Lyophilization approach to improve long-term stability of LGA-PEI nanoparticles for drug delivery

Osvaldo *Vega-Martínez*, ENG., M.S.
Chief Scientific Officer
+1 (346) 327-1186 / +506 8314-0692
osvaldo@speratum.com
www.speratum.com

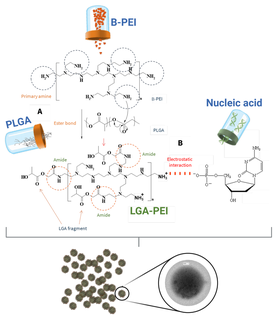
Speratum Biopharma Inc.

Laura Chaves-Martínez[1], Yendry Regina Corrales-Ureña[2], Osvaldo Vega-Martínez[1], Jian Ming Lü[3] and Christian Marin-Müller[1]

[1]*Speratum Biopharma, Inc., Houston,TX 77021-2024, USA*
[2]*National Laboratory of Nanotechnology, SJ 1174-1200, CRI*
[3]*Baylor College of Medicine, Houston, TX 77030, USA*

We aimed to enhance the long-term stability of LGA-PEI-based nanoparticles by employing a lyophilization strategy using trehalose or sucrose as cryo/lyoprotectants in a histidine-based buffer.

BACKGROUND



We have developed a novel polymer named **LGA-PEI**, in which polylactic-co-glycolic acid (PLGA) is depolymerized into lactic-co-glycolic acid (**LGA**) units, which are then covalently linked to the primary amines of polyethylenimine (**PEI**).

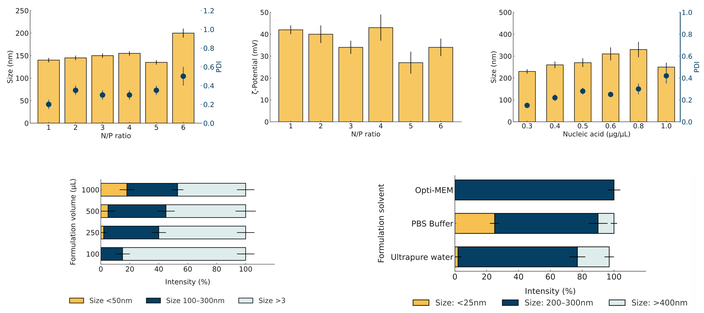
By combining these two polymers, we have created a potent drug delivery system with reduced toxicity. When mixed with nucleic acids, **LGA-PEI** forms nanoparticles through condensation, driven by electrostatic interactions between the remaining amine groups of PEI and the phosphate groups of the nucleic acids.

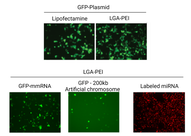
- ***PEI** is highly effective at condensing nucleic acids into nanoparticles for efficient delivery; however, its high density of positively charged amines renders it inherently toxic..*
- ***PLGA** is non-toxic and already FDA-approved for human use, but its negative charge limits its ability to interact effectively with nucleic acids.*

This interaction protects the nucleic acids from degradation and facilitates their cellular uptake

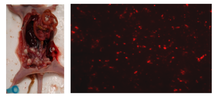
The nanotechnology has been optimized and thoroughly characterized both in terms of its synthesis process and its interaction with nucleic acids.

When combined with various RNA or DNA payloads, it demonstrates high loading efficiency, strong transfection efficacy, and low cytotoxicity in cell cultures, along with effective delivery to major organs and a favorable safety profile in mouse models.





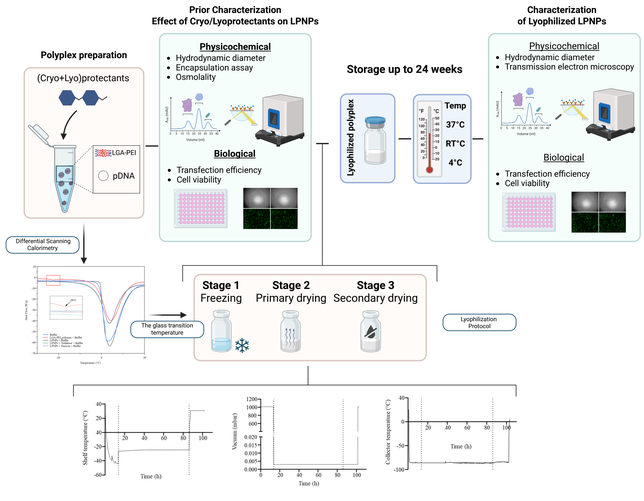
LGA-PEI Nanoparticles Containing RFP Plasmid iv administered three times over five days.



Parameter (Units)	Untreated	LGA-PEI (2.5 mg/kg)
Albumin (g/dL)	2.4 ± 0.1	2.2 ± 0.2
Globulin (g/dL)	2.6 ± 0.3	2.5 ± 0.1
Albumin/Globulin (ratio)	0.94 ± 0.07	0.88 ± 0.12
Total protein (g/dL)	5.0 ± 0.5	4.6 ± 0.1
Alkaline phosphatase (U/L)	42 ± 7	49 ± 10
Alanine transaminase (U/L)	56 ± 21	53 ± 8
Lipase (U/L)	848 ± 103	845 ± 81
Amylase (U/L)	1565 ± 164	1557 ± 77
Creatine (mg/dL)	0.08 ± 0.1	0.10 ± 0.08
Urea nitrogen (mg/dL)	0.20 ± 0.14	0.18 ± 0.1
Total bilirubin (mg/dL)	21 ± 2	20 ± 1
Glucose (mg/dL)	171.3 ± 29.3	156.8 ± 7.7

METHODS

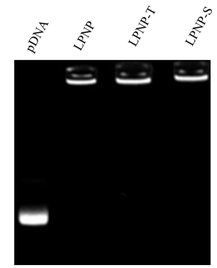
LPNP: LGA-PEI-based nanoparticles
LPNP-T: LPNP + Trehalose +Histidine buffer
LPNP-S: LPNP + Sucrose + Histidine buffer

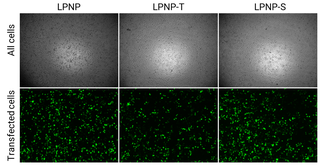


RESULTS OF PRIOR CHARACTERIZATION

The addition of trehalose or sucrose as cryo/lyoprotectants in a histidine-based buffer did not alter the established physicochemical and functional properties of LPNPs. Notably, this combination increased formulation osmolality toward physiological levels, potentially improving *in vivo* compatibility without compromising nanoparticle integrity or performance.

Formulation	Hydrodynamic diameter (nM)	Osmolality (mOsmol/kg)	Transfection efficiency (%)	Cell viability (%)
LPNP	159±19	45±2.5	54±11	90±3
LPNP-T	186±22	267±3.5	51±4	90±2
LPNP-S	146±7	335±3.2	48±3	91±6

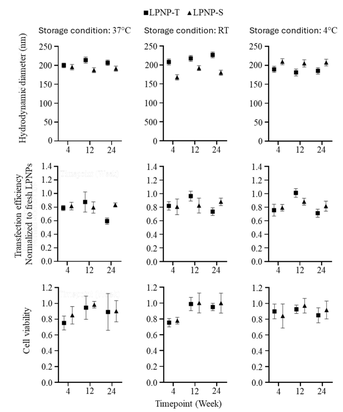


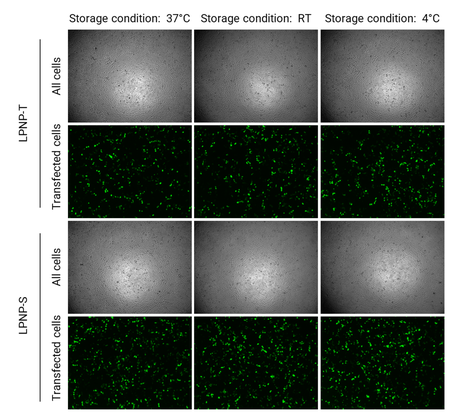


RESULTS OF LYOPHILIZED LPNP CHARACTERIZATION

The long-term stability of LPNPs was significantly enhanced using a histidine-buffered formulation containing trehalose or sucrose as cryo/lyoprotectants. These excipients preserved the physicochemical integrity, transfection efficiency, and low cytotoxicity of lyophilized LPNPs even during prolonged storage at or above room temperature, underscoring their robustness under suboptimal conditions and supporting the potential for simplified handling and distribution logistics.

Additionally, when stored in aqueous form at 4 °C, LPNPs formulated with trehalose or sucrose in a histidine-based buffer also retained their physicochemical and biological properties for up to 24 weeks, demonstrating that lyophilization is not strictly required to achieve extended stability under refrigerated conditions (*data not shown*).





References

Lü, J. M. *et al.Nanomedicine***11**, 1971–1991 (2016)
Lü, J.-M. *et al.Pharmaceuticals***14**, 841 (2021)

LGA-PEI offers a safer and more scalable alternative to viral and lipid-based non-viral delivery systems. However, it faces challenges related to aggregation during prolonged storage and transport, which limit its feasibility as a clinically relevant therapeutic agent.

This study demonstrates that LGA-PEI nanoparticles can be effectively stabilized for long-term storage in both aqueous and lyophilized forms without compromising functionality, underscoring their strong potential as clinically viable, scalable, and logistically flexible platforms for nucleic acid delivery.