“Proximity Activated” smart nanoparticle for the delivery of siRNA to metastatic tumor cells

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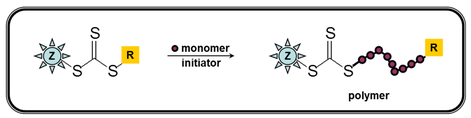
ABSTRACT

Permeability-glycoprotein (P-gp) over expression in breast cancer cells desensitizes the tumor to chemotherapeutics and can lead to the development of multiple drug resistance (MDR), significantly worsening patient chance of survival. siRNA presents a powerful tool for silencing P-gp, but in vivo delivery barriers such as endosomal trafficking and off-target cytotoxicity must be overcome to make the treatment feasible. MMP-7 plays a significant role in tissue breakdown and cell migration, and its over expression is a hallmark of tumor progression into metastasis. In this study, an MMP-7 responsive peptide and polyethylene glycol (PEG) cloak were incorporated onto a previously designed smart polymeric nanoparticle (SPN) that contains a cationic corona for condensing siRNA and pH-responsive, endosomolytic core. The cationic corona of the SPN can trigger nonspecific cell uptake in normal tissues. The PEG cloak shields the positive surface charge of the SPNs until being cleaved in MMP-7 rich tumor environments, allowing “proximity activated” delivery of siRNA.

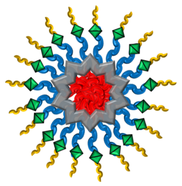
INTRODUCTION

Reversible Addition Fragmentation Chain Transfer (RAFT)



- RAFT is a controlled, free radical polymerization technique that provides easy routes to higher order architectures and allows for desirable control over polymer molecular weight and polydispersity.

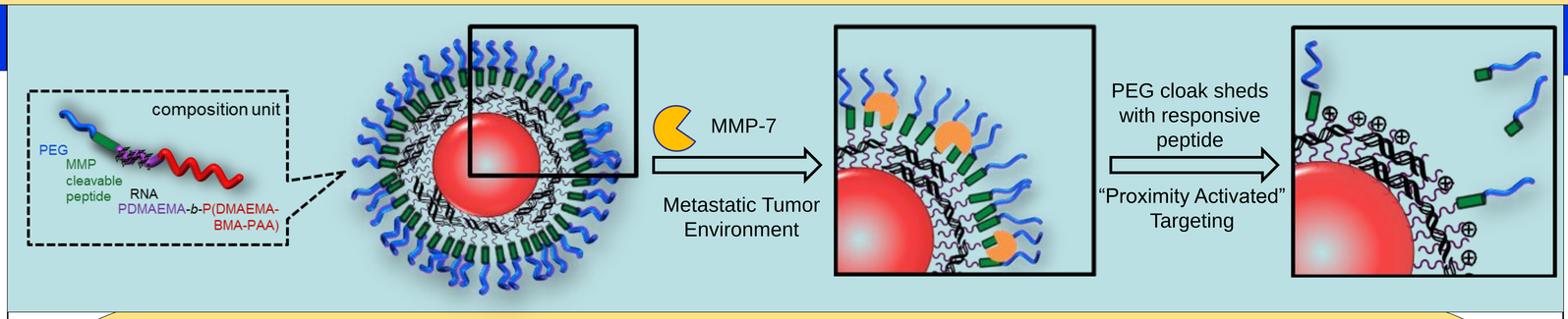
Micelles



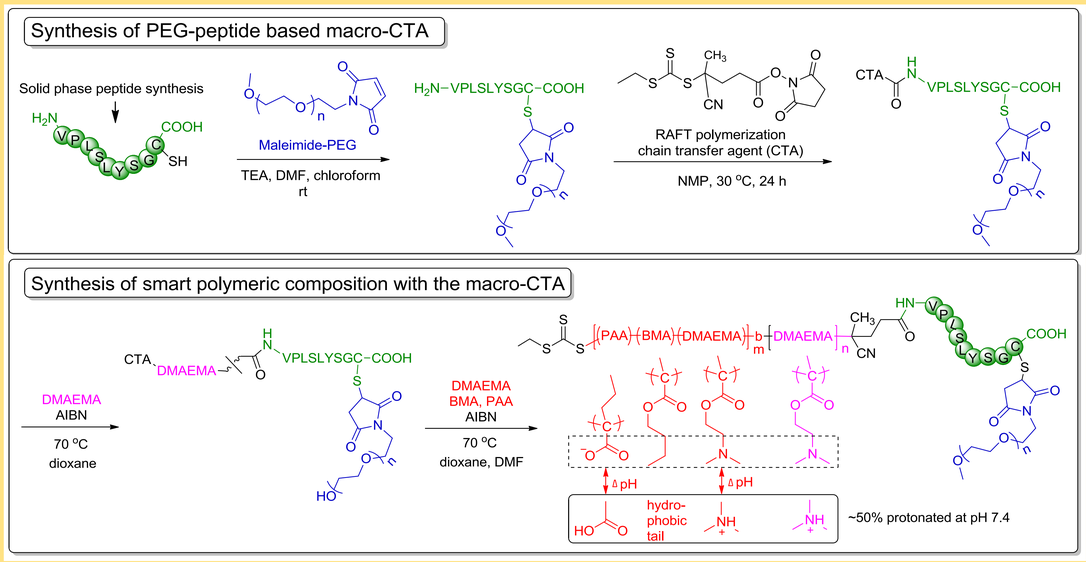
- Micelle formation is governed by natural hydrophobic/hydrophilic interactions.

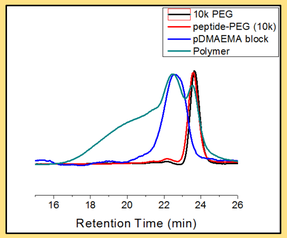
Characterization Equipment

- Gel Permeation Chromatography (GPC) is a type of size exclusion chromatography that separates particles in a solution based on size.
- Dynamic Light Scattering (DLS) applies a light source to solutions and then observes the time-dependant fluctuation in scattering intensity.

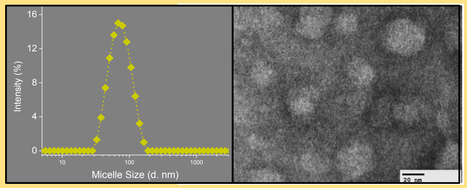


Methods: Smart Nanoparticle Synthesis & Characterization





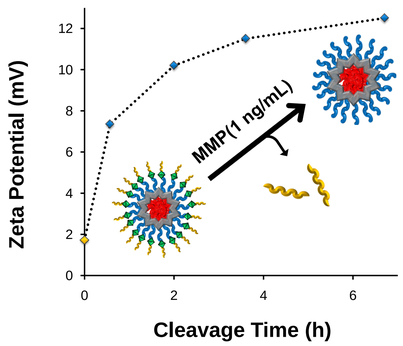
- GPC characterization helps confirm proper higher-order architecture of the polymer design



- DLS and transmission electron microscopy (TEM) were used to characterize micelle size. DLS showed micelles to have a diameter of 76nm in aqueous solution, and TEM showed a diameter of ~40nm for dehydrated micelles.

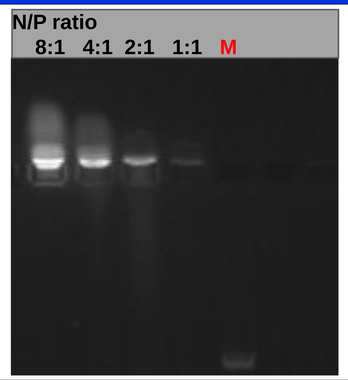
Results: Effective siRNA Delivery by Smart Nanoparticle

Zeta Potential Measurement of micelles over time



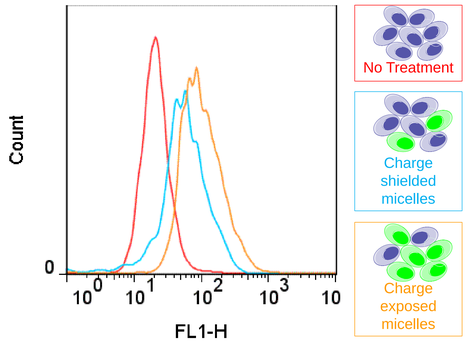
- A micelle solution was prepared and treated with MMP-7. Zeta potential measurements were taken on DLS at time points up to 24 hours. Zeta potential increased 6-fold over the time period due to the gradual removal of the PEG cloak of the nanoparticle. These results confirm peptide cleavability in MMP-7 rich environments.

siRNA Condensation on Micelle



- Gel Electrophoresis was run to determine the optimum N/P (NH_3^+/PO_4^-) ratio for siRNA condensation onto micelles. Samples of 8:1, 4:1, 2:1, and 1:1 were all prepared. As shown, the 4:1 ratio condensed most effectively.

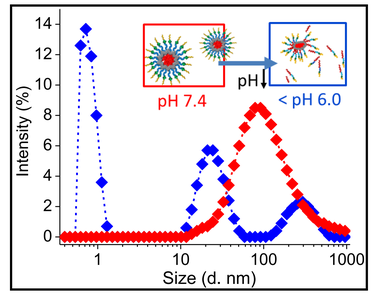
Cellular uptake of siRNA



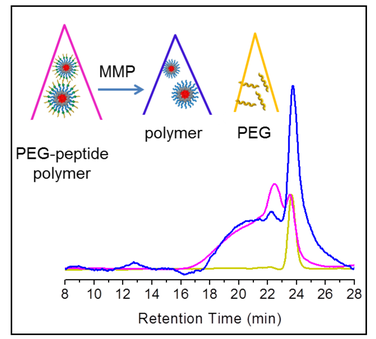
- Flow cytometry was conducted to monitor siRNA uptake in cells treated with this smart nanoparticle design before and after MMP-7 exposure. Micelles were condensed with siRNA, exposed to MMP-7, and then used to treat breast cancer cells. Preliminary results show more uptake after MMP-7 exposure time.

DISCUSSION

- DLS measurements confirm expected micelle size and pH-responsiveness of this smart nanopartcle design.



- The smart nanoparticle design also exhibited expected MMP-7 responsiveness and cellular uptake; as shown by zeta potential measurements, GPC, and flow cytometry.



- These results indicate the potential of this “Proximity Activated” carrier to enable tumor-specific delivery of siRNA in order to overcome MDR and re-sensitize breast cancers to standard chemotherapeutic regimes

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