Targeted Delivery of Nano-pores into Cancer Cells Using pHLIP® Coated Liposomes

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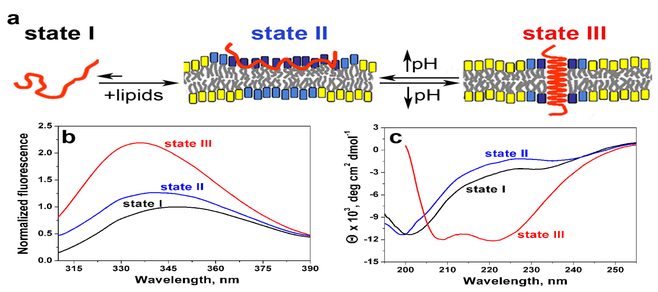
Abstract

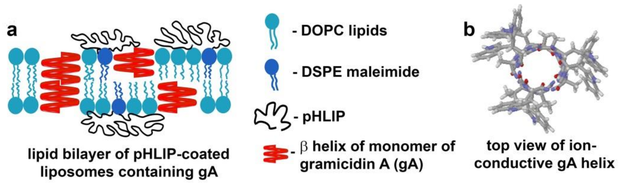
Extracellular acidity is a universal biomarker for carcinoma and a number of other pathological conditions. It also plays a significant role in cell functioning and proliferation in pathological cells. By exploiting this extracellular acidity, we were able to formulate a novel pH-sensitive nanomedicine for therapeutics & diagnostics using pHLIP® technology. The formulated pHLIP® coated small, unilamellar vesicles have a superior stability and prolong shelf life to deliver hydrophobic agents to pathological sites by safer and more effective means. The right balance of ions in intracellular and extracellular spaces is vital for regular cell functioning. This ion balance helps to maintain the required membrane potentials, holding everything within the cell. Any variations in this vital balance of ions could induce the cell death in both healthy and pathological cells. We employ pHLIP® coated small, unilamellar vesicles to deliver nano-pores to cancer cells to disrupt the monovalent cation balance and induce apoptosis. In this work, *gramicidin A* was used to form monovalent cation conductive nano-pores.

pHLIP-pH (Low) Insertion Peptide

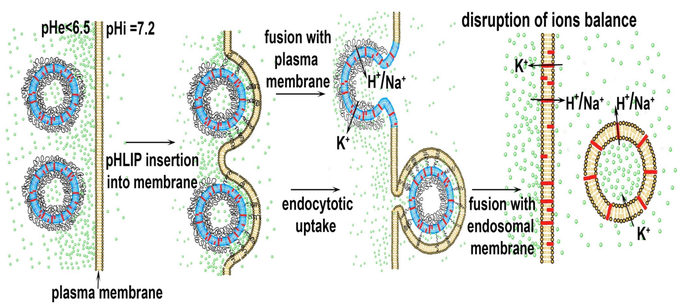
G G E Q N P I Y W A R Y A D W L F T T P L L L L D L A L L V D A D E G T C G



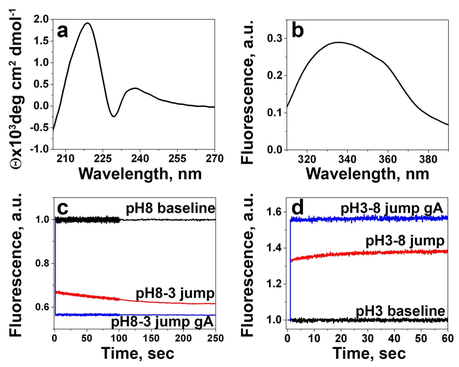
A segment of liposome (a) and top view of gA helix (b)



Schematic presentation of interactions of lipid bilayer of pHLIP-coated liposomes containing gA with plasma membrane of a cell, which leads to the transferring of gA channels (nano-pores) to cellular membranes and disruption of monovalent cations balance (presentation of liposome in the endosome is schematic and not in a scale).

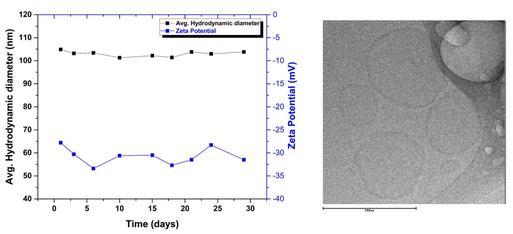


Biophysical characterization of liposomes containing gramicidin A



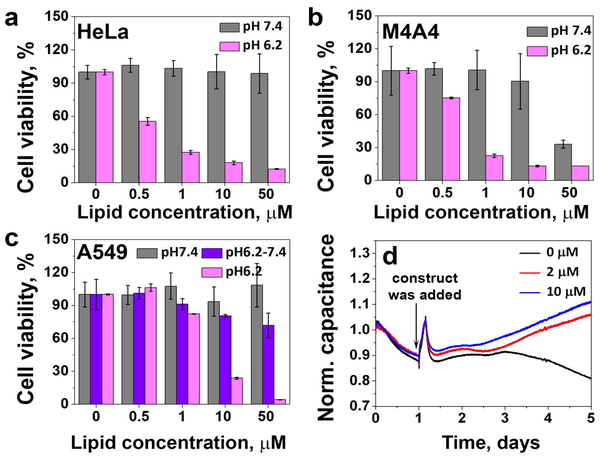
The circular dichroism (a) and tryptophan fluorescence (b) of the DOPC liposomes containing 2% gA were are shown c-d) The DOPC liposomes containing 2% FITC-DHPE lipids and containing no gA (red line) or 2% gA (blue line) in phosphate buffer pH 8 or pH 3 were mixed with HCl or NaOH (dead time of mixing was 5 ms) to induce transition from pH 8 to 3 (c) or transition from pH 3 to 8 (d), respectively. The changes of FITC emission were observed in real time in the stopped-flow apparatus. The baseline signal obtained by mixing of liposomes at pH 8 or pH 3 with buffer of the same pH is shown by black lines.

Size and Zeta potentials of gA liposomes



The size and the zeta potential of liposomes were measured by the dynamic light scattering (DLS) in 10 mM phosphate, 150 mM NaCl, pH 7.4 buffer over 30 days. For cryo-electron microscopy, samples were imaged using JEOL 2100 TEM with an accelerating voltage of 200 kV at magnifications of 40,000x.

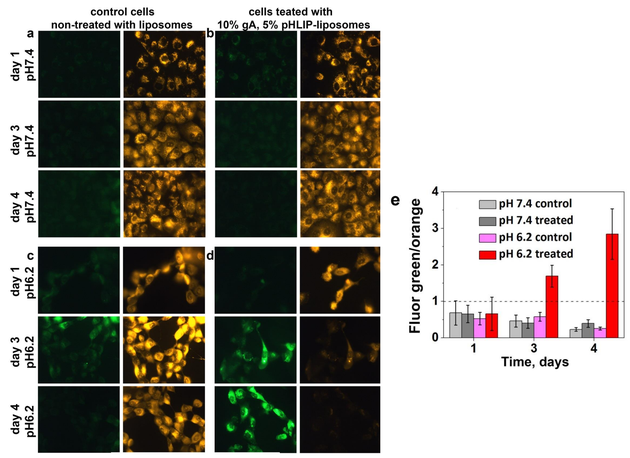
Cyto-toxicity Assays and kinetics



(a) M4A4, human breast ductal carcinoma, (b) A549, human lung carcinoma (c) cells grown in standard media of pH 7.4 and adapted for low pH growth of 6.2 were treated with the DOPC pHLIP-coated liposomes containing 10% gA and 5% pHLIP for 18 hrs at pH 7.4 or 6.2 in DMEM followed by the replacement of media with DMEM containing 10% FBS of the same pH as treatment or DMEM of pH7.4, 10% FBS (blue columns). MTS assay was performed on 4-th day after the treatment. d) About 25,000 of A549 cells were seeded in an 8W10E+ ECIS array in DMEM at pH 6.2, next day cells were treated for 3 hrs with different concentrations of DOPC pHLIP-coated liposomes containing 10% gA and 5% pHLIP liposomes in DMEM at pH 6.0 followed by addition an equal volume of DMEM containing 20% FBS at pH 6.2. The kinetics of inhibition of cell proliferation was monitored by changes of capacitance within 5 days.

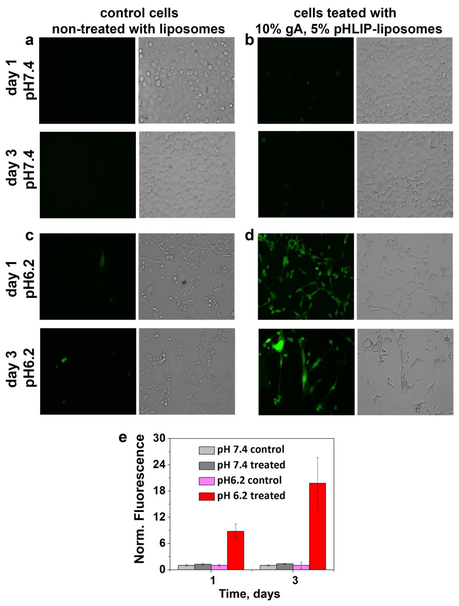
Mitochondria depolarization assay

Mito PT Assay shows that gA pHLIP liposomes induce apoptosis in cells. In treated cells green:orange fluorescence ratio is significantly higher than control cells.



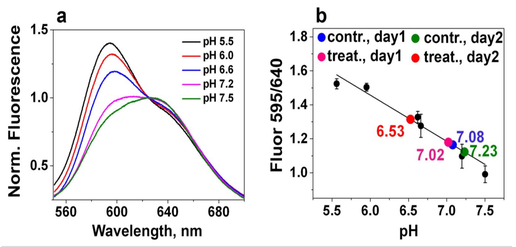
Depolarization of mitochondria of A549 cells is characterized by changing color from orange to green of JC-9 fluorescence. The cells images were taken at excitation/emission wavelengths centered at 490 nm /520 nm (green) or 540 nm/580 nm (orange), respectively, at different days after cells treatment with liposomes (a-d). The changes in average ratio of green to yellow signals are shown on panel e.

Monitoring changes of intracellular concentration of Na^+



A549 cells non-treated or treated for 1 day with DOPC liposomes with 10% gramicidin and 5% pHLIP at pH 6.2 and pH 7.4 were incubated with CoroNa™ Green next and 3-rd days after the treatment followed by washing and imaging of cells. The representative fluorescent and phase-contrast images of cells are shown (a-d). The average per cell fluorescence intensity changes for control and treated cells are shown on panel e.

Monitoring changes of intracellular pH



First, calibration curve was established: A549 cells grown at pH6.2 were incubated with SNARF-5F followed by washing with 50 mM phosphate/citric acid solution of pH 5.5, 6.0, 6.6, 7.2 and 7.5. To equilibrate extracellular and intracellular pH, ionophore nigericin was added to cells and fluorescence spectra of SNARF-5F were recorded (a). Ratio (595/640 nm) of SNARF-5F fluorescent signal was plotted for each respective pH (black dots) and linear fit was performed to establish calibration curve (b). The fluorescence spectra of SNARF-5F was obtained on A549 cells non-treated and treated with the DOPC pHLIP-coated liposomes containing 10% gA and 5% pHLIP at pH 7.4 or 6.2 for 3 hrs followed by addition an equal volume of DMEM containing 20% FBS. The SNARF-5F emission ratio of 595/640 nm was calculated and the corresponding intracellular pH was obtained from the pH calibration curve (color dots on panel b).

Discussion & Conclusion

pH-selective delivery of conductive pores to destroy balance of ions, which leads to cell death, could be considered as potential novel way of treatment of acidic solid tumors. Also, an example of selective delivery and proper assembly of gA channels in cellular membranes, opens opportunity for delivery of various membrane peptides and proteins to the cellular membranes, which might find wide application in biotechnology and medicine.

References

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Acknowledgements

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