pHLIP-coated niosomes as novel delivery systems for cancer therapy

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The aim of this work was the preparation and characterization of pHLIP-coated niosomes as novel delivery systems for cancer therapy. Acidity is considered as universal cancer biomarker and pHLIP is used as an acidity-targeting probe [1].
pHLIP (pH Low Insertion Peptide) is a polypeptide able to insert across a membrane . This event is driven by a drop of pH from neutral or high (>7.4) to slightly acidic (7.0–6.5 and less) pH values [1].
Therefore, the presence of pHLIP on vesicles surface would enhance their cell internalization in a pH dependent fashion [2].
During the preparation of different vesicular systems (Tween 20 and Span 20 niosomes), the targeting agent, after its conjugation with DSPE-Maleimide or N-(1-Pyrenyl) Maleimide, was added (Figure 1).
A physicochemical characterization was carried out in terms of dimensions, ζ-potential and morphology (cryo-TEM, Figure 2). For colloidal stability at different temperatures, the vesicle formulations were stored at 4 and 25°C for 30 days. The effect of plasma on vesicle stability was also evaluated. Results showed that pHLIP-coated niosomes exhibit nanoscale size, spherical and unilamellar structure and stability during the time of experiments.
In some samples, pHLIP presence was confirmed through anisotropy studies. Indeed, the anisotropy value increases with pHLIP concentration. Furthermore, fluorescence studies showed that these samples are able to release a model probe (calcein).
In other samples pHLIP presence was confirmed by UV measurements. Afterwards, in vitro and in vivo experiments were carried out. In particular, cell proliferation assays, fluorescence microscopy (Fig. 3), quantification of cellular uptake and fluorescent imaging of organs were conducted.
In vitro experiments showed no samples toxicity and good cell internalization at low pH; in vivo experiments highlighted the tumor targeting capability of vesicular carriers (Figure 3).
Therefore, according to these studies, pHLIP-coated niosomes resulted promising delivery systems of diagnostic and therapeutic agents towards solid tumors.

References

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[2] Yao L., Daniels J., Dayanjali W. et al, *pHLIP®-Mediated Delivery of PEGylated Liposomes to Cancer Cells*, *J. Controlled Release*, **2013**, *167*, 228-237

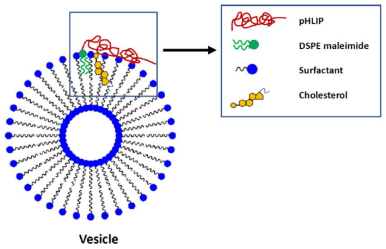

Figure 1

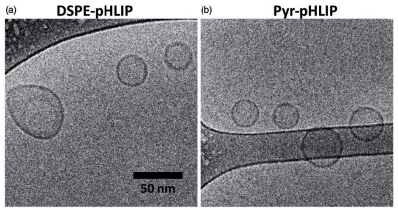

Figure 2 Cryogenic TEM image of the (a) DSPE-pHLIP (5 mol%) , (b) Pyr-pHLIP (5 mol%) coated Span20 (45 mol%) and cholesterol (50 mol%) niosomes. The images are obtained at 25,000_ magnification

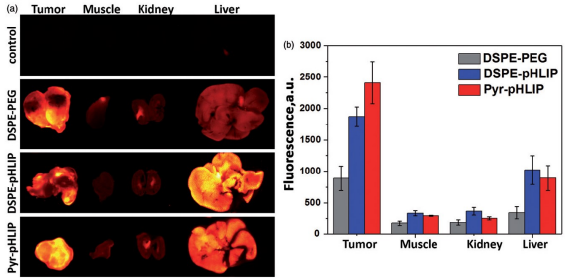

Figure 3 (a) The representative rhodamine fluorescence images and (b) mean surface fluorescence of tumor (cut in half), muscle, kidney (cut in half) and liver obtained by ex vivo imaging after collection of organs and tissues 24 hours after I.V. administration of pHLIP and PEG coated niosomes are shown (the autofluorescence signal is subtracted). The color coded fluorescent images shown on panel (a) are obtained at the same settings of the imaging instrument, the same exposure time (15 sec) and all of them were processed exactly the same way. Control is the organs collected from the mouse with no injection of fluorescent niosomes and it represents level of auto fluorescence signals in tissue.