4-2016

Ionic Driven Embedment of Hyaluronic Acid Coated Liposomes in Polyelectrolyte Multilayer Films for Local Therapeutic Delivery

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1) SIGNIFICANCE

- When designing disease treatments, there are several disadvantages of introducing free drug to a patient:
  - Burst Kinetics, toxic off site effects, and low efficacy due to systematic dilution
- Drug delivery attempts to solve these problems by controlling the spatial (location) and temporal (timing) characteristics of drug release!
- Substrate mediated delivery, the release of drug from a surface or object, offers a unique way in which to control these characteristics.
  - Substrate mediated delivery of cargo has shown great promise in applications including drug and gene eluding films/scaffolds [1,2], coatings for stents [3] and other implantable devices [4]

2) POLYELECTROLYTE MULTILAYERS (PEMs) AND LIPOSOMES NANOPARTICLES

- PEMs are thin polymer films which are constructed of layers of oppositely charged polyelectrolytes.
  - Simple to assemble on biomedical surfaces
  - Have been used to entrap small molecule drugs [5], proteins [6], and nucleic acids [7,8]
- Liposomes nanoparticles are the most researched, tested, and commercialized drug delivery carrier.
  - Can entrap hydrophilic and hydrophobic cargo
  - Easy to functionalize and excellent biocompatibility
- PEM entrapped liposomes have been explored, but maintaining the stability of the liposomes in the PEM structure has been the primary advancement to this point.

3) PROJECT OBJECTIVE

In this project, we report the engineering of a novel liposome (HALNP) embedded PEM substrate (HALNP-PEM) for the local and sustained delivery of therapeutic cargo.

- Entrapment is verified and release kinetics are analyzed.
- A cell driven mechanism of release for HALNPs is hypothesized.
- Doxorubicin (DOX) is delivered to 21M-T metastatic breast cancer *in vitro.*

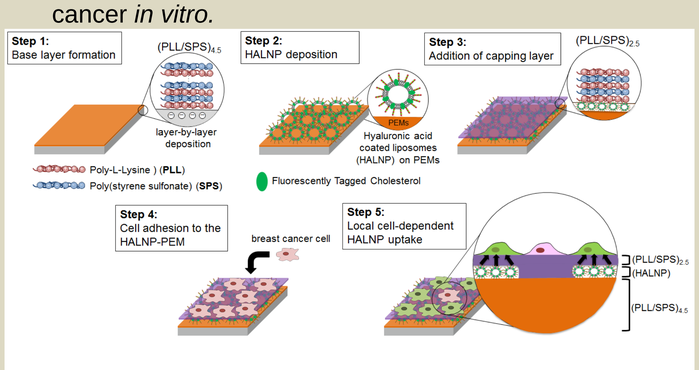

Fig. 1. Project Objective Overview

4) IONICALLY EMBEDED PARTICLES ARE STABLE, RELEASE FROM PEM IS MINIMIZED BY CAPPING POLYMER

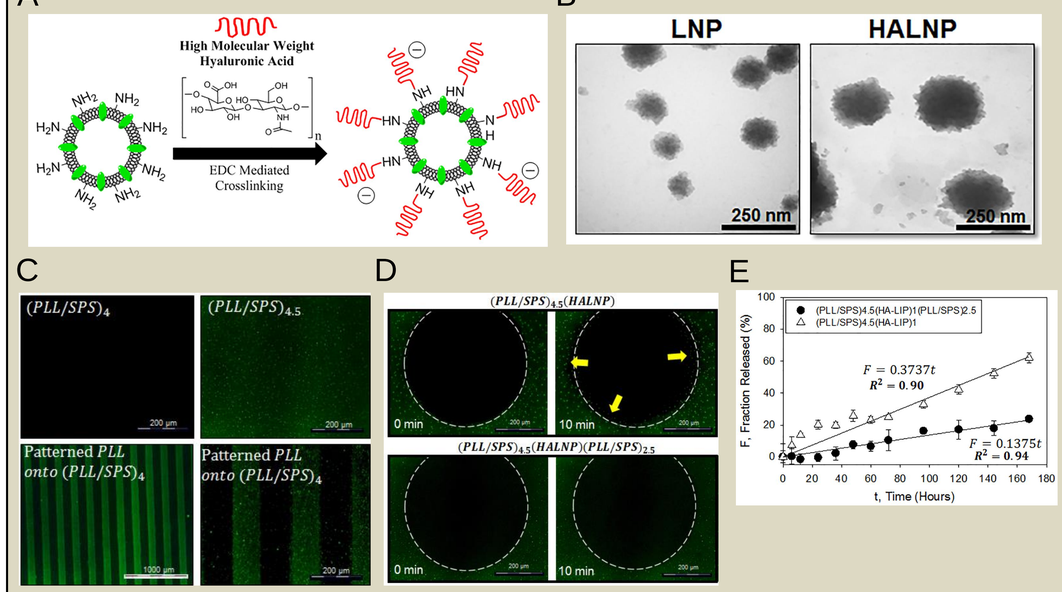

Fig. 2. Hyaluronic Acid (HA) decorated lipid nanocarrier fabrication overview and characterization. Fluorescent Microscopy and release studies demonstrate ionically driven HALNP entrapment, and minimized HALNP release by addition of capping polymer layers. (A) Lipid nanoparticles (LNPs) were surface functionalized with HA (HALNPs) via EDC facilitated amide bond formations. (B) Transition Electron Microscopy (TEM) images of particles pre- and post- HA addition. (C) Preferential deposition of HALNPs on PLL over SPS topped surfaces. Capillary Force Lithography (CFL) was used to create PLL patterns on $(PLL/SPS)_4$ and to demonstrate the level of spatial control for nanoparticle adsorption. (D) Fluorescent Recovery after Photobleaching (FRAP) Analysis pre and post the addition of the capping layer for 10 minutes (yellow arrows point out particles that have moved). (E) HALNP nanocarrier release profile from the non-capped [(PLL/SPS)4.5(HALNP)] and capped [(PLL/SPS)4.5(HALNP)(PLL/SPS)2.5] HALNP-PEM platforms.

5) FLUORESCENTLY TAGGED HALNPs ARE TAKEN UP FROM SUBSTRATE BY 21MT-1 CELLS

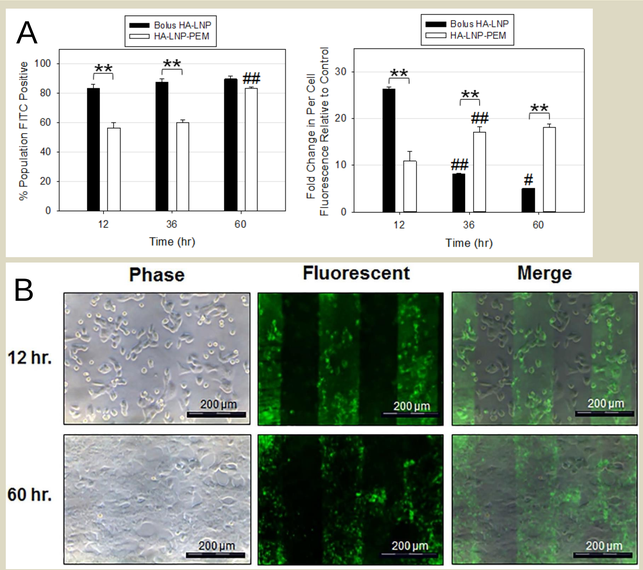

Fig. 3. 21MT-1 localized uptake of fluorescently labeled particles. (A) Flow Cytometry Analysis of percent cell population FITC positive and per cell fluorescence directly comparing nanoparticle uptake between the HALNP-PEM and HALNP bolus systems (*P < 0.05, **P < 0.005; n = 3; #denotes significance between a specific sample type and the preceding time point following the same significance scale as the stars). (B) Phase Contrast and Fluorescent Microscopy visual investigation of nanoparticle intracellular delivery on the HALNP-PEM platform. CFL was used to pattern HALNPs beneath the capping layers. Note that only the cells attached directly above the particles become fluorescent.

6) HALNPDOX-PEM DELIVERS ACTIVE THEREPUTIC TO 21MT-1 CELLS

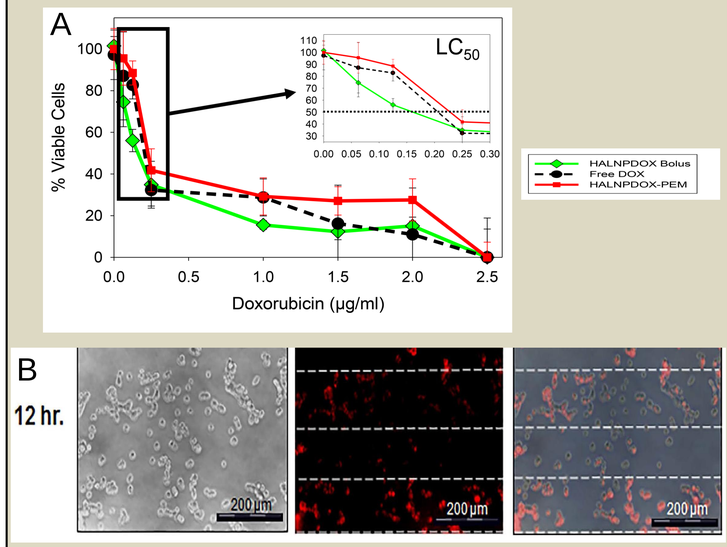

Fig. 4. DOX Toxicity Assay and 21MT-1 localized uptake of DOX Therapeutic. (A) Doxorubicin potency assay comparison between Free Dox, DOX encapsulated in HALNPs (HALNPDOX) in the bolus form, and HALNPDOX nanoparticles embedded into the PEM platform (HALNPDOX-PEM). Standard MTT protocol was used to determine the percent viable cells at 24 hours. (B) Localized delivery of DOX therapeutic. Note that cells attached directly above DOX loaded HALNPs become predominantly fluorescent.

7) CONCLUSIONS

- HALNP-PEM system entraps and stabilizes drug loaded liposomes through ionic interactions.
- The locally driven uptake of fluorescent and drug loaded nanoparticles supports the hypothesis of a cell mediated degradation mechanism of the PEM substrate.
- HALNPs are taken up by attached cells, and drug loaded substrate embedded particles show competitive efficacy.

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9) RELATED PULBICATIONS

- Hayward, S. L., Francis, D. M., Kholmatov, P., & Kidambi, S. (2016). Targeted Delivery of MicroRNA125a-5p by Engineered Lipid Nanoparticles for the Treatment of HER2 Positive Metastatic Breast Cancer. *Journal of Biomedical Nanotechnology J Biomed Nanotechnol, 12*(3), 554-568.

10) ACKNOWLEDGEMENTS

We thank Dr. Hamid Band and Dr. Vimla Band (University of Nebraska Medical Center, Omaha, NE, USA) for kindly providing 21MT-1 breast cancer cells. This work was funded by the startup funds from UNL, Layman's Award, MRSEC Seed Grant, and UNL Interdisciplinary Award. This work was supported by University of Nebraska Medical Center INBRE program and the University of Nebraska-Lincoln UCARE program.