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Short communication

Novel niosome formulation design for gene delivery

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Introduction

Niosomes are vesicular systems like Liposomes, developed in 1970s for cosmetic industry by Loreal®. They are prepared using non-ionic surfactants instead of phospholipids. Niosomes have some advantages over Liposomes. They are stable over a wide pH range, as their primary ingredient non-ionic surfactants are cheaper than phospholipids, have a longer shelf life than liposomes and require simpler storage conditions (Bartelds et al., 2018). Cholesterol is used to provide rigidity of niosome vesicles as a secondary component. Similar to non-ionic surfactants, cholesterol is a non-toxic ingredient also. Aforementioned specialties of niosomes are making them attractive as a drug/gene delivery systems. Non-viral gene therapy methods are preferred over viral methods nowadays because of safety issues like insertional mutagenesis and immunogenicity (Hackett et al., 2013). In this study, a novel niosome formulation for pDNA delivery was developed.

Materials and methods

Formulation Design

Span 60 is chosen as a main surfactant because its high encapsulation efficiency is well known (Bartelds et al., 2018). Cholesterol is used as stabilization agent (Leite et al., 2018). Menthol was used as penetration enhancer (Joshi et al., 2017). Cetylpyridinium chloride role is as cationic charge inducer. Final ingredient curcumin is added to formulation to enhance the effect of plasmid DNA by working synergistically (Grynkiewicz & Ślifirski, 2012). All chemicals was purchased from Sigma Aldrich, USA.

Niosome Preparation via Thin Film Hydration Method

Thin Film Hydration method adopted to form niosomal vesicles. Briefly, formulation ingredients weight in a 50 mL round bottom flask. Mixture was dissolved in chloroform. Organic solvent was evaporated at 60°C, with constant stirring rate under vacuum with rotary evaporator (Heidolph, Germany). Formed inner thin film hydrated with 20 ml ultrapure water. Final suspension was sonicated in a bath sonicator (Elmasonic, Germany) to obtain smaller vesicles. Visually stable formulations characterized with DLC (Dynamic Light Scattering) technique (Malvern Zetasizer Nano ZS, Malvern Instruments, UK).

WST-1 Analysis

Cytotoxicity of optimized final formulation was evaluated on L929 mouse fibroblast cells (FDA regulation: ASTM F895-11). 10000 cells/well were seeded on 96 well plates. Cells were cultured in DMEM media supplemented with 10% FBS (fetal bovine serum) and 100 U/mL penicillin/streptomycin. Cells were treated with increasing amounts of formulation applied into the wells and WST-1 (Abcam, USA) analysis was performed 24th hour following the exposure. Briefly, WST-1 reagent added to the culture media and incubated 3 hours. Cell viability was calculated according the absorbance readings at 440 nm. Non-treated cells are considered as 100% viable. All treatment groups were applied in triplicates.

Results and discussion

The molar ratios used for formulation screenings are as follows: Span 60: 15 to 1, Cholesterol: 3 to 1, Cetylpyridinium chloride: 3 to 1, Menthol: 3 to 1, Curcumin: 3 to 1.

Stable formulations are detected in pre-formulation trials. Figure 1 shows the visual stability of pre-formulations. The final formulation was obtained after the formulations that did not form a thin film layer and showed

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stability problems such as coagulation and creaming were eliminated. DLS measurements were applied for further characterization (Table 1).

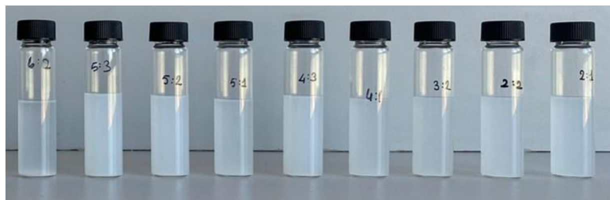

Fig. 1. Representative image of stability study (Span 60:Cholesterol as molar ratios)

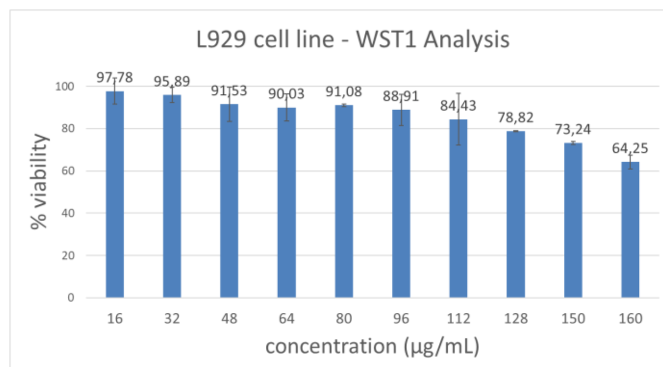

Fig. 2. WST-1 cell viability assay

Table 1. Physical properties of niosomal vesicles

Span 60 : Cholesterol (molar rat.)	Vesicle Size ± SD (nm)	PDI	Zeta Potential ± SD (mV)
10:3	483.8 ± 153.3	0.812	-62.7 ± 2.45
8:3	200.9 ± 3.179	0.368	-53.0 ± 1.34
8:1	546.0 ± 82.59	0.511	-61.9 ± 1.60
7:3	414.2 ± 36.56	0.559	-47.9 ± 1.10
7:1	586.1 ± 4.658	0.636	-61.2 ± 1.62
6:2	602.5 ± 95.45	0.596	-58.5 ± 2.42
5:3	380.5 ± 63.88	0.438	-59.4 ± 2.93
5:2	203.6 ± 14.09	0.351	-52.2 ± 2.75
5:1	299.4 ± 5.605	0.244	-62.8 ± 2.40
4:3	501.0 ± 182.2	0.461	-60.7 ± 1.08
4:1	311.2 ± 28.54	0.429	-60.8 ± 2.33
3:2	265.6 ± 7.856	0.374	-38.5 ± 1.70
2:2	383,4 ± 20,22	0,249	-57,6 ± 4,97
2:1	294.3 ± 19.78	0.418	-63.9 ± 2.73

The final formulation containing the other aforementioned ingredients was obtained in a similar way (data not shown). Highest stability was obtained for 2:1:1:0.1:2 ratio of Span 60: Cholesterol : CPC : Menthol : Curcumin. DLS measurements show that the formulation has 449.2 ± 58.8 nm vesicle size and 45.3 ± 1.27 mV zeta potential.

Optimal concentration was detected as 150 µg/mL during WST-1 cytotoxicity study (figure 2). It is observed that cell viability decreases below 70% at doses above this concentration.

Conclusion

This study was focused on development of a novel niosomal formulation for gene delivery. DLS measurement results show that the final formulation has appropriate characteristics as genetic material carrier. Appropriate doses for cell culture studies were determined to be below 150 µg/mL in WST-1 study. The niosome formula may be a good candidate carrier for further development as gene delivery system.

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