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Comparison of Freeze-fracture- with Cryo-Electron Microscopy on Molecular Assemblies Suitable 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. Cryofixation techniques such as freeze-fracture electron microscopy (ff-em) and cryo-electron microscopy (cryo-em) are powerful techniques to monitor the self-assembling of lipid- as well as protein-based drug and gene carries.

Cryo-em has a slightly higher resolution compared to ff-em (2nm for periodical structures) and allows monitoring the inner volume of drug and gene carriers. However, it is restricted to smaller carriers and sub-cellular structures, to low concentrated/viscose dispersions, and has to struggle with beam damage. These drawbacks are irrelevant for ff-em. Ff-em is not only a powerful technique to characterize nanometer and micrometer-size drug/gene carriers but also the method of choice to study their fate related to drug/gene load, application milieu, and during interaction with cells [1-3]. Furthermore it allows distinguishing between bilayer and non-bilayer structures.

Using ff-em we studied the morphology of a wide variety of drug and gene carriers such as multilamellar liposomes (MLV), lipid-stabilized gas bubbles, depofoam particles, cochleate cylinder, niosomes, small unilamellar liposomes (SUV), micelles, and cationic liposome/DNA complexes (CLDC) [1-4]. Because of their small size, spherical micelles (<50nm) and small liposomes (<100nm) accumulate in pathological areas and are excellent carriers for poorly water-soluble drugs [5,6]. Depending upon helper lipid, ionic strength, and gene component CLDC adopt polymorph structures such as spaghetti/ meatballs, map-pins, as well as honeycomb structures [7,8]. Parallel studies of transfection activity and morphology of CLDC showed that 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 [8].

Furthermore the fracture behavior of lipid-based macromolecular assemblies indicates their adoption of bilayer or non-bilayer structures. While bilayer vesicles such as liposomes display convex *and* concave fracture faces, monolayer-coated gas bubbles show concave, and hard-core particles such as micelles convex fracture faces respectively.

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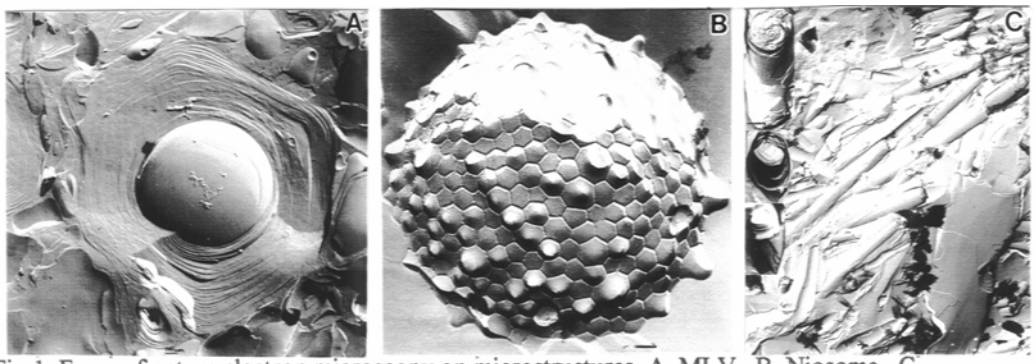

Fig.1. Freeze-fracture electron microscopy on microstructures. A. MLV. B. Niosome. C. Cochleate cylinder. Bar scale represents 100nm.

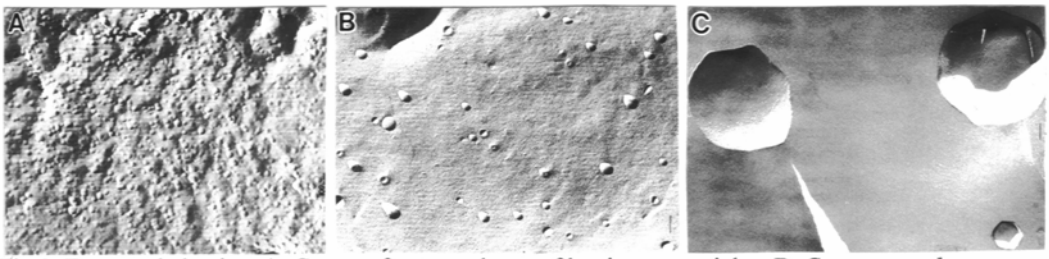

Fig.2. Fracture behavior. A. Convex fracture planes of hard-core particles, B. Concave *and* convex fracture planes of SUV, C. Concave fracture faces of lipid coated gas bubbles. Bar scale=100nm.

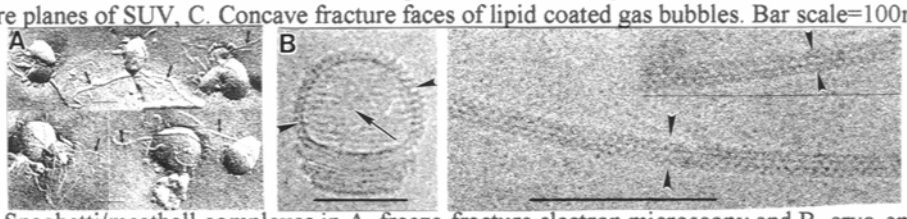

Fig.3. Spaghetti/meatball complexes in A. freeze-fracture electron microscopy and B. cryo-em. Bar scale=100nm. Fig.3B. courtesy of Christoph Böttcher.

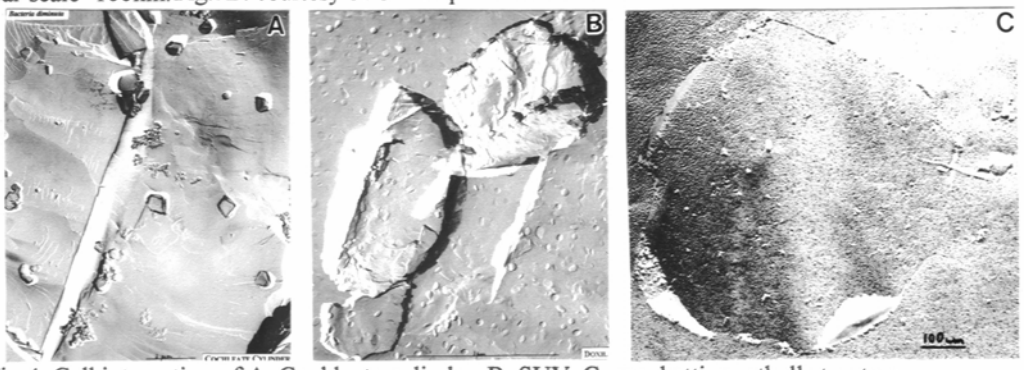

Fig.4. Cell interaction of A. Cochleate cylinder, B. SUV, C. spaghetti-meatball structure. Fig.4A. + 4B. bar scale=1μm. Fig.4C. bare scale=100nm.

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