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Freeze-fracture Electron Microscopy on Nano- and Microparticles Used for Theranostics and in Vaccines

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Nano- and microparticles are frequently used as carrier systems for delivering of macromolecules at cellular level. Nowadays these macromolecules do not only represent therapeutics such as anticancer and anti-fungal agents and informational molecules such as DNA, plasmids, or antisense oligonucleotides. Currently they also include diagnostics and antigens for vaccination. The potency of such nano- and micro-particles is frequently depending upon their morphology adopted in a biological relevant environment.

Freeze-fracture electron microscopy (ff-EM) as a cryo-fixation, replica TEM method is a powerful technique to monitor self-assembling of lipid-, polymer-, as well as protein/peptide-based nano- and microcarriers, encapsulating drug-, gene-, vaccine, antimicrobial- and imaging molecules [1-5]. Since the resolution of ff-EM is (in our hands) 2 nm for periodical structures we are able to characterize such carriers on a nano-scale resolution. Furthermore, beam-damage resistant replica can be produced from micro-meter size objects, allowing us to study nano-scale events in micro-scale biological and artificial assemblies [6]. Freeze-fracture-EM allows not only the characterization of nano- and microcarriers suitable for theranostics, but also is the method of choice to study their fate related to their pay load, application milieu, and during their interaction with cells. Furthermore, ff-EM allows observing superstructure transformation from bilayer to non-bilayer structures such as micelles and hexagonal as well as cubic lipid phases [7].

Using ff-EM we studied the morphology of a wide variety of nano- and microcarriers suitable to encapsulate a large variety of theranostics [1-6]. Currently, however, we are focused on carriers for diagnostics and vaccines, including nano-carries such as quantum dots (free (Fig.1) and coupled to drug-loaded immunoliposomes (Fig.4) [8], polymeric immunomicelles loaded with iron oxide NP (Fig. 2) [9], gold nanoparticles (Fig. 3), and ^{1}H MR contrast agents (Fig.5) [8]. Recently, we explored Influenza virus-derived virosomes (Fig.6+7) and measles vaccine powders (Fig. 8) by ff-EM [10].

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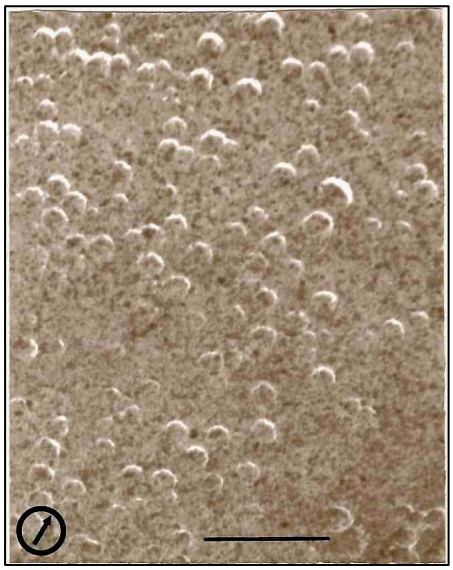

Fig.1: Quantum Dots

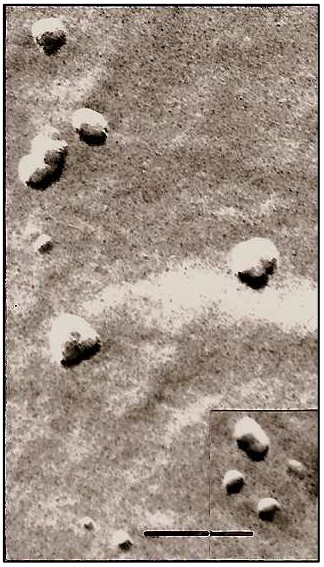

Fig.2: Polymeric Micelles

Fig.3: Gold Nanoparticles

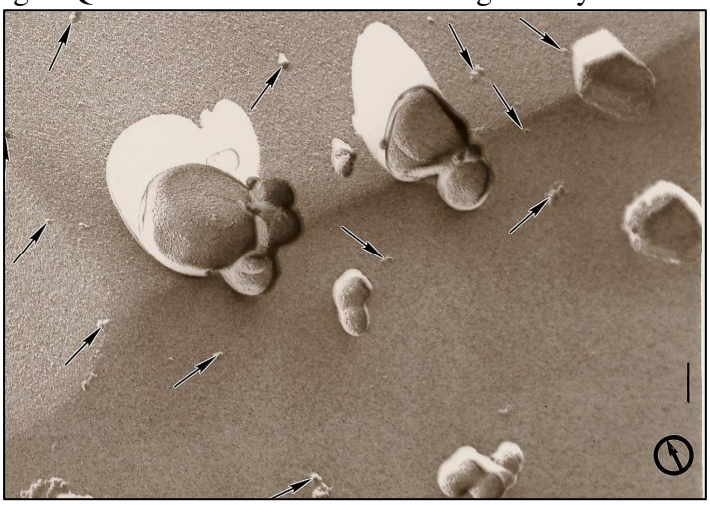

Fig.4: Drug-loaded QD-coupled Immunoliposomes

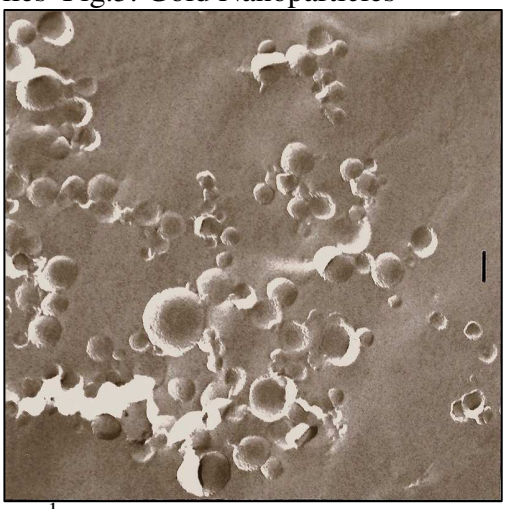

Fig.5: ^{1}H MR thermosensitive Liposomes

Fig.6: Influenza Virus

Fig.7: Virosomes

Fig.8: Measles Powder

The bars represent always 100nm. Shadow directions are running from bottom to top.

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