Seeing Multifunctional Nano-& Microparticles Suitable for Therapy & Imaging by Freeze-fracture Electron Microscopy

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ABSTRACT

The potency of nanoparticles, loaded with drugs, genes, and/or diagnostics, is frequently depending upon their morphology adopted in a biological relevant environment. Freeze-fracture electron microscopy is a powerful techniques to monitor the self-assembling of lipid-, polymer-, as well as protein/peptide-based drug and gene carries on a nano-size resolution scale. Freeze-fracture electron microscopy allows not only the characterization of nano- and micoparticles suitable for therapy and imaging but also is the method of choice to study their fate related to their pay load, application milieu, and during their interaction with cells. Using freeze-fracture elelctron microscopy we studied the morphology of a wide variety of nano- and microparticles suitable for contrast enhancement as well as drug and gene delivery. This study includes quantum dots, micelles (spherical-, disc-, and worm-type micelles), small unilamellar liposomes, multilamellar liposomes, niosomes, lipid-stabilized gas bubbles, cochleate cylinder, depofoam particles, and drug crystals. Additionally we recorded the interaction of selected drug/gene carries with bacteria or cultured skin cells.

Keywords: nano- and microparticles, drug- and gene delivery, quantum dots, freeze-fracture electron microscopy.

1 METHOD

For freeze-fracture electron microscopy the multifunctional nano- and microparticles suitable for therapy and/or imaging were shock-frozen using sandwich technique and liquid nitrogen-cooled propane. Using this technique a cooling rate of 10,000 Kelvin per second is reached avoiding ice crystal formation and artifacts possibly caused by the cryofixation process. The cryo-fixed samples were stored in liquid nitrogen for less than 2 hours before processing. The fracturing process was carried out in JEOL JED-9000 freeze-etching equipment and the exposed fracture planes were shadowed with Pt for 30 sec in an angle of 25-35 degree and with carbon for 35 sec (2kV/ 60-70mA, $1x10^{-5}$ Torr). The replicas produced this way were cleaned with concentrated, fuming HNO_3 for 24 hours followed by repeating agitation with fresh chloroform/methanol (1:1 by vol.) at least 5 times. The replicas cleaned this way were examined at a JEOL 100 CX electron microscope.

2 RESULTS AND DISCUSSION

As a cryofixation -, replica -, and transmission electron microscopy technique, the resolution limit of freeze-fracture electron microscopy is determined by the particle size of the evaporation layer forming the replica. In our hands it is 2nm for periodical structures [1-3].

2.1 Nano- & Microparticles for Drug- & Gene Delivery

While multilamellar vesicles (MLV) display a multitude of bilayers and diameters of several micrometers, small, unilamellar vesicles (SUV) have only one bilayer and can be as small as 15 nm in diameter [1-4]. Because of the their small size liposomes, spherical and disc-type micelles (usually 5-50nm), demonstrate spontaneous accumulation in pathological areas with leaky vasculature, such as infarct zones and tumors and turn out to be excellent carrier for poorly water soluble drugs [4, 5].

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 [6]. 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 [1,2]. DepoFoam particles are prepared by a double emulsification process and display a chambered inner volume and diameters of several micrometers.

Cationic Liposome/DNA Complexes (CLDC) are able to adopt structures such as spaghetti/meatball–type structure, map-pin, as well as honeycomb structure [7,8]. 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 [8].

Our cell interaction studies of selected drug/gene carriers show size/curvature-dependent uptake mechanisms.

2.2 Nano- & Microparticles for Diagnostics

Semiconductor quantum dots (QDs) are a new class of promising fluorescent probes for many biological and biomedical applications due to their advantages over conventional dyes. QDs have been incorporated into immunoliposome nanocomplexes to develop novel molecular imaging agents for applications in cancer diagnosis and treatment. Using freeze-fracture electron microscopy we visualized carboxyl QDs (11nm), naked and complexed with liposomes as well as with HER-2-tageting immunoliposomes.

Suspensions of lipid-stabilized microbubbles are frequently used for biomedical applications such as ultrasound contrast enhancement, ultrasound-promoted drug and gene delivery, and blood substitution. Since lipid-stabilized gas bubbles display concave fracture planes only, freeze-fracture electron microscopy provides the proof for monolayer coated gas bubbles where the hydrophilic lipid headgroups are reaching into the water phase and their hydrophobic tails are directed into the gas phase.

2.3 References

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