Transcutaneous Co-Delivery of Therapeutic Agents Using Liposomes to Treat Cancer

SYNOPSIS

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ABSTRACT

Co-delivery of more than one therapeutic agent is a promising strategy to improve the therapeutic outcome of cancer therapy. Recently, a better understanding of the fact that cancers arise from the genetic disorders in cell signaling prompted the use of combination therapy involving drug and gene. Among the various nanocarriers explored for the topical delivery of nucleic acids, liposomes have emerged as the most preferable candidates. In the present work, different liposomal formulations were investigated for the transcutaneous co-delivery of either two anticancer drugs or an anticancer drug with siRNA for the treatment of breast and skin cancer respectively.

Initially, we have focussed on transcutaneous co-delivery of tamoxifen and imatinib mesylate using flexible temperature-sensitive liposomes for the treatment of breast cancer. Tamoxifen is a selective estrogen receptor modulator widely used for adjuvant hormonal therapy of estrogen receptor positive breast cancer. Tamoxifen and imatinib co-encapsulated liposomes were prepared using DPPC, MPPC, Span 80 (70:15:15 w/w) and characterised for average particle size, zeta potential, drug encapsulation efficiency, elasticity, in-vitro drug release at different temperatures and storage stability. Further, the impact of this co-delivery system on breast cancer cells were studied using MCF-7 (estrogen receptor positive) and MDA-MB-231 cells (estrogen receptor negative). Skin permeation studies using porcine skin were carried out to investigate the impact of temperature on permeation parameters of the temperature-sensitive liposomes. Results of in-vitro drug release studies showed more than 80% release of both drugs within 30 minutes at 40^{o}C. Cell viability studies showed enhanced cell growth inhibition upon combination therapy compared to individual therapeutic agents in case of both cell lines.

Later, we have investigated the liposomal co-delivery of curcumin and STAT3 siRNA by non-invasive topical iontophoretic application to treat skin cancer. Curcumin was encapsulated in cationic liposomes and then complexed with STAT3 siRNA. The liposomal nanocomplex was characterized for particle size, zeta-potential, drug release and stability. Human epidermoid (A431) cancer cells were used to study the cell uptake, growth inhibition and apoptosis induction of curcumin loaded liposome-siRNA complex. Topical iontophoresis was applied to study the skin penetration of nanocomplex in excised porcine skin model. Results showed that curcumin loaded liposome-siRNA complex was rapidly taken up by cells preferentially through clathrin mediated endocytosis pathway. The co-delivery of curcumin and STAT3 siRNA using liposomes resulted in significantly ($p<0.05$) greater cancer cell growth inhibition and apoptosis events compared with neat curcumin and free STAT3 siRNA treatment. Furthermore, topical iontophoresis application enhanced skin penetration of nanocomplex to penetrate viable epidermis.

Further, in-vivo studies were carried out to evaluate the efficacy of transcutaneous co-delivery of curcumin and STAT3 siRNA in mouse melanoma model using cationic liposomes. Co-administration of the curcumin and STAT3 siRNA using liposomes significantly ($p<0.05$) inhibited the tumor progression as measured by tumor volume and tumor weight compared with either liposomal curcumin or STAT3 siRNA alone. Furthermore, the iontophoretic administration of curcumin loaded liposome-siRNA complex showed similar effectiveness in inhibiting tumor progression and STAT3 protein suppression compared with intratumoral administration. Taken together, cationic liposomes can be developed for topical iontophoretic co-delivery of small molecule and siRNA for effective treatment of skin diseases.

Chapter 1:

Introduction and Objectives

This chapter deals with detailed review of transcutaneous drug delivery systems for delivery of small and macro-molecules. Initially, we discussed about cancer, especially breast cancer and skin cancer in detail as these two cancer cells could be accessed through transcutaneous delivery of therapeutic agents. Further, the scope and challenges in co-delivery of therapeutic agents in cancer chemotherapy is discussed in detail. As our focus was on transcutaneous delivery of chemotherapeutic agents, a thorough literature review on anatomy and physiology of skin, various routes of skin penetration and challenges & techniques for enhancing skin permeation of therapeutic agents was included. We also discussed about various nanocarriers like lipid based, polymeric and metal nanocarriers for transdermal drug delivery. Later, a detailed discussion about liposomes, including the various advantages of liposomes in transdermal drug delivery is also included. Based on the literature review, various gaps in existing research were identified and objectives of the study have been defined at the end of the chapter.

Chapter 2

Transcutaneous co-delivery of tamoxifen and imatinib mesylate using temperature-sensitive liposomes to treat breast cancer

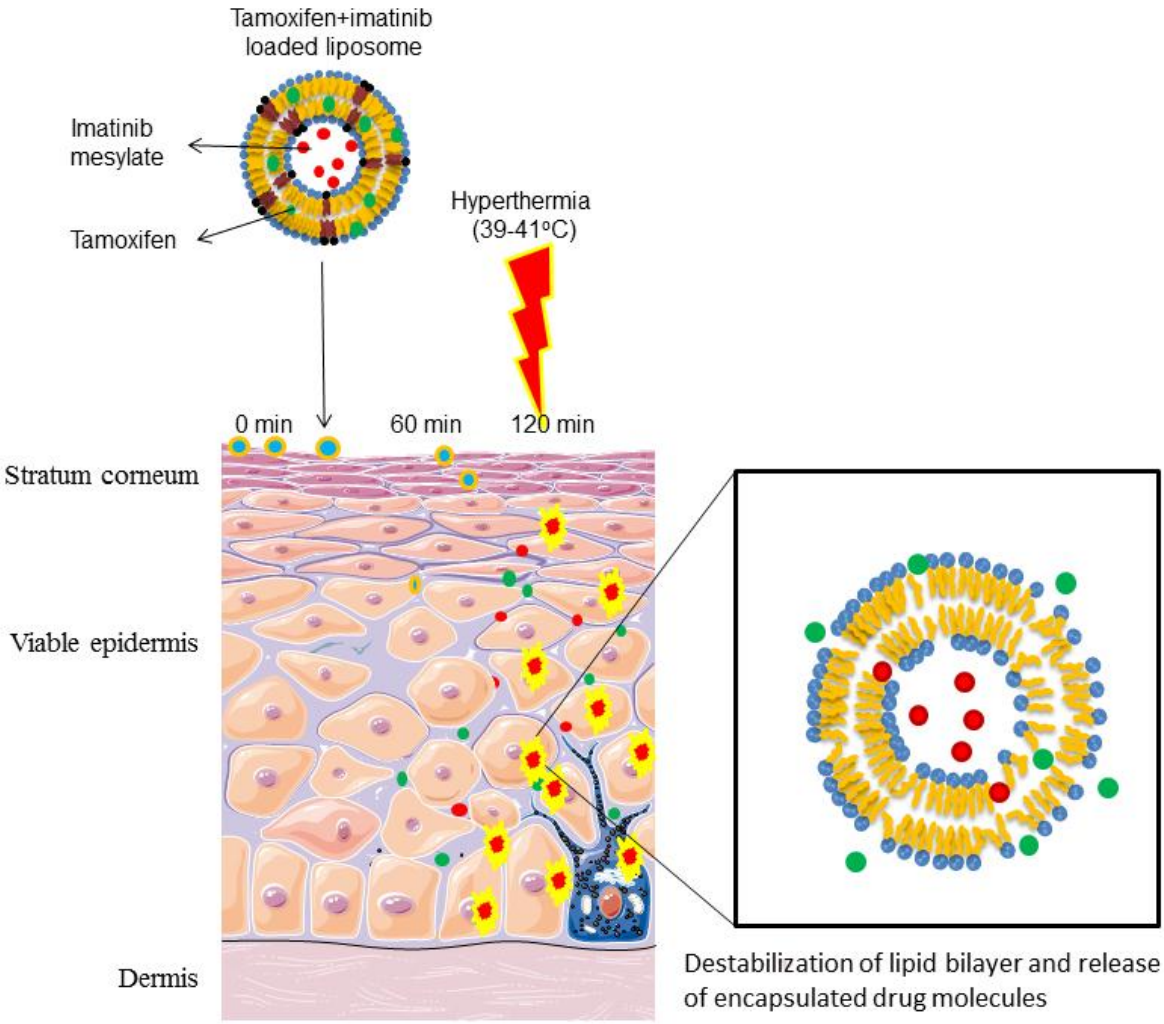

Fig.1. Schematic representation of the transcutaneous co-delivery of tamoxifen and imatinib mesylate using temperature-sensitive liposomes.

In this chapter, we investigated the feasibility of using temperature-sensitive liposomes for the transcutaneous co-delivery of tamoxifen and imatinib mesylate for the treatment of breast cancer. Co-delivery of chemotherapeutic agents using nanocarriers is a promising strategy for enhancing therapeutic efficacy of anticancer agents. Here, we have focussed on transcutaneous co-delivery of tamoxifen and imatinib mesylate using flexible temperature-sensitive liposomes for the treatment of breast cancer. Tamoxifen and imatinib co-encapsulated liposomes were prepared using DPPC, MPPC, Span 80 (70:15:15 w/w) and characterised for average particle size, zeta potential, drug encapsulation efficiency, elasticity, in-vitro drug release at different temperatures and storage stability. Further, the impact of this co-delivery system on breast cancer cells were studied using MCF-7 (oestrogen receptor positive) and MDA-MB-231 cells (oestrogen receptor negative). Skin permeation studies using porcine ear skin were carried out to investigate the impact of temperature on permeation parameters of the temperature-sensitive liposomes. The average particle size and zeta potential of dual drug loaded liposomes were found to be 168.50±7.20 nm and 16.70±3.60 respectively with encapsulation efficiency of more than 70% for both the drugs. Results of in-vitro drug release studies showed more than 80% release of both drugs within 30 minutes at 40^{o}C. Both MCF-7 and MDA-MB-231 cells showed uptake of coumarin loaded liposomes within 30 minutes. Cell viability studies showed enhanced cell growth inhibition upon combination therapy compared to individual therapeutic agents in case of both cell lines. Exposure of formulation incubated cells at 40^{o}C for 15 minutes resulted in further increase in percentage cell death compared with those incubated at 37^{o}C. Results of these studies suggests that transcutaneous co-delivery of tamoxifen and imatinib mesylate using temperature-sensitive liposomes can be used as a potential strategy for the effective management of breast cancer.

Chapter 3

Co-delivery of curcumin and STAT3 siRNA using deformable cationic liposomes to treat skin cancer

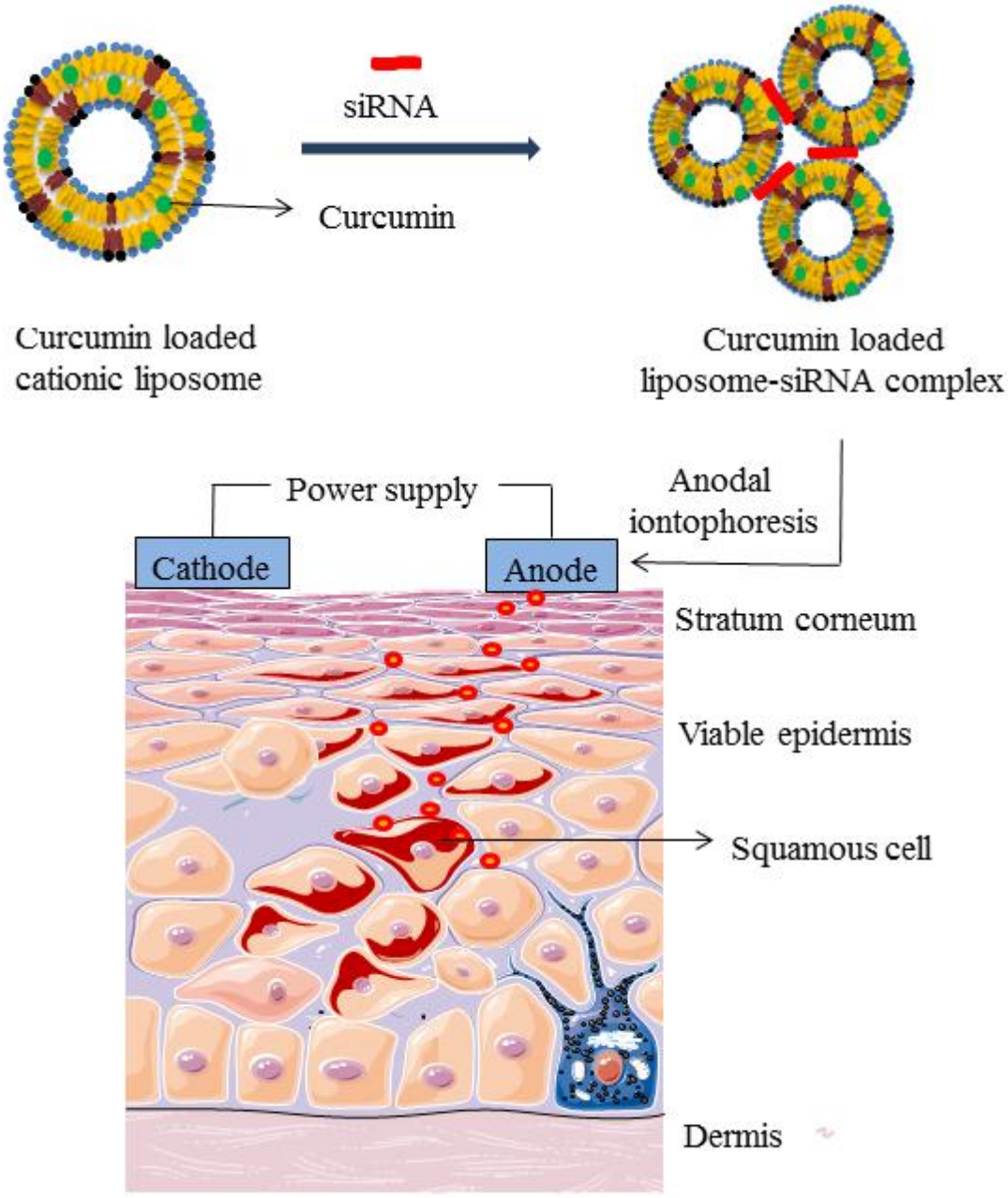

Fig.2. Schematic representation of the transcutaneous co-delivery of curcumin and STAT3 siRNA using flexible cationic liposomes.

In this chapter, we investigated the feasibility of using flexible cationic liposomes for the transcutaneous co-delivery of curcumin and STAT3 siRNA for the treatment of skin cancer. Skin cancer is one of the most widely prevalent cancer types with over expression of multiple oncogenic signaling molecules including STAT3. Curcumin is a natural compound with effective anti-cancer properties. The objective of this work was to investigate the liposomal co-delivery of curcumin and STAT3 siRNA by non-invasive topical iontophoretic application to treat skin cancer. Curcumin was encapsulated in cationic liposomes and then complexed with STAT3 siRNA. The liposomal nanocomplex was characterized for particle size, zeta-potential, drug release and stability. Human epidermoid (A431) cancer cells were used to study the cell uptake, growth inhibition, apoptosis induction and STAT3 protein suppression of curcumin loaded liposome-siRNA complex. Topical iontophoresis was applied to study the skin penetration of nanocomplex in excised porcine skin model. The average particle size and zeta potential of curcumin loaded liposome-siRNA complex was found to be 195.0±9.0 nm (PDI-0.240±0.005) and 58.8±6.0 mV respectively. Gel electrophoresis results indicated complexation of curcumin loaded cationic liposomes with STAT3 siRNA. Results showed that curcumin loaded liposome-siRNA complex was rapidly taken up by cells preferentially through clathrin mediated endocytosis pathway. The co-delivery of curcumin and STAT3 siRNA using liposomes resulted in significantly ($p<0.05$) greater cancer cell growth inhibition and apoptosis events compared with neat curcumin and free STAT3 siRNA treatment. Furthermore, topical iontophoresis application enhanced skin penetration of nanocomplex to penetrate viable epidermis. In conclusion, cationic liposomal system can be developed for non-invasive iontophoretic co-delivery of curcumin and siRNA to treat skin cancer.

Chapter 4

Transcutaneous iontophoretic co-delivery of curcumin and STAT3 siRNA using flexible cationic liposomes: Proof of concept in mouse skin cancer model

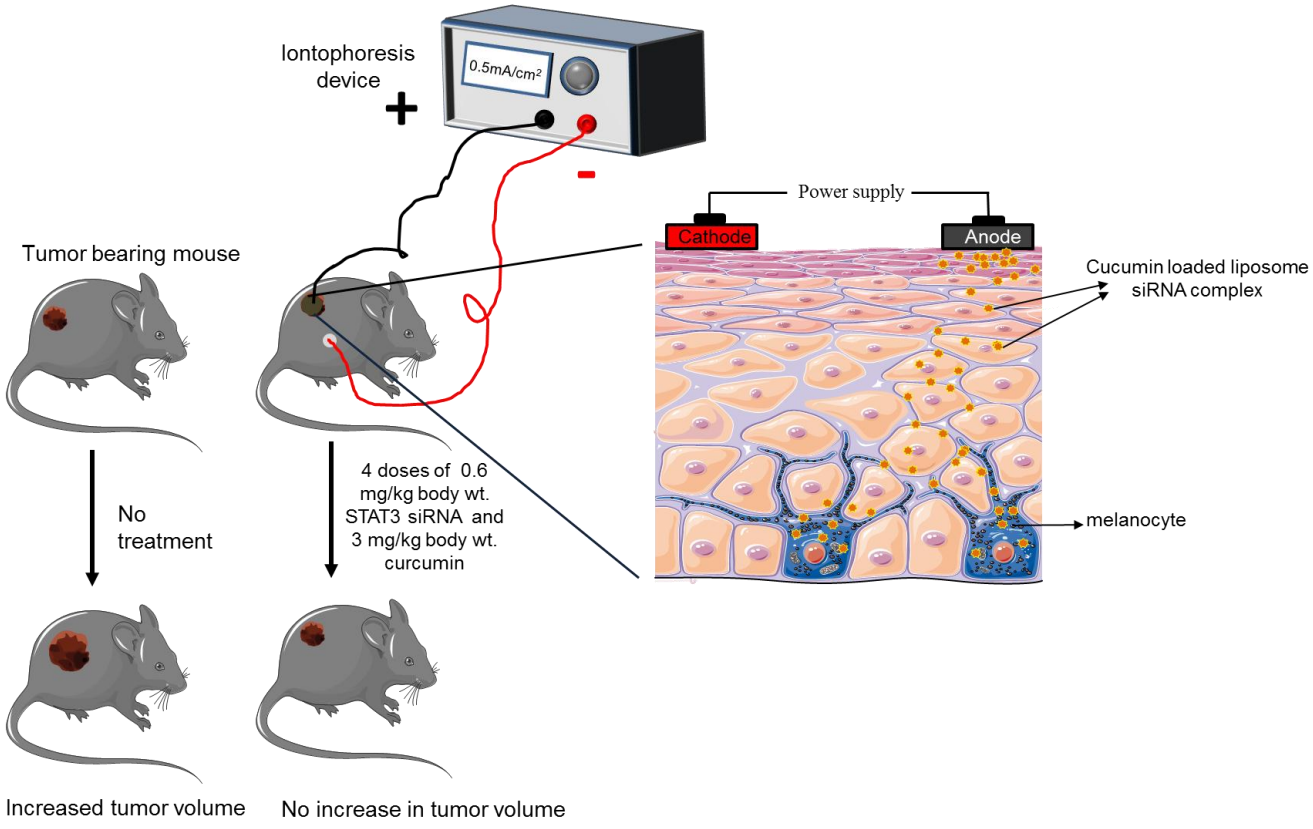

Fig.3. Schematic representation of iontophoretic transcutaneous co-delivery of curcumin and STAT3 siRNA using cationic liposomes to treat mice melanoma.

In this chapter, we investigated the feasibility of using flexible cationic liposomes for the iontophoretic transcutaneous co-delivery of curcumin and STAT3 siRNA to treat mice skin cancer. Curcumin was encapsulated in DOTAP-based cationic liposomes and then complexed with STAT3 siRNA. This nanocomplex was characterized for the average particle size, zeta-potential and encapsulation efficiency. The cell viability studies in B16F10 mouse melanoma cells have shown that the co-delivery of curcumin and STAT3 siRNA significantly ($p<0.05$) inhibited the cancer cell growth compared with either liposomal curcumin or STAT3 siRNA alone. The curcumin loaded liposomes were able to penetrate up to a depth of 160 µm inside the skin after iontophoretic (0.47 mA/cm^2) application. The *in vivo* efficacy studies were performed in mouse model of melanoma skin cancer using C57/BL6 mice. Skin cancer was induced on mice by subcutaneous injection of mice melanoma cells (B16F10). Divided the tumor bearing mice into 10 groups of five animals each and each groups were treated with different formulations. Co-administration of curcumin and STAT3 siRNA using liposomes significantly ($p<0.05$) inhibited the tumor progression as measured by tumor volume and tumor weight compared with either liposomal curcumin or STAT3 siRNA alone. Furthermore, the iontophoretic administration of curcumin loaded liposome-siRNA complex showed similar effectiveness in inhibiting tumor progression and STAT3 protein suppression compared with intratumoral administration. Results of western blotting and immunohistochemistry studies indicated significant STAT3 protein suppression in those groups treated with iontophoretic co-delivery of curcumin and STAT3 siRNA. Taken together, cationic liposomes can be utilized for topical iontophoretic co-delivery of small molecule and siRNA for effective treatment of skin diseases.

Chapter 5

Summary and conclusions

In the present work, we have investigated the feasibility of transcutaneous co-delivery of two micro-molecules, tamoxifen and imatinib mesylate using temperature-sensitive liposomes to treat breast cancer. Additionally, cationic liposomes containing surface active agents were investigated for the co-delivery of a small molecule, curcumin along with a macro-molecule, STAT3 siRNA for the treatment of skin cancer.

Temperature-sensitive liposomes made up of DPPC, MPPC and span 80 co-encapsulated tamoxifen and imatinib mesylate with sufficient loading and encapsulation efficiency. Inclusion of span 80 in the liposomal composition enhanced the elasticity of liposomes and thereby improved skin permeation. Average particle size of liposomes remained less than 200 nm, ideal for transdermal application and the nano-carriers were found to be stable for more than one month when stored at 2-8^{o}C. Temperature dependent release studies showed more than 80% release of both tamoxifen and imatinib from the liposomes at temperature above 39^{o}C.

These liposomes were taken up by both MCF-7 and MDA-MB-231 cells within 30 minutes of incubation. Cell viability studies showed that co-delivery of tamoxifen and imatinib significantly enhanced cell growth inhibition of MCF-7 and MDA-MB-231 cells compared to individual agents. Liposomes heated at 40^{o}C to release the drugs outside the cells showed enhanced cell growth inhibition compared to those at 37^{o}C. Skin permeation studies showed significant increase in the amount of drug retained in SC and viable epidermis after application of mild hyperthermia.

Further, to improve the skin permeation by iontophoresis and to incorporate negatively charged siRNA, cationic liposomes were prepared using DOTAP, DOPE and sodium cholate. The encapsulation efficiency of curcumin within cationic liposomes was more than 80% and

sodium cholate significantly improved the elasticity of liposomes. N/P ratio of 10:1 resulted in sufficient complexation of siRNA with curcumin loaded cationic liposomes. The liposome-siRNA complex remained stable for more than one month when stored at 2-8^{o}C.

Cell uptake studies showed that curcumin loaded liposome-siRNA complex was taken up by A431 cells within 30 minutes, predominantly through clathrin mediated pathway. Cell viability, apoptosis and in-vitro gene silencing studies showed significant enhancement in cell growth inhibition and protein suppression by the co-delivery of curcumin and STAT3 siRNA compared to individual agents. Skin permeation studies showed significant enhancement in skin permeation upon iontophoresis compared to passive application.

In-vivo studies showed significant reduction in tumor weight and tumor volume by iontophoretic application of curcumin loaded liposome-siRNA complex compared with individual agents. Iontophoresis significantly reduced tumor progression compared with passive application of the same formulations. Results of in-vivo gene silencing and immunohistochemistry studies showed significant STAT3 suppression in those animals treated with iontophoretic application of curcumin loaded liposome-siRNA complex compared with individual agents.

Overall, this study demonstrates that liposomes can be utilized for topical iontophoretic co-delivery of small and macro-molecules for the treatment of various cancers including skin and breast cancer.