Testing Fabrication Conditions to Optimize Properties of Peptide-Loaded Nanoparticles

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Background

The use of biologics, *i.e.* peptide therapeutics, has increased drastically over the past decade, however, there are still barriers to their use:

ADVANTAGES	DISADVANTAGES
• Potency • Specificity • Biocompatibility	• Poor cellular uptake • Vulnerable to proteolytic degradation

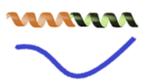
Goal: Improve the translatability of therapeutic peptides using electrostatically complexed nanoparticle delivery vehicles.

Introduction/Methods

Research question: How do the following parameters affect nanoparticle size, polydispersity, and cell uptake?

- **Ionic Strength** – Molarity of buffer solution
- **Concentration** – Concentration of initial solutions
- **Charge Ratio** – Molar ratio of carboxylates to primary amines
  - (NH_2^+ on the peptide):(COO^- on the polymer)
- **Lyophilization** – freeze-drying and reconstituting with DI water
  - Key for increasing shelf life/long term storage possibilities

Materials:

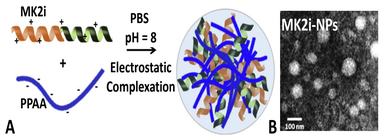


-MK2i: MAPKAP Kinase 2 inhibitor peptide (YARAAARQARAKALARQLGVAA)

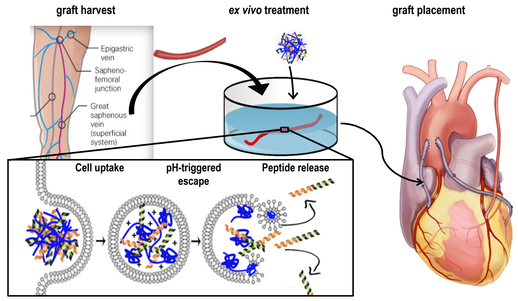
- PPAA: Endosomolytic polymer, Poly(propyl acrylic acid)

Nano-Polyplex Formation:[1][2]

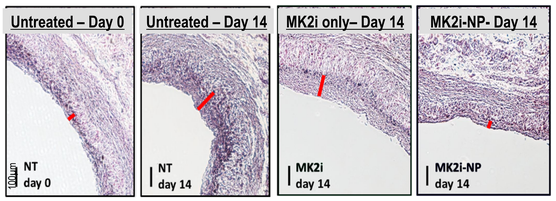
Nanoformulation to Enhance Intracellular Bioavailability



Clinical Delivery Concept[2]



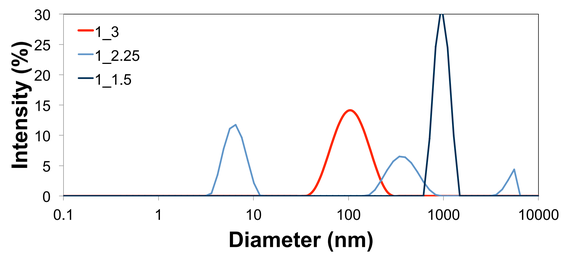
MK2i-NPs Prevent Thickening of Venous Walls, Reducing the Potential for Clotting and Vein Graft Failure:[2]



Human saphenous veins were treated *ex vivo* at 100 μM MK2i dose for 2 hours and maintained in organ culture for 14 days. Red bars demarcate intimal thickness.

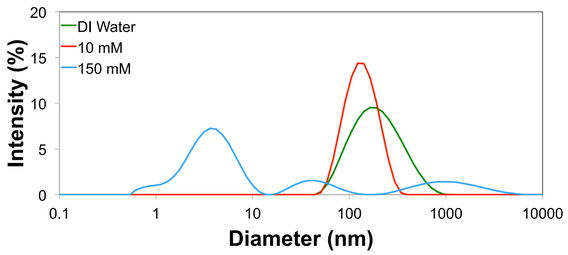
Results

1. A Charge Ratio of 1:3 is Optimal for MK2i-NP Formation:



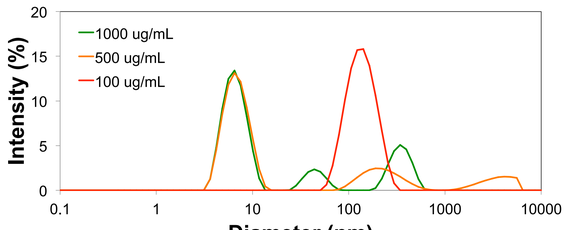
- Conditions: 10 mM PBS, 100 μM [MK2i-NPs]
- At charge ratios <1:3, particle size increases, aggregation occurs, and the peak at ~6 nm indicates complexation failure

2. Less Aggregation is Observed at Lower Ionic Strength:



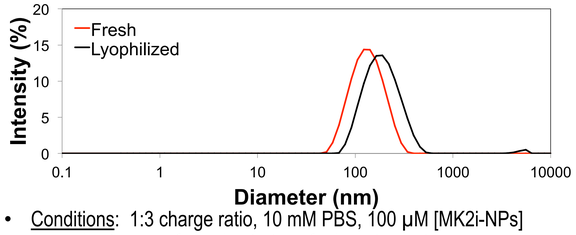
- Conditions: 1:3 charge ratio, 100 μM [MK2i-NPs] , 0-150 mM PBS
- As Ionic Strength increases, particle polydispersity increases and unreacted solutes remain in the solution.

3. Increasing Solute Concentration Inhibits NP Formation:



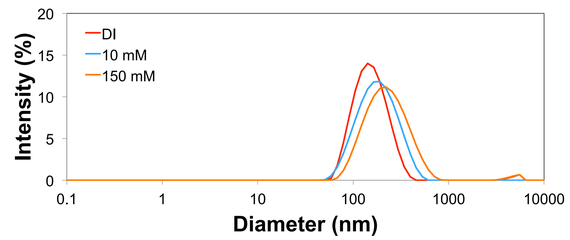
- Conditions: 1:3 charge ratio,10 mM PBS, 100-1000 μM [MK2i-NPs]
- Concentration inversely affects size/polydispersity

4. Lyophilization Does Not Significantly Alter Particle Size:



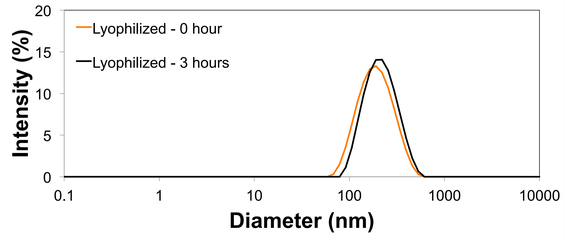
- Conditions: 1:3 charge ratio, 10 mM PBS, 100 μM [MK2i-NPs]
- Lyophilization is a feasible strategy for long-term storage
- This approach is highly translatable as it will extend the shelf life of the particles and allow for simplified distribution.

5. Lyophilized Particles are Less Sensitive to Ionic Strength:



- Conditions: lyophilized, 1:3 charge ratio, 100 μM [MK2i-NPs]
- Lyophilized particles show a similar trend to fresh particles, with ionic strength being directly proportional to size and polydispersity.

6. Lyophilized NPs are Stable for 3 hours after Reconstitution:

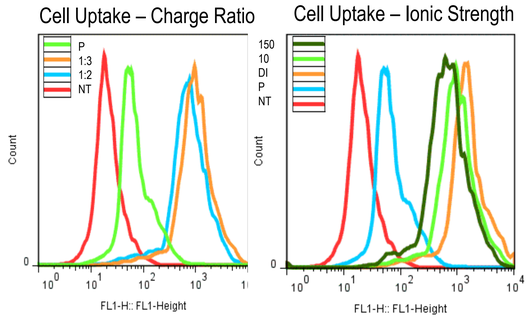


- Conditions: lyophilized, 10 mM PBS,100 μM [MK2i-NPs]
- Lyophilized MK2i-NPs are stable 3 hrs after reconstitution

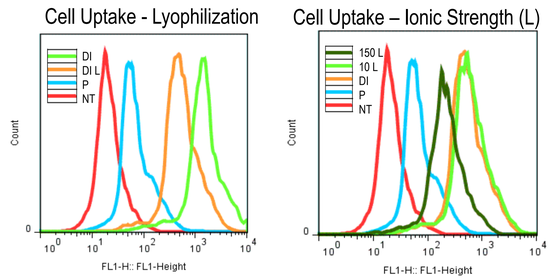
Cell Uptake Results

7. Cell Uptake Increases as Ionic Strength Increases and as the Charge Ratio Approaches 1:3

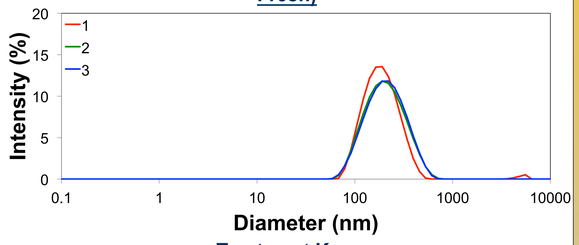
**Note, key for treatment groups is located in top right panel



8. Lyophilization Decreases Cell Uptake, and Trends with Ionic Strength are Similar to that of Fresh Particles



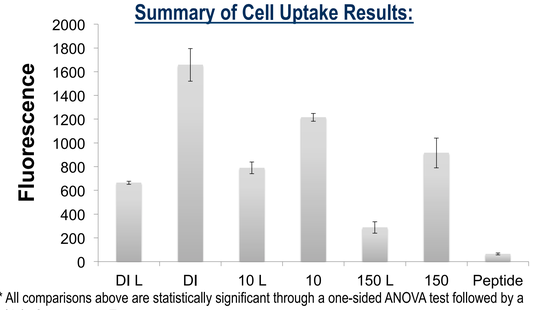
9.) Reproducibility of MK2i NP's (1:3, 100ug/mL, 10 mM PBS, Fresh)



Treatment Key:

- DIL/10 L/150L – particles prepared in DI H_2O /10mM PBS/150 mM PBS and lyophilized/reconstituted in DI H_2O
- DI/10/150 – particles prepared in DI H_2O/10mM PBS/150 mM PBS
- Peptide (P) – pure MK2i peptide dissolved in 150 mM PBS

Summary of Cell Uptake Results:



*** All comparisons above are statistically significant through a one-sided ANOVA test followed by a Multiple Comparisons Test

Conclusions & Future Directions

Conclusions:

1) Nanoparticle synthesis size and polydispersity improves as
   - The charge ratio approaches 1:3
   - Ionic strength of the buffer used for synthesis decreases
   - The sample is lyophilized
   - Concentration of solutes is minimized
2) Cell uptake improves as
   - Ionic Strength decreases
   - The sample is not lyophilized

Future Directions:

- Use micromixer system for more efficient mixing during synthesis
- Add excipients during lyophilization to preserve bioactivity
- Investigate delivery of other biologics (proteins, nucleic acids, etc.)

Works Cited

1. Evans BC, Hocking KM, Kilchrist KV, Wise ES, Brophy CM, Duvall CL (2015). "Endosomolytic Nano-Polyplex Platform Technology for Cytosolic Peptide Delivery To Inhibit Pathological Vasoconstriction." ACS Nano **9**(6): 5893-5907.

2. Evans BC, Hocking KM, Osgood MJ, Voskresensky I, Dmowska J, Kilchrist KV, Brophy CM, Duvall CL (2015). "MK2 Inhibitory Peptide Delivered in Nanopolyplexes Prevents Vascular Graft Intimal Hyperplasia." Science Translational Medicine **7**(291): 291-295.

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