The Use of Microfluidic Mixing Devices for Minimizing Polyplex Nanoparticle Size and Increasing Tumor Penetration

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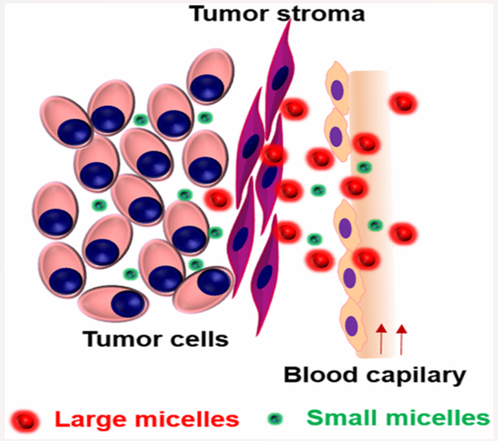
Introduction



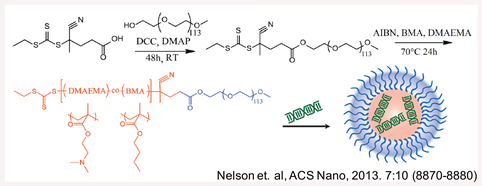
Wang, J. et. al, ACS Nano, 2015. 9.7 (7195-7206)

Background:

- Researchers are currently exploring the delivery of small interfering RNA (siRNA) for gene silencing in malignant tumors as an alternative to drug delivery.
- Previous work has shown that larger nanoparticles containing siRNA are less able to penetrate tumor tissues as effectively as smaller ones.

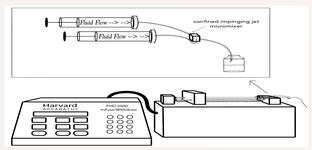
Hypothesis: The use of a micro-mixer can improve the size, batch-to-batch consistency, and tumor penetration abilities of polyplex nanoparticles.

Materials and Methods



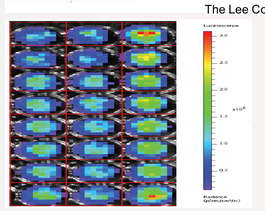
Nelson et. al, ACS Nano, 2013. 7:10 (8870-8880)

- RAFT polymerization for PEG-DB synthesis
- Electrostatic interactions between polymer and siRNA drives micelle formulation and encapsulation

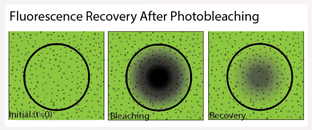


The Lee Company

- Lee Visco-Jet confined impinging micromixer used to mix nanoparticles
- Ability to increase turbulence and particle interaction
- Variable flow-rate settings used to determine optimal conditions



- Ribogreen assay used to study encapsulation efficiency
- Luciferin gene knockdown assay done on MDA MB231 breast cancer cells to measure cytotoxicity and bioactivity of nanoparticles.



- FRAP test done on nanoparticles using FITC labeled DNA to test tumor diffusivity of nanoparticles in 50% Matrigel/ 50% PBS.

Results: Nanoparticle Size

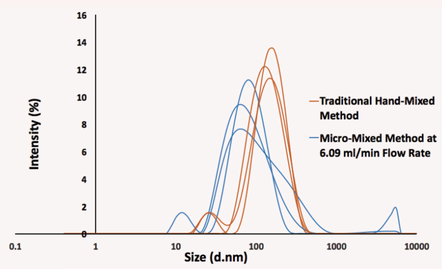


Figure 1: DLS determined Intensity vs. Size analysis of PEG-DB nanoparticles created using a hand-mixed and micro-mixed method (n=3). Micro-mixer generally produced nanoparticles of smaller diameter size.

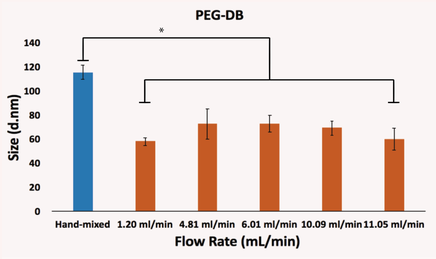


Figure 2: Averaged (n=3) sizes of PEG-DB nanoparticles micro-mixed at various flow rate settings. Flow rates determined through Reynold's number calculations. Small nanoparticles can be obtained using various micro-mixed flow rates.

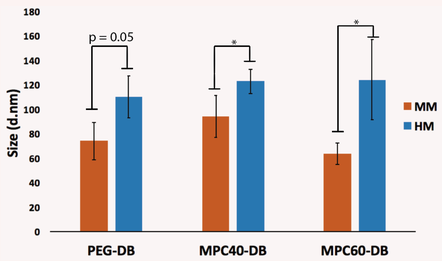


Figure 3: Averaged (n=3) sizes of PEG-DB polyplexes compared with that of zwitterionic polyplexes using hand-mixed and micro-mixed formulation methods. For all polymers, micro-mixing methods lead to decreased polyplex size.

Results: Encapsulation Efficiency

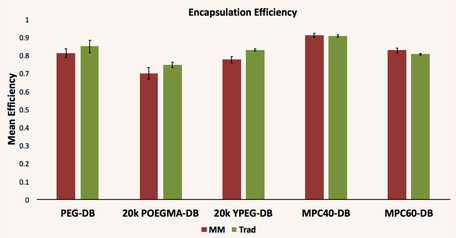


Figure 5: Comparison of averaged (n=3) encapsulation efficiencies of micro-mixed and hand-mixed polyplexes. A negligibly small difference exists between methods, though high encapsulation achieved for both.

Results: Toxicity, Activity, Diffusivity

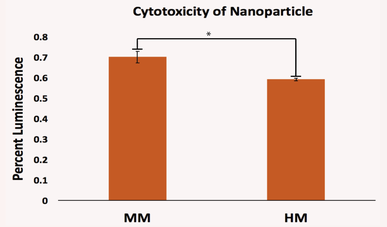


Figure 6: Averaged (n=4) percent luminescence of micro-mixed and hand-mixed PEG-DB carrying luciferase siRNA. Significant (p= 0.0003) decrease in percent luminescence in cells treated with hand-mixed particles indicate increased cell death.

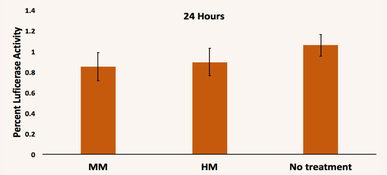


Figure 7: Averaged (n=4) percent bioactivity in cells treated with micro-mixed and hand-mixed PEG-DB particles carrying luciferase siRNA. Although cells treated with micro-mixed particles show slightly lower percent activity, this difference is not significant.

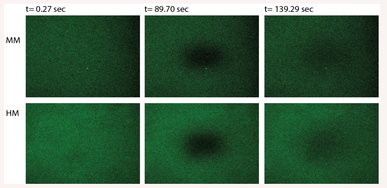
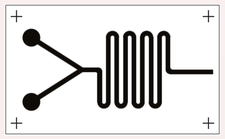


Figure 8: Images of FITC labeled nanoparticle diffusivity in Matrigel before, during, and after photo bleaching. Results show no significant difference in recovery time between hand-mixed and micro-mixed particles.

Conclusion and Future Works

- Confined impinging jet micromixer use minimizes polyplex size for superior tissue penetration, optimizes batch-to-batch consistency of particle formulations, and decreases nanoparticle cytotoxicity.
- There is no sacrifice of encapsulation efficiency when using the micromixer for nanoparticle formulation.
- Further studies include examining:



  - the effects of excipient additions for increased long-term stability of nanoparticles.
  - diffusivity differences between nanoparticle formulations.
  - the effect of 100% Matrigel use on optimizing micromixing complexation.
  - the effects of Herringbone geometry micro-mixer on particle size.

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