system synthesized in this study revealed good entrapment efficiency of SPMs, nanometer-sized particles, as well as optimal stability over time, justifying their use further as new potential anti-inflammatory agents for the treatment of various inflammatory diseases including atherosclerosis.

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References:

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