International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:6, No:6, 2012

# Topical Delivery of Thymidine Dinucleotide to Induce p53 Generation in the Skin by Elastic Liposome

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***Abstract*—**Transcription factor p53 has a powerful tumor suppressing function that is associated with many cancers. However, p53 of the molecular weight was higher make the limitation across to skin or cell membrane. Thymidine dinucleotide (pTT), an oligonucleotide, can activate the p53 transcription factor. pTT is a hydrophilic and negative charge oligonucleotide, which delivery in to cell membrane need an appropriate carrier. The aim of this study was to improve the bioavailability of the nucleotide fragment, thymidine dinucleotide (pTT), using elasic liposome carriers to deliver the drug into the skin. The study demonstrate that dioleoylphosphocholine (DOPC) incorporated with sodium cholate at molar ratio 1:1 can archived the particle size about 220 nm. This elastic liposome could penetration through skin from stratum corneum to whole epidermis by confocal laser scanning microscopy (CLSM). Moreover, we observed the the slight increase in generation of p53 by western blot.

***Keywords*—**Elastic liposome, Tymidine dinucleotide, p53, Topical delivery

## I. Introduction

RECENT researches demonstrated that numerous types of cancers were associated with specific gene [1], [2]. Normal cells contain two genome, one is oncogenes which cause the cell to divide in an unregulated situation when a single altered copy leads mutation; the other is antioncogenes which provide a protected mechanism, also called tumor suppressor gene [3]. In brief, the logic of oncogenes and antioncogenes is opposite, which regulate the formation of cancer. The p53 tumor suppressor gene is well known for genetic alteration in cancer. The function has been identified that p53 protein will accumulation or activation when DNA damaged. P53 is considerated as a critical regulator of the cell cycle as it allows either DNA repair or apotosis.

However, gene mutation occurs with not only genetic deficient but also exogenous factor. Several skin disease including basal cell carcinoma and squamous cell carcinoma are relative with mutation of p53, and the site of mutation is basis to distinguish the skin cancers [4]. Skin cancer induced by UV-radiation may trigger the apoptosis in a p53-dependent maner and the formation of the sunburn cells (apoptotic keratinocytes). Numerous studies indicated that trigger p53 induction stopped the cell to divide in an unregulated situation [5]. Thymidine dinucleotide (pTT), a fragment of the oligonucleotide, which activates the p53 transcription factor and p53 protein and triggers in a cascade of DNA repair enzymes from the in vitro and in vivo evidences, and it focus on UV-radiated mutagenesis and carcinogenesis [6].

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Typically, the oligonucleotide has high molecular weight, negative charge and high water solubility properties, which causes problem when transporting through the cellular membrane to generalize the specific reaction in nucleus [7]. According to the above, the bioavailability of pTT is low when delivering pTT through cellular membrane by passive diffusion. The aim of the study was to improve the bioavailability of nucleotide employee elastic liposome by skin administration route. Skin, the outermost superficial organ of the body, is an attractive target site for delivering/transferring drugs, active ingredients, and gene fragments. However, the upper layer of skin is the stratum corneum (SC) which is nature barrier makes the permeability not effective [8]. Past investigations demonstrated liposome could as potential carrier to deliver active pharmaceutical ingredients [9]. On the other hand, conventional liposomes were not efficiently delivered across to skin because it not penetrates to the deep skin, and retards at the upper layer of the SC [10]. Ceves's group developed novel elastic vesicles could improve the penetration problem in terms of quantity and depth [11]. Elastic liposomes are similar to conventional liposomes but with the incorporation of an edge activator in the lipid bilayer structure to provide elasticity [12]. Generally, elastic vesicles are classified two types including transferosomes/ethosomes and detergent-based types. Transferosomes/ethosomes are mainly composed of phospholipid and high concentration of hydroalcohols or hydroalcohols [13]. Detergent-based types are often composed of span 60, span 80, tween 80, span 80, sodium cholate, sodium deoxycholate and stearylamine [14], [15].

The aim of this study was to improve the bioavailability of the nucleotide fragment, thymidine dinucleotide (pTT), using elasic liposome carriers to deliver the drug into the skin. The physicochemical properties were characterization in terms of the particle size, size distribution and zeta potential. Delivery of pTT-loaded elastic lipoosme was observed cross section of skin tissue and visualized by confocal laser scanning microscopy (CLSM). The p53 generation from pTT was examined by western blot assay.

## II. Experimental

### A. Materials

pTT with or without 5'fluorescein was obtained from Protech Technology (Taipei, Taiwan). Sodium cholate was purchased from Sigma Chemical (St Louis, MO). 1,2-Dioleoyl-sn-glycero--3-phosphocholine (DOPC, with a purity of 95%) was purchased from Avanti Polar Lipids (Alabaster, AL). Other chemicals used in the study were of reagent grade.

### B. Preparation of elastic liposomes

Elastic Liposomes were prepared by conventional thin-film hydration method. 1,2-Dioleoyl-sn-glycero-3-phosphoethanol-

amine (DOPE) and sodium cholate with different molar ratio were dissolved in 5 mL of chloroform: methanol (2:1, v/v) solution in round-bottomed flasks. Organic solvent traces were evaporated by a rotary evaporator above the transition temperature of the lipid to make a lipid coarse suspension. The solvent traces were removed under a vacuum overnight. The thin film was fully hydrated with double-distilled water containing pTT (100 μmol/L) above lipid transition temperature for 20 min. The vesicle suspension was dispersed by a probe-type sonicator (VCX 600; Sonics and Materials, Newtown, CT) at 25 W for 10 min.

### *C. Physicochemical characterization*

The vesicle size and zeta potential of the liposomes were measured by laser light scattering (LLS) with a helium-neon laser at 630 nm (Nano ZS1 90; Malvern, Worcestershire, UK). Liposomal suspensions were directly measured. The polydispersity index (PdI) was used to measure the size distribution. All vesicle sizes and zeta potentials were measured at 25°C. Measurements were repeated three times per sample for three samples.

### *D. Confocal Laser Scanning Microscopy (CLSM) study*

In order to understand the elastic liposome located in the different skin layers, pTT-loaded elastic liposome was employed in vitro model to observe. Moreover, fluorescein (green) was labeled at 5'-pTT as fluorescence marker to visualize by CLSM. After incubation on the Franz diffusion cell for 24 h, nude mouse skin sample was sliced in sections of 12 μm thickness, perpendicular to the skin, with the help of cryomicrotome. The cross-section was investigated for the amount of pTT in the different skin layers by using the CLSM Leica TCS SP2 confocal microscope (Leica Microsystems, Wetzlar, Germany). Optical excitation was carried out with a 488 nm argon laser beam and fluorescence emission was detected at 543 nm and with software version 2.61.

### *E. Western Blot Analysis*

Generally, pTT convert to p53 should be in in vivo situation. The study was employed a glass cylinder with an available area of 0.785 cm2 was placed with glue on the dorsal skin in in vivo nude mouse model. pTT-fluorescein loaded-elastic liposome (100 μL) was added to the cylinder for 24 h. At the end of the incubation period, mouse was sacrificed. The suspension of elastic liposome was removed, and the skin was wiped ten times with a cotton cloth. The treated area was excised, and instantly frozen at -80°C. Skin specimen was added lysis buffer and then homogenized by using a homogenizer to isolated total protein. Protein concentrations were determined by Bradford method. Total cellular proteins (100 μl) were separated over 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. After transfer, the gel was stained with Coomassie Blue to verify even loading as visualized by the residual high molecular weight proteins. Membranes were blocked in 0.05% Tween-20/PBS with 5% milk. Antibody reactions were performed with p53 antibodies as specific target, and β-actin and GAPDH as control [16].

## III. Results And Discussion

Generally, oligonucleotides present hydrophilic property which makes the limitation of penetration into skin or cellular membrane. In the present study demonstrated that topical delivery of pTT by using elastic liposome which can deliver into skin more deeply. We established the properties of pTT-loaded elastic liposome and the effect on skin reached depth by CLSM. Moreover, p53 generated from pTT also detectable by western blot.

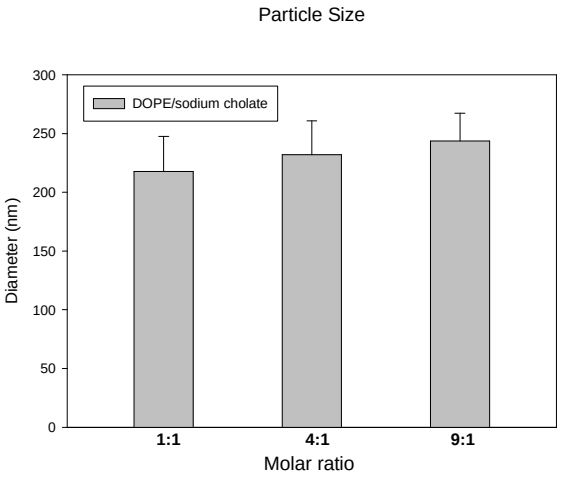
### *A. Physicochemical properties of elastic liposomes*

Elastic liposome ingredients mainly contain phospholipid and edge activator which could be more effective than conventional liposome in enhancing skin penetration of various active pharmaceutical ingredients. The edge activators are often a single chain surfactant that destabilizes lipid bilayers of the vesicles and increases deformability of the bilayer [17].

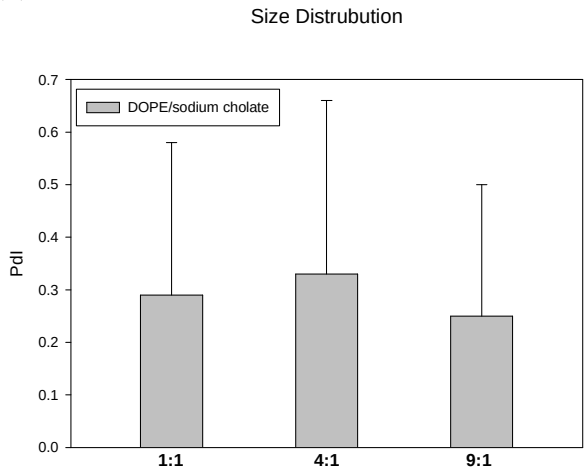
DOPC and sodium cholate was selected as phospholipid material and edge activator, respectively. The particle size, PDI, and zeta potential of elastic liposomes with pTT were measured by an LLS system, and the results are shown in figure 1. Data on the particle size showed corresponding increases depending on the molar ratio between lipid and edge activator. The DOPC/sodium cholate molar ratio of 1:1 represented the smallest particle about 220 nm. Moreover, results of the polydispersity index (PdI; representing the distribution of particle size) were less than 0.4, which represent the well distribution of particle sizes. The zeta potential of DOPC/sodium cholate vesicles was presented higher negative surface charge.

Sodium cholate, a water-soluble substrate, is used as cell lysis, or isolation of membrane proteins and lipids. On the other hand, Sodium cholate was as anionic bile-acid detergents which can effective for liposome preparation. The results indicated sodium cholate increase make the size smaller. DOPE was added as helper lipid in all formulations because of its well documented ability to form columnar inverted hexagonal liquid-crystalline structures, which improve the fusogenic properties of lipidic vectors [18], [19].

(A)



(B)




(C)

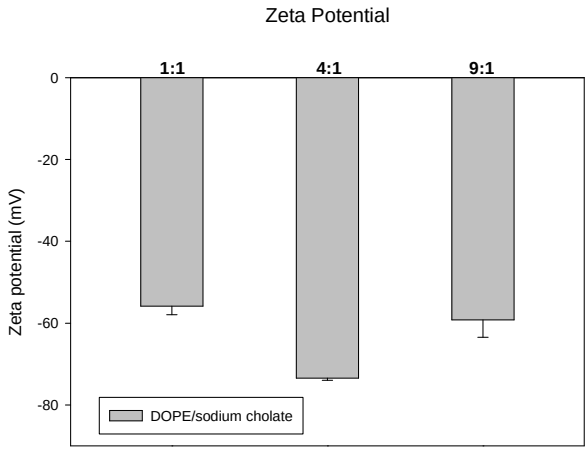



Fig. 1 Physicochemical characteristics of liposomes with or without encapsulated pTT- fluorescein (A) particle size (B) size distribution (C) zeta potential

*B. pTT localization by CLSM study*

We employed an in vitro CLSM technique to understand where pTT was localization. Generally, skin has small virtual pores (20–40 nm) that limit to passage through intercellular spaces in the outer skin layers [20], [21]. In the present study, the diameter of elastic liposome was about 220 nm which larger than the penetration pathway. However, CLSM demonstrated that the fluorescein visualized from stratum corneum to whole epidermis, as shown in figure 3. Past research also indicated that elastic liposome such as transfersomes and ethosome squeeze through pores in stratum corneum less than one-tenth the liposome's diameter, although sizes up to 200–300 nm can penetrate intact skin [22]. The ultradeformable ability depends on their physicochemical properties: composition, preparation method. The main contribution were employed DOPC as phospholipid and sodium cholate as edge activator.

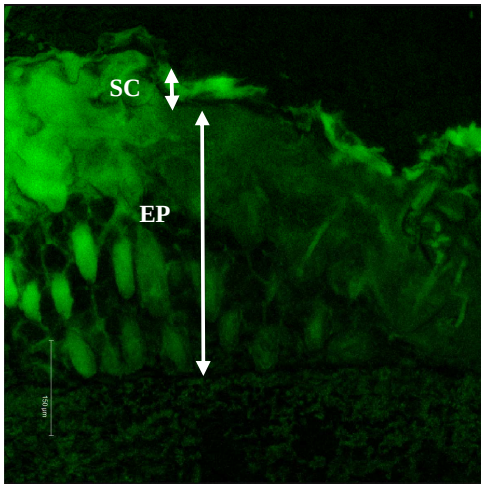



Fig. 2 CLSM images of a cross-section of nude dorsal skin incubated in a Franz diffusion cell with pTT- fluorescein elastic liposomes for 24 h. Scale bar represents 150 nm
SC: stratum corneum; EP: epidermis

*C. p53 protein generation study*

Thymidine dinucleotide (pTT) is an oligonucleotide that can activate the p53 transcription factor and trigger the signal transduction cascade. In brief, the p53 gene acts as a regulator of anti-oncogenes or as a tumor suppressor due to its ability to maintain normal growth control and genomic stability [23]. We employed an in vivo study apply elastic liposome for 4 h to dorsal skin of nude mouse and extract the total protein of treated areas to understand the p53 generation. The western blot study was compared to a negative control (pTT dissolved in double-distilled water) and a positive control (pTT dissolved in DMSO/PG: 7:3).The result of western blot showed the positive control group higher than elastic liposome. Elastic liposome group seems slight band in the last column, and we presume the phenomena due to the inefficient the application time.

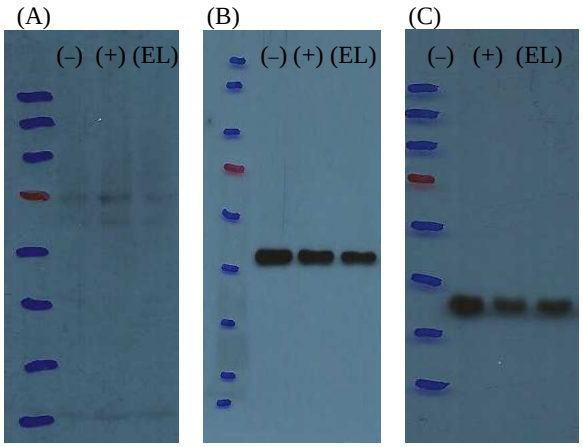



Fig. 3 pTT-loaded negative control, positive control and elastic liposome were applied once topically to the dorsal skin of nude mouse. Protein loading was assessed by probing the membrane with (A)p53 (B)β-actin (C) glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
(–)C: negative control; (+)C: positive control; EL: elastic liposome

## IV. Conclusion

In summary, elastic liposome containing pTT was characterized in this study. We confirmed that application of elastic liposome carriers in nude mouse skin can improve the penetration of oligonucleotide-pTT by CLSM study. The results indicated that the application time play a particular role in generated p53. Furthermore, to increase the generation of p53 by applies pTT-elastic liposome needs to be elucidated.

## Acknowledgment

We deeply appreciated the National Science Council, Taiwan for the financial support (NSC 99-2320-B-264-001-MY2) in this study. We are also grateful to Prof. Jia-You Fang, Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, Taiwan, for using Malvern instrument to conform the physicochemical properties.

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