

Letter to the Editor

PRODUCT-DELIVERING LIPOSOMES SPECIFICALLY TARGET HAIR FOLLICLES IN HISTOCULTURED INTACT SKIN

Dear Editor:

There is an important need in finding a way to directly influence hair growth, color and appearance, especially for the treatment of alopecia in humans. The existing approaches are expensive, labor-consuming and relatively short-lasting (surgical transplantation) or relatively low-efficient (the use of different creams and lotions with biologically-active substances).

The use of biologically-active stimulators of hair growth seems to be the more natural and attractive approach, especially at the stage when hair-follicle cells still exist but hair growth, for unknown reasons, is affected. The relatively low efficacy of this approach can possibly be explained by the inability of stimulators to penetrate the cellular membrane of hair-follicle cells and to enter inside cells where their action is needed.

Liposomes which are artificial phospholipid vesicles can be successfully used for the delivery of different low-molecular-weight water-soluble and oil-soluble compounds into different cells (Gregoriadis, 1988; Schmidt, 1986). At the same time no available data exist on liposome-mediated drug delivery into hair follicle cells. This is probably due to the absence of experimental cultures of appropriate cells or skin samples.

Previously we developed relatively long-term histoculture (Hoffman, 1991) of whole human and mouse skin, utilizing a collagen-sponge-gel-supported in vitro three-dimensional histoculture system, such that long-term effects of various agents can be studied with regard to toxicology, penetration and hair growth (Li et al., 1991). We have been able to culture both white (Li et al., 1992a) and black mouse skin (Li, et al., 1992b) with hair growing for periods of at least 10-16 days respectively, and human scalp tissue with hair follicle cells proliferating for at least 40 days (Li et al., 1992c). We have been able to determine cytotoxicity and viability with fluorescent dyes identifying dead and living cells, including cells in hair follicles, three-dimensionally in the histocultured skin in the living state, in particular with confocal microscopy (Li et al., 1991). A key aspect of the intact skin histocultured in our system is the presence of the major types of cells, including hair follicles, as well as growing hair. The histoculture model for skin may be an effective replacement for animal systems and superior to the dispersed and reconstituted skin equivalent cell system used previously (Naughton et al., 1989) for measurements of the effect of manufactured products, drugs and pollutants on skin and hair growth.

The use of three-dimensional histoculture in conjunction with confocal microscopy opens the opportunity to follow fine details of product-delivering liposome interactions with hair-follicles at the cellular level. As a result, optimal liposome compositions can be

established as well as the conditions for the delivery of the liposomal content into target cells. We describe here the specific affinity of product-delivering liposomes for hair follicles in histocultured skin.

Pieces of shaved outbred white-haired-mouse or nude-mouse skin (approx. $2 \times 5 \times 2$ mm) were harvested under a dissection microscope and then histocultured on collagen-gel supported sponge as described earlier (Li et al., 1991). Liposome interaction with the skin was initiated after 24 hours of histoculture.

Liposomes were prepared by sonication of phosphatidylcholine (PC) (25 mg) emulsion in phosphate buffered saline (PBS) containing the fluorescent dye calcein (20 mg/ml). Liposomes were separated from the non-entrapped dye by gel-filtration on a Sepharose 4B column diluted with phosphate buffered saline (PBS). The amount of the entrapped dye was measured spectrofluorimetrically. Two types of PC were used: egg PC (EPC) and dipalmitoyl PC (DPPC). Due to their phase transition temperatures liposomes made of DPPC are in a gel phase at 37° C while liposomes prepared from EPC are in a liquid-crystalline state.

Mouse skin histocultures were incubated for 20 minutes with liposomes or with a solution of "free" calcein dye at the same concentration as it was in the liposome preparation. After the tissues were thoroughly washed, the specimens were analyzed with a Biorad MRC 600 laser confocal microscope with a BHS filter block, which excites the tissue at 488 nm and passes the light emitted at 520. These parameters are close to the excitation and emission maxima reported for calcein (Haugland, 1989). It is also important to note, that there is no autofluorescence of tissue when these emission and excitation wavelengths are used.

Figure 1 A shows the skin histocultures after incubation with liposome-entrapped dye with specific delivery of the dye to the hair follicles. The histocultured skin incubated with free calcein solution exhibits relatively low fluorescence with no preferential staining of any particular skin structure as seen in the confocal micrographs in Figure 1 B. As demonstrated by these images, liposome-entrapped dye in contrast to free dye becomes associated with hair follicles. It is important to note that the image in Figure 1 B was with the same parameters of aperture and gain control as Figure 1 A.

In order to understand whether liposomes are preferentially targeting hair follicles we have used gel-phase liposomes, which are known for their stability in living systems. As seen in Figure 1 C and 1 D these liposomes are associated preferentially with hair follicles evident both when liposomes were labeled with calcein (Figure 1 C) or when the liposomes were fluorescently labeled with NBD-phosphatidylcholine (Haugland, 1989) (Figure 1 D).

Based on the results of the experiments, we conclude: 1) liposomes seem promising as a preferential delivery vehicle to hair

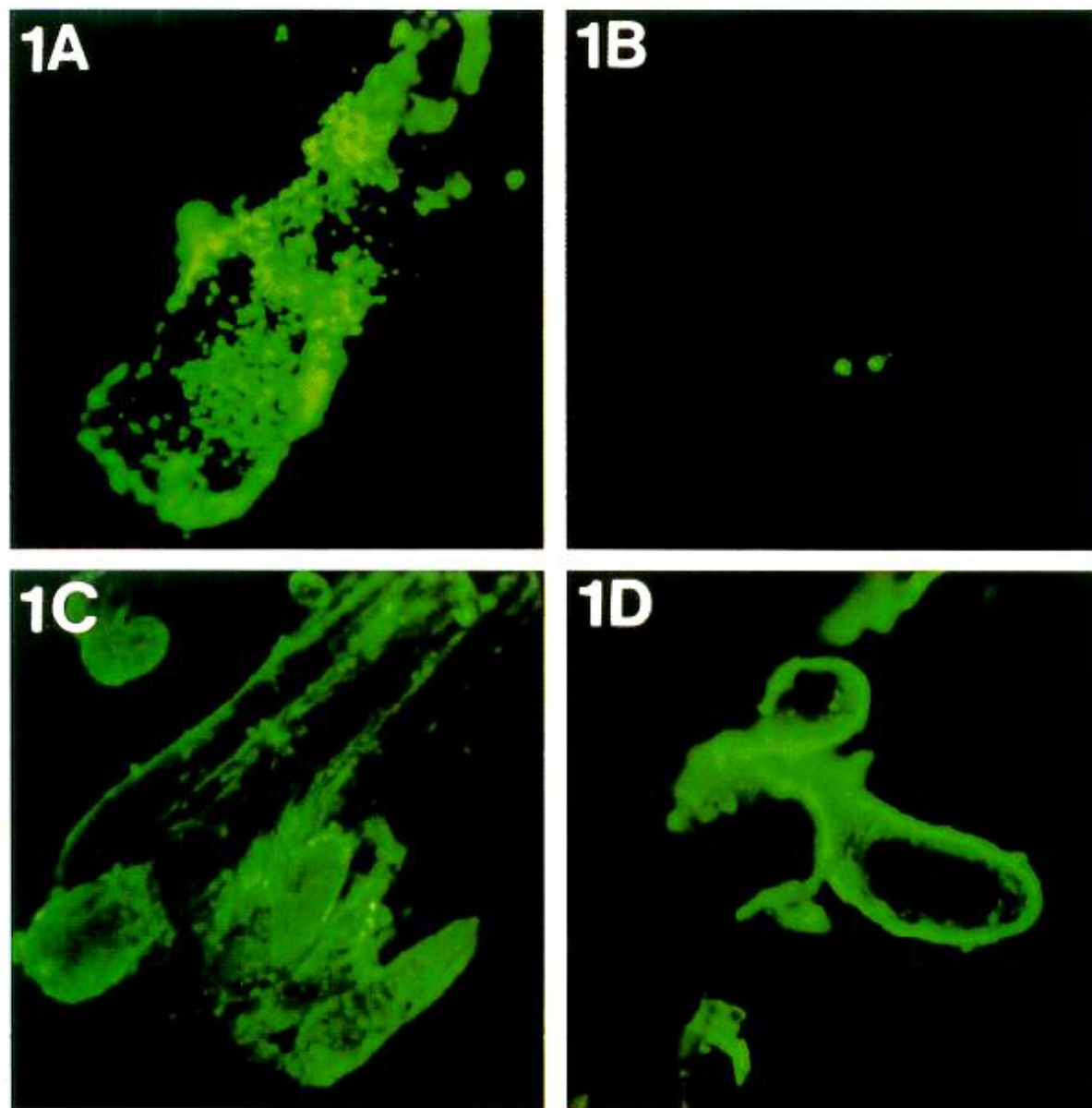


FIG. 1. A, Mouse skin histocultures treated with calcein-entrapped egg phosphatidylcholine liposomes (liquid-crystalline). Note the high efficiency of the delivery of the green fluorescent dye preferentially into hair follicles. B, Mouse skin histocultures treated with the green fluorescent hydrophilic dye calcein without liposomes which is at the same concentration as was entrapped into the liposomes in Figure 1 A (control). Note that the green staining is very weak and there is no preferential delivery of the stain to skin structures. C, D, Mouse skin histocultures treated with fluorescent-labeled gel dipalmitoylphosphatidylcholine liposomes. Note the preferential localization of the liposome-associated markers on the surface of hair follicles. C, Calcein-labeled liposomes; D, NBD-phosphatidylethanolamine labeled liposome. Images obtained by confocal scanning laser microscopy. Magnification $\times 500$.

follicles; 2) depending of the goal, different type of liposomes can be used to deliver entrapped substances into the follicle or to its surface; 3) confocal laser microscopy is a highly efficient instrument to study hair follicles in the histocultured skin specimens stained with fluorescent dyes; and 4) the histoculture-liposome system coupled with confocal scanning laser microscopy and other means of analysis such as measurement of hair growth itself (Li et al., 1992a, 1992c) could serve as an effective screen for agents which perturb the hair cycle such as hair-growth stimulators.

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