A Novel Mixing Device For the Reproducible Manufacture of Non-Viral Gene Therapy Formulations

INTRODUCTION

- Production of non-viral gene therapy formulations requires the controlled mixing of GTAs with plasmid DNA (pDNA).
- Variability in mixing procedures between laboratories and individuals can greatly impact formulation efficacy.
- Large-scale production of non-viral gene therapy formulations suitable for clinical applications will require a reliable and reproducible mixing methodology.
- We have developed and tested a novel pneumatic mixing device for the standardised production of non-viral gene therapy formulations.

RESULTS

Electron micrographs of experimental formulations

- Mixing studies were performed using two important non-viral GTAs that show promise for aerosol gene delivery:
  - GL67A (GL67:DOPE:DMPE-PEG$_{5k}$) is a cationic lipid that forms complex multi-lamellar lipoplexes with pDNA.
  - PEI: a highly cationic polymer that forms small discrete polyplexes with pDNA.

Physical characteristics of generated complexes

- pCIKLux/GL67 and pCIKLux/PEI complexes prepared over a range of mixing rates from 0.5 ml/s to 10 ml/s or by hand mixing.
- pCIKLux/GL67 and pCIKLux/PEI complexes prepared over a range of mixing rates from 0.5 ml/s to 10 ml/s or by hand mixing.
- Complexes prepared using the LMD2 demonstrate equivalent size/charge characteristics to complexes prepared by hand.
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Mixing studies were performed using two important non-viral GTAs that show promise for aerosol gene delivery:

1. Gene Medicine Group, Nuffield Department of Clinical Laboratory Sciences, University of Oxford, John Radcliffe Hospital, Oxford, UK.
2. VGXI, Houston, TX, USA.
3. Genzyme Corporation, Framingham, MA, USA.

CONCLUSIONS

- The LMD2 is a fully automated mixing device that allows reliable and reproducible production of non-viral gene therapy formulations.
- Suitable for a range of different non-viral GTAs.
- Easily scaleable for clinical manufacture.
- Allows standardised production of non-viral gene therapy formulations for clinical applications.

pCIKLux/GL67 complexes demonstrate equivalent size/charge characteristics to complexes prepared by hand mixing.

pCIKLux/GL67 and pCIKLux/PEI complexes prepared over a range of mixing rates from 0.5 ml/s to 10 ml/s or by hand mixing.

Mixing rate (ml/s)

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<th>Mixing rate (ml/s)</th>
<th>GL67A</th>
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Data represents mean ±SEM for n=3 separate experiments.

Absence of shear degradation using the LMD2

- Plasmid DNA is highly sensitive to shear degradation during manufacturing processes.
- pCIKLux integrity analysed by gel electrophoresis after complexation with GL67A or PEI using the LMD2.
- No evidence of increased shear related damage following complex preparation using the LMD2 compared to standard hand mixing.

Physical characteristics measured using dynamic light scattering (Zetasizer Nano, Malvern Instruments, UK).

- pCIKLux/GL67 and pCIKLux/PEI complexes prepared over a range of mixing rates from 0.5 ml/s to 10 ml/s or by hand mixing.

- Physical characteristics measured using dynamic light scattering (Zetasizer Nano, Malvern Instruments, UK).

- In vivo performance of LMD2 formulations

  - 10 ml pCIKLux/GL67 complexes generated using the LMD2 at a mixing rate of 2.5 ml/s.
  - Aerosolised to BALB/c mice (n=6) using whole body aerosol exposure chamber and pneumatic nebuliser.
  - Luciferase expression in lung tissues analysed 24 hr later.

- Complexes prepared using the LMD2 demonstrate equivalent in vivo gene expression to complexes prepared by hand mixing (p>0.05 Student’s t-test).