Hybrid Cancer Therapeutics

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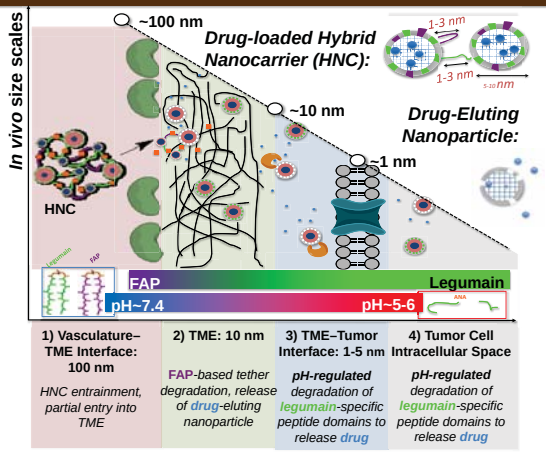
Motivation & Approach:

In nano-scale drug delivery targeting cancer cells, a major **challenge** is design of a system that will effectively breach the many size-selective interfaces along the delivery path. These are between the

- Vasculature
- Tumor microenvironment (TME)
- Tumor cell membrane
- Tumor intracellular space

Current approaches include organic particles that are conjugated to specific ligands which may be limited by chemical instability, immune response, and variable porosity.



With these challenges in mind, the aim is to design a system that will…

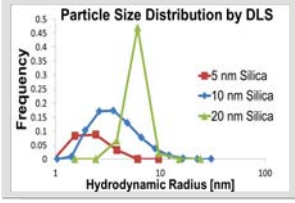
- Remain stable and prevent premature release of drug by using pH-dependent peptides to covalently bond to particles and form HNCs.
- Utilize loaded HNCs on the order of 100 nm to deliver drug to intracellular space once cleaved from HNCs.

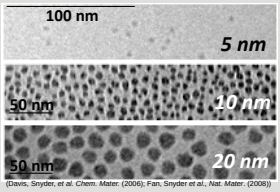
The resulting hybrid particle-peptide structure will allow for…

- Cascade approach to drug release that is driven by size-selective permeability through TME to the cell interior.
- Control of drug release through particle size, porosity, and architecture.

Particle Synthesis and Characterization:

Silica particles of various size were synthesized by hydrolysis reaction in the presence of lysine and were characterized by both DLS and TEM.



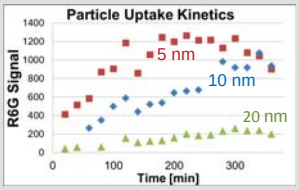


Particle Uptake in HEK Cells:

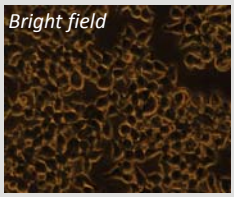
Kinetics data on silica particle uptake was used to…

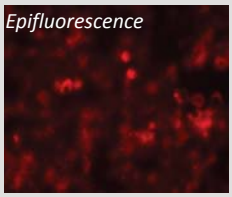
- Determine the relative rate that particles of 5, 10, and 20 nm would diffuse into the cell environment.
- Study the nature of uptake (passive diffusion, cell metabolic mechanisms.)

Flow cytometry results indicate that 5 nm particles would be most suitable for drug delivery due to their superior ability to permeate HEK cell membrane.



Uptake was also confirmed with fluorescent microscopy of HEK cells. Particles with physically incorporated R6G dye were added to cells, which were then centrifuged to remove excess dye.

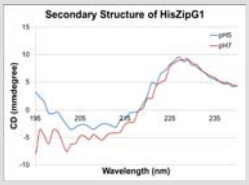




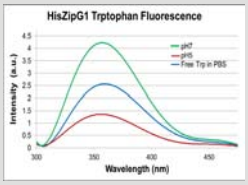
Peptide Characterizations:

Encapsulating Peptide: HisZipG1 (SWHWHGPWHWHS)

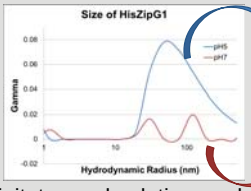
HisZipG1 shows evidence in favor of a beta structure that is independent of pH, but CD alone cannot confirm this due to the tryptophan-rich nature of the peptides. We are using FT-IR and Raman to determine this currently, which do not have the sensitivity to tryptophans that CD does.

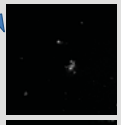


The environment is not changing as a function of pH due to the stability in trp fluorescence. The aggregates formed that are free in solution do not form in such a way that the trp environment is changing.

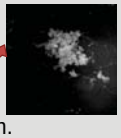


The DLS data indicate that at pH 5, there is a clear, homogeneous solution with particles of approximately 40-80 nm, whereas at pH 7, there is an evidence for macroscopic precipitates and gelation, and this solid phase is in equilibrium with a liquid phase containing aggregates near 40 and 100 nm.





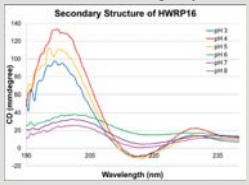
At pH 5, there is no clear evidence of aggregation.



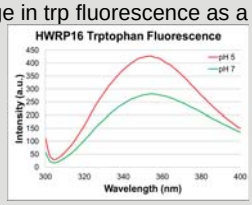
At pH 7, the aggregate size is apparent, which is roughly 400 nm in diameter.

Tethering Peptide: HWRP16 (EAHARAWANAHAEAWA)

HWRP16 peptide shows strong alpha and beta at low pH and primarily beta at high pH, which is a consistent transition with the pH change caused by histidine protonation-deprotonation.

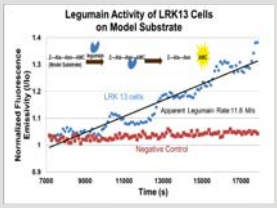


Lack of change in trp fluorescence as a function of pH suggests stability in local environment. The peptides do not self-associate as a function of pH. The changes in the secondary structure depend only on the monomer.



Legumain-Induced Peptide Cleavage

Legumain is a cysteine protease that cleaves peptides strictly at Asn in the P1 position of the peptide bond. Z-Ala-Asn-AMC is a model substrate to measure AMC fluorescence upon proteolytic release from the substrate. The apparent rate for the legumain in LRK13 cells (lung cancer cells) is calculated as 11.8 M/s. This study helps us to model the tumor interface.



Summary:

- Self-assembly of HisZipG1 peptide at high pH shows promise for particle incorporation into gel-based delivery system.
- Particle size has been controlled to address drug delivery according to *in vivo* length scale.
- The comparison between the apparent rates of legumain in LRK13 cells and the reported value is performed to demonstrate that LRK13 cells exhibit specific and strong legumain activity.

Acknowledgements:

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