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Freeze-fracture Electron Microscopy on Nanostructures for Drug & Gene Delivery

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The potency of drug/gene-loaded carriers is frequently depending upon their morphology adopted in a biological relevant environment. Freeze-fracture electron microscopy is not only a powerful technique to characterize drug/gene carrier on a nanometer resolution scale but also the method of choice to study their fate related to drug/gene load, application milieu, and during interaction with cells [1-3]. Using freeze-fracture electron microscopy we studied the morphology of a wide variety of drug and gene carriers such as Multilamellar Vesicles (MLV}, Small Unilamellar Vesicles (SUV), niosomes, cochleate cylinder, depofoam particles, and Cationic Liposome/DNA Complexes (CLDC). Furthermore we investigated the structural transformation of cationic liposomes caused by certain helper lipids, the ionic strength of the incubation media, as well as the gene encapsulated and correlated the morphology to their transfection activity under in vitro as well as in vivo conditions. Additionally we recorded the interaction of selected drug/gene carries with bacteria or cultured skin cells.

While MLV display a multitude of bilayers and diameters of several micrometers, SUV have only one bilayer and can be as small as 15 nm in diameter (FIG. 1.1 and 1.2). Niosomes are made of non-ionic detergents and look normally very similar to liposomes. Under certain conditions, however, they are able to adopt geodetic sphere structure (FIG. 1.3) [4]. Cochleate cylinders are made of negatively charged lipids and form, when calcium ions are added, large cigar-type cylinders, several micrometer in width and several tens of micrometers in length (FIG. 1.4) [1,2]. DepoFoam particles are prepared by a double emulsification process and display a chambered inner volume and diameters of several micrometers (FIG. 1.5). Depending upon helper lipid, ionic strength, and gene component CLDC are able to adopt structures such as spaghetti/meatball–type structure (FIG. 2.1), map-pin (FIG. 2.2), as well as honeycomb structure (FIG. 2.3) [5,6]. Parallel studies of transfection activity and morphology of CLDC revealed a fundamental difference between *in vitro* and *in vivo* transfection activity. Lipid precipitates displaying honeycomb structure are associated with high transfection rates under *in vitro* conditions. *In vivo* transfection activity seems to be associated with small complexes such as map-pin structure [6]. Our cell interaction studies of selected drug/gene carriers show size/curvature-dependent uptake mechanisms.

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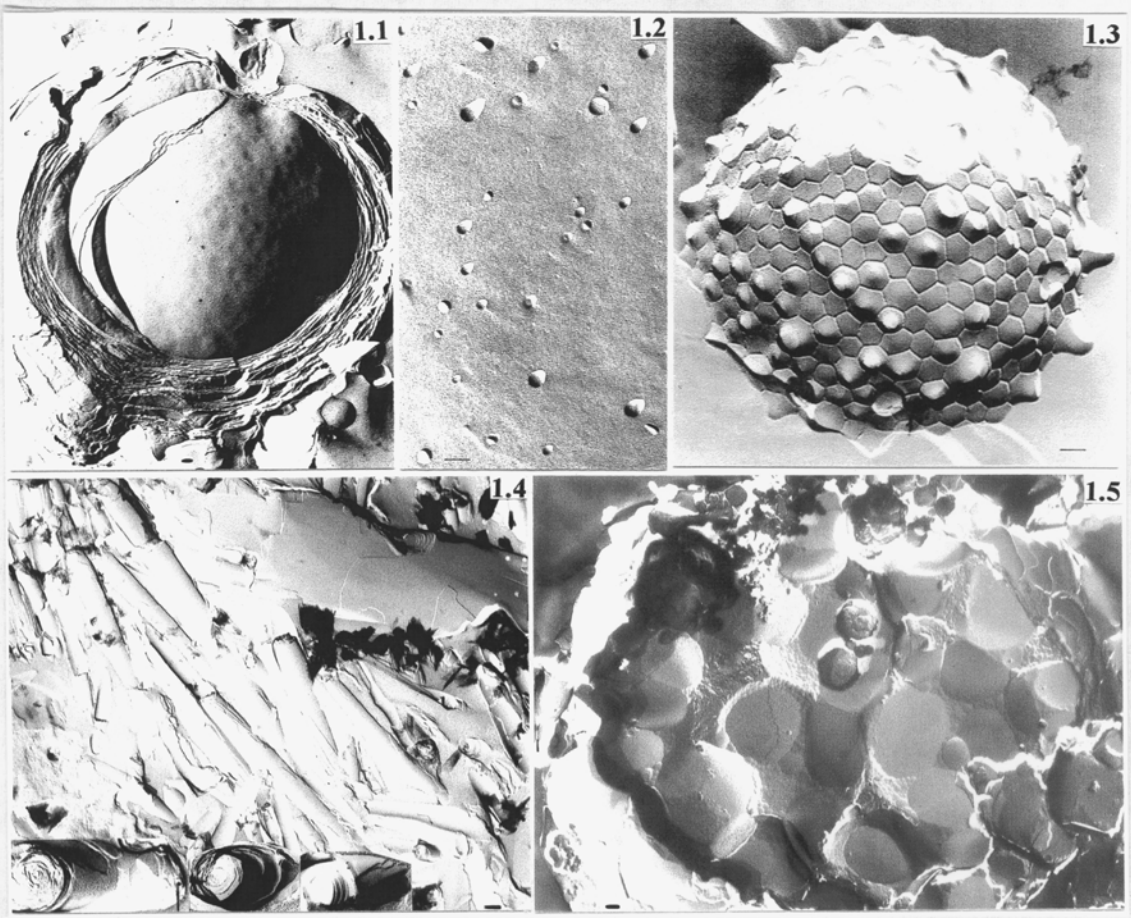

FIG. 1. Freeze-fracture electron micrographs of selected drug carriers.

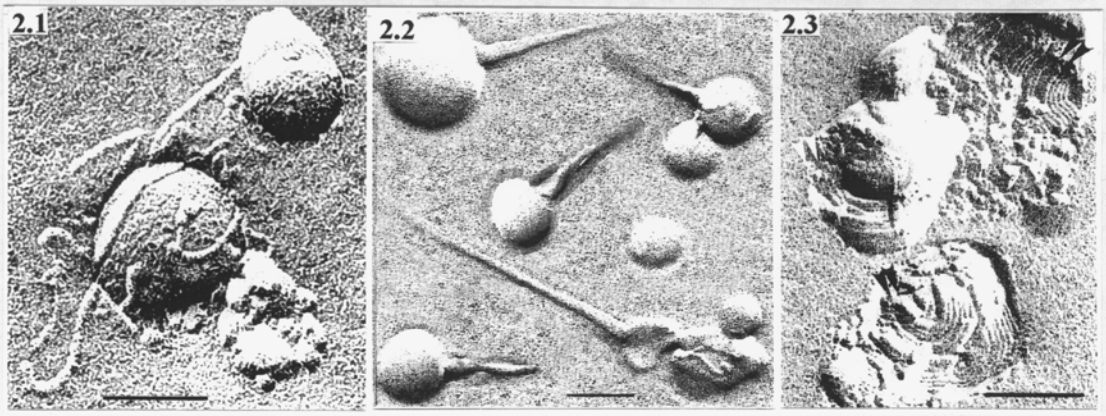

FIG. 2. Structural polymorphism of CLDC as lipid-based, non-viral vectors.

The bar represents 100 nm on all electron micrographs and the shadow direction is running from bottom to top.

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