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Principles of rational design of thermally targeted liposomes for local drug delivery

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Abstract

Drug release from 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) liposomes occurs close to the main transition temperature T_m = 41 °C. The exact release temperature can be adjusted by additional lipids, which shift T_m. A major issue is drug leakage at 37 °C. We here describe a novel approach with improved drug retention yet rapid release. To obtain spherical, smooth liposomes we included: i) 2 mol% cholesterol, to soften bilayers (Lemmich et al 1997), ii) lipids, which due to their spontaneous curvature stabilize the negative and positive curvatures of the inner and outer leaflets of unilamellar liposomes. In addition to differential scanning calorimetry (DSC) and fluorescence spectroscopy, the lipid mixtures were analyzed by a Langmuir balance for their elastic properties and lipid packing, aiming at high elasticity modulus C_S^{-1}. Maxima in C_S^{-1} coincided with minima in the free energy of lateral mixing. These liposomes have reduced drug leakage, yet retain rapid release.

Key words: Liposomes; Doxorubicin; Phase transition; Elasticity; Controlled drug release

The use of liposomes as drug carriers continues to be intensively investigated. This approach introduces several advantages to the administration of chemotherapeutic agents, which are inherently toxic and irritating and should ideally only act in the tumor. An increased permeability of the tumor capillaries results in an augmented extravasation, resulting in a passive accumulation of liposomes with subsequent slow release of the contained drug.[1] However, passive targeting also involves accumulation of liposomes into healthy tissues,[2] as well as insignificant extravasation into poorly-vascularized tumor regions.[3]

Temperature induced local drug release represents an attractive new approach,[4,5] with local heating of target tissues achieved by techniques such as magnetic resonance imaging guided high intensity focused ultrasound (MRIgHIFU) or radiofrequency probes, in combination with so-called thermosensitive liposomes.[6,7] The latter term is somewhat unfortunate

Abbreviations: Chol, cholesterol; Cryo-TEM, cryogenic transmission electron microscopy; D/L, drug/phospholipid molar ratio; DLS, dynamic light scattering; DMEM, Dulbecco's Modified Eagle's Medium; Dox, doxorubicin; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DSC, differential scanning calorimetry; DSPE-PEG2k, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt); Edf, edelfosine,1-O-octadecyl-2-methyl-*sn*-glycero-3-phosphocholine; HBS, Hepes buffered saline,20 mM Hepes 7.4 pH,150 mM NaCl; Hepes, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; LUV, large unilamellar vesicles; lysoPC, 1-acyl-*sn*-glycero-3-phosphocholine; MLV, multilamellar vesicles; MRIgHIFU, magnetic resonance imaging guided high intensity focused ultrasound; NBD-PC, 1-hexanoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino] hexanoyl]-*sn*-glycero-3-phosphocholine; PDI, polydispersity index; T-jump, temperature jump; T_m, phospholipid main phase transition temperature; T_R, temperature of drug release; T-scan, temperature scan; TTL, thermally targeted liposomes; Z_{av}, average hydrodynamic particle diameter.

The authors declare no conflicts of interest.

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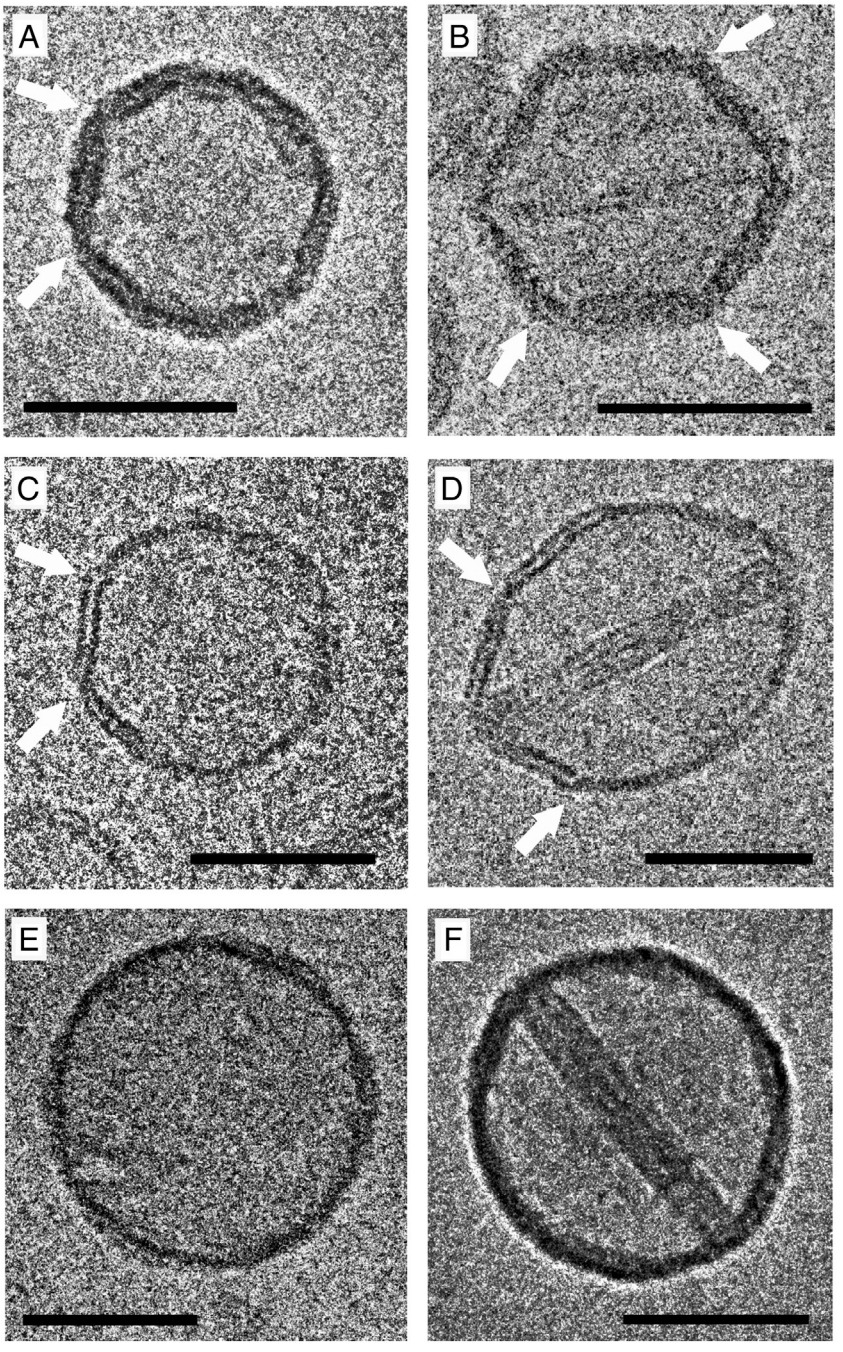

Figure 1. Cryo-TEM images of TTL composed of **(A and B)** DPPC:1-stearoyl-lysoPC:DSPE-PEG2k (86:10:4 molar ratio), **(C and D)** DPPC:Edf:DSPE-PEG2k (86:10:4 molar ratio), and **(E and F)** DPPC:Edf:DPPE:Chol:DSPE-PEG2k (86:6:4:2:4 molar ratio) as such (left hand panels), or encapsulating Dox (right hand panels). White arrows point the edges of planar facets and scale bars represent 50 nm.

and misleading as one of the characteristics of all liposomes and lipid membranes is the sensitivity of e.g. lipid packing and acyl chain order to an increase in temperature. Accordingly, a more accurate term would be thermally targeted liposomes (TTL), which, upon entering a tissue that is maintained at elevated temperature, will undergo a thermally triggered change in their permeability barrier properties, releasing any encapsulated material retained at body temperature of 37 °C. At present MRIgHIFU is perhaps the most promising approach for thermal targeting because of its non-invasive nature and possibility for real time monitoring of tissue temperatures allowing precise maintenance of the desired target tissue temperature.[8]

The use of TTL for thermally induced local drug release requires the liposome bilayer to be stable and retain the encapsulated drug while in circulation at 37 °C. Yet, upon reaching the target tissue maintained at the predetermined temperature T_R, the contained drug should be released. Optimally, drugs such as Dox should be rapidly released intravascularly during their transit