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

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INTRODUCTION

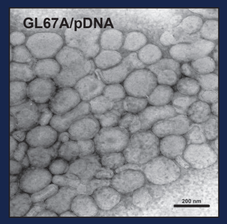
- Production of non-viral gene therapy formulations requires the controlled mixing of GTAs with plasmid DNA (pDNA)
- Variability in mixing procedures between laboratories and individuals can greatly impact formulation efficacy
- Large-scale production of non-viral gene therapy formulations suitable for 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

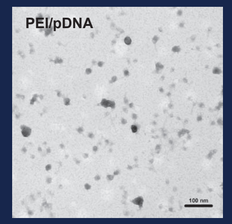
RESULTS

Dimensions: w : d : h = 20 x 12 x 45cm

The LMD2 pneumatic mixer device

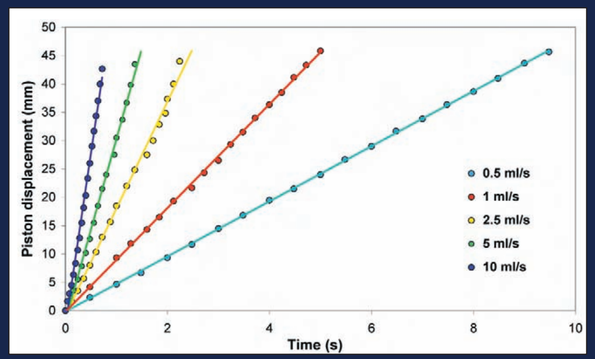
- Fully adjustable pneumatic syringe-driver powered via 50 psi compressed air source
- Incorporates dual lumen syringe containing pDNA and GTA in separate compartments
- Activation drives both reagents through 8-element HDPE static mixer to ensure adequate mixing
- Fully scaleable for large volume clinical mixing





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$_{5000}$) is a cationic lipid that forms complex multi-lamellar lipoplexes with pDNA
- 25 kDa branched polyethylenimine (PEI) is a highly cationic polymer that forms small discrete polyplexes with pDNA



Data represents mean ±SEM for n=3 separate measurements

Reproducible mixing using the LMD2

- LMD2 mixer was loaded with GL67A and the luciferase expression plasmid pCIKLux (5.6 kb)
- Complexes were prepared using a range of applied mixing rates from 0.5 ml/s to 10 ml/s
- Vertical displacement of LMD2 plunger during activation measured using high-speed video analysis
- Linear displacement indicates constant and reproducible mixing conditions at all rates tested

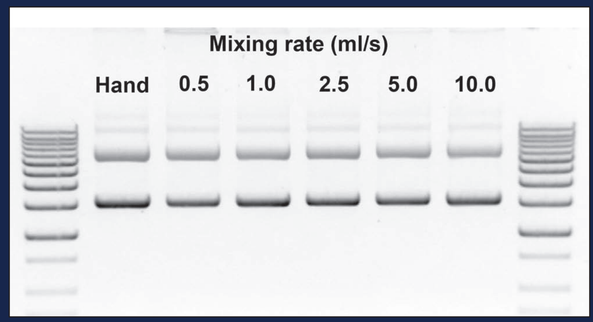
Mixing rate (ml/s)

		Hand	0.5	1.0	2.5	5.0	10.0
GL67A*	Size (nm)	265 ± 18	287 ± 13	278 ± 13	285 ± 19	306 ± 16	315 ± 22
	Charge (mV)	+3.8 ± 0.2	+3.5 ± 0.3	+3.6 ± 0.4	+3.4 ± 0.3	+4.1 ± 0.5	+4.1 ± 0.4
PEI**	Size (nm)	75 ± 4	73 ± 2	70 ± 1	73 ± 1	80 ± 1	95 ± 2
	Charge (mV)	+47.3 ± 0.7	+47.1 ± 0.9	+48.3 ± 0.4	+48.8 ± 0.3	+49.9 ± 0.4	+48.4 ± 0.8

Data represents mean ±SEM for n=3 separate measurements.
*GL67A : pDNA ratio 0.6mM : 0.8mM. **PEI : pDNA at N : P ratio of 10 : 1.

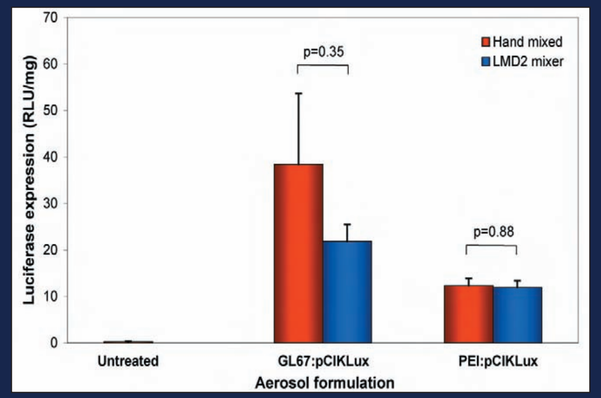
Physical characteristics of generated complexes

- 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
- Physical characteristics measured using dynamic light scattering (Zetasizer Nano, Malvern Instruments, UK)
- pCIKLux/GL67 complexes demonstrate equivalent size/charge characteristics to complexes prepared by hand
- pCIKLux/PEI complexes increase in size at higher mixing rates due to formation of aggregates



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



Data represents mean ±SEM for n=3 separate experiments.
GL67A : pDNA ratio 6mM : 8mM. PEI : pDNA at N : P ratio of 10 : 1.

In vivo performance of LMD2 formulations

- 10 ml pCIKLux/PEI or 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 ($p>0.05$ Student's t-test)

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

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