Topical Iontophoretic Co-delivery of Curcumin and STAT3 siRNA Using Liposomes to Treat Skin Cancer
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Purpose
The objective of this study was to prepare and characterize curcumin loaded cationic liposome-STAT3 siRNA complex and to investigate the efficiency of this formulation in-vitro on human epidermoid carcinoma cells (A431 cells).

Methods
Curcumin loaded cationic liposomes containing DOTAP, DOPE, sodium cholate and C6 ceramide (50:30:10:10 w/w) were prepared by thin film hydration method. Liposomes were then complexed with STAT3 siRNA at N/P ratio of 10:1. The nanocomplexes were characterized using Zetasizer and polyacrylamide gel electrophoresis. In vitro evaluations including cytotoxicity, cellular uptake and mechanism of uptake, STAT3 protein suppression and apoptosis assay were performed in A431 cells. Passive and iontophoresis assisted skin permeation of curcumin loaded liposome-Cy3 siRNA complex was performed on excised porcine ear skin model.

Results
A lipid to curcumin ratio of 10:1 was found to show greatest encapsulation efficiency. The average particle size and zeta-potential of curcumin loaded cationic liposome-siRNA complex (Cur-lip-siRNA) were found to be 195.0±9.0 nm (PDI, 0.240±0.005) and 58.8±6.0, respectively. Gel electrophoresis results revealed complexation of siRNA with cationic liposomes. Co-delivery of siRNA and curcumin using Cur-lip-siRNA (33 μg curcumin and 0.5 nM siRNA) showed greatest cell growth inhibition of 72.9±2.3% compared with liposomal curcumin (44.9±3.1%) and liposome-siRNA (53.4±2.7%) treatment (Fig. 1). A431 cell uptake studies showed no cell associated fluorescence after treatment with free curcumin or siRNA, whereas Cur-lip-Cy3 siRNA complex showed significant cell associated fluorescence within 30 minutes. The cells pretreated with chlorpromazine showed lesser fluorescent intensity compared to those pretreated with methyl-β-cyclodextrin indicating the predominant cell uptake pathway for curcumin loaded liposome-siRNA complex was clathrin mediated mechanism. Cur-lip-siRNA showed greatest apoptotic activity with 13.80±1.88% early apoptotic and 67.27±3.02% late apoptotic cells. Similarly, Western blotting showed 56.4% STAT3 protein suppression after treatment with Cur-lip-siRNA. It was found that passive application of Cur-lip-Cy3 siRNA did not reach the viable epidermis and was retained within stratum corneum. Meanwhile, 4 h iontophoresis application enhanced the skin permeation of Cur-lip-siRNA to reach the viable epidermis (Fig. 2).

Conclusion
Cationic liposomes containing sodium cholate can be developed as a potential delivery system for topical co-delivery of curcumin and STAT3 siRNA to treat skin cancer.