CRS/2025
ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 14-18, 2025

NanoGeneSkin
Dr. Bentley's Lab

Immunoliposomes as a targeted delivery system for enzyme replacement therapy in Fabry disease

Luiz Henrique Mesquita Souza, Daniel Giuliano Cerri, Lívia Vieira Depieri, Maria Vitória Lopes Badra Bentley

University of São Paulo, School of Pharmaceutical Sciences of Ribeirão Preto, Brazil
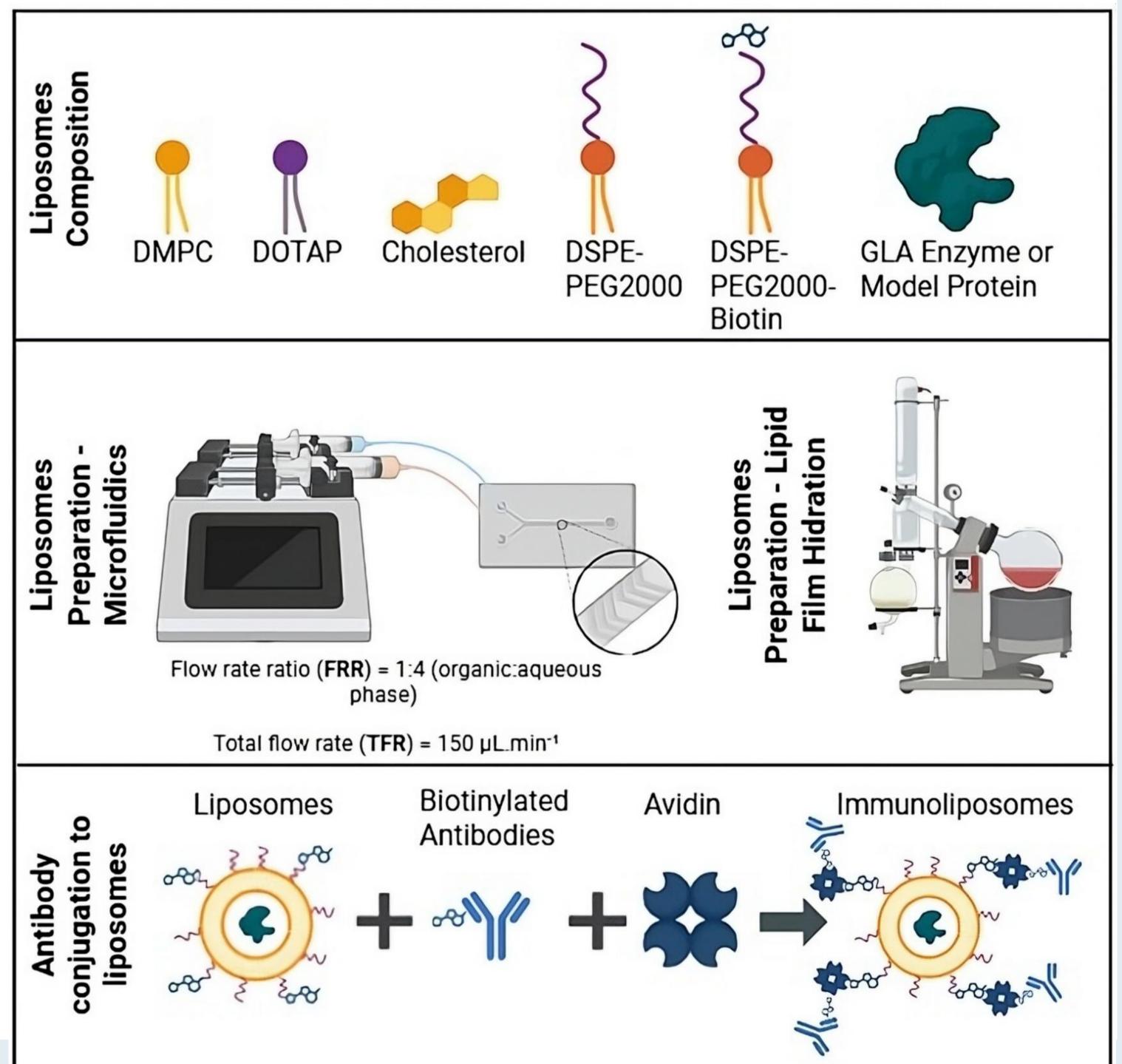
lhmesquita.souza@usp.br
vbentley@usp.br

NanoGeneSkin Bentley's Laboratory

INTRODUCTION

Fabry disease is a lysosomal storage disease that mainly affects cardiac tissue [1]. It causes the storage of glycolipids, such as globotriaosylceramide, in lysosomes due to a deficiency of the enzyme α-galactosidase A (GLA) [2]. The current standard of care, enzyme replacement therapy (ERT), involves intravenous infusions of recombinant human GLA. However, ERT has significant limitations, including poor tissue uptake, a short half-life, immunogenicity, and the need for large enzyme doses. Consequently, ERT is often unable to completely restore kidney and heart physiology, leaving many patients with severe symptoms and increased mortality. Liposomes have been extensively studied as nanocarriers for enzyme delivery. Functionalization with polyethylene glycol (PEG) prolongs circulation half-life, while conjugation with antibodies creates immunoliposomes for specific tissue targeting and cellular uptake [3]. This work proposes the development of an immunoliposome to deliver GLA specifically to cardiomyocytes. The nanocarrier will be functionalized with both PEG and a monoclonal antibody targeting integrin alpha-7 (ITGA-7), a receptor that is highly expressed on cardiomyocytes and upregulated in Fabry disease [4]. This formulation aims to utilize receptor-mediated endocytosis, ensuring the efficient delivery of the encapsulated GLA directly to the lysosomes and overcoming the critical limitations of conventional ERT.

METHODS

PEGylated liposomes were prepared by both lipid film hydration and microfluidic methods. Using BSA-Alexa Fluor™ 647, as a model protein, the encapsulation was performed with a protein concentration of 16 µg.mL-1, at a ratio of 19.63 µg of protein per µmol of lipid. Following separation from free protein via size exclusion chromatography (SEC), encapsulation efficiency was determined by fluorescence measurement after lysing the liposomes. Antibody functionalization is being performed via biotin-avidin conjugation.

Liposomes Composition: DMPC, DOTAP, Cholesterol, DSPE-PEG2000, DSPE-PEG2000-Biotin, GLA Enzyme or Model Protein

Liposomes Preparation - Microfluidics / Liposomes Preparation – Lipid Film Hidration
Flow rate ratio (**FRR**) = 1:4 (organic:aqueous phase)
Total flow rate (**TFR**) = 150 µL.min-1

Antibody conjugation to liposomes: Liposomes + Biotinylated Antibodies + Avidin → Immunoliposomes

References: [1] Azevedo et al., International Journal of Molecular Sciences, 2021; [2] Yuasa et al., Journal of Echocardiography, 2017; [3] Di et al., Advanced Drug Delivery Reviews, 2020; [4] Cabrera, et al., Advanced healthcare materials, 2016

RESULTS

The synthesized liposomal batches demonstrated excellent physicochemical properties, including a homogenous particle size distribution with a low polydispersity index (PDI). Successful surface modification with a polyethylene glycol (PEG) layer was confirmed by the observed shift in ζ-potential from a positive surface charge to a value approaching neutrality. This provides clear evidence that the "stealth" PEG layer effectively shields the liposome's core charge. Furthermore, implementing a microfluidic synthesis method yielded a significant improvement in encapsulation efficiency and drug loading compared to conventional techniques (**Table 1**).

During purification by SEC, initial experiments revealed that the liposome fractions (eluting in fractions 4-6) and the free protein (eluting from fraction 7 onwards) were poorly resolved. To improve this resolution, the column length was increased (**Figure 1**).

Table 1. Physicochemical characteristics of liposomes produced by lipid film hydration or microfluidics. Data includes particle size, polydispersity index (PDI), and ζ-potential before purification by size-exclusion chromatography (SEC), alongside post-purification measurements of size, PDI, encapsulation efficiency, and drug loading.

	Formulations	Before SEC			After SEC (Fraction 5)			
		Size (nm)	Pdl	ζ Potential (mV)	Size (nm)	Pdl	Encapsulation Efficiency (%)	Drug Loading (%)
Lipid Film Hydration	Non-PEGylated (n=2)	119.1 ± 2.9	0.121 ± 0.006	+ 34.7 ± 2.4	-	-	-	-
	PEGylated (n=2)	113.4 ± 2.5	0.106 ± 0.015	+ 8.2 ± 2.3	-	-	-	-
	PEGylated + BSA-647 (n=2)	110.7 ± 3.0	0.156 ± 0.065	- 0.3 ± 0.9	103.7 ± 4.8	0.058 ± 0.005	7.97 ± 0.55	0.25 ± 0.02
Microfluidics	PEGylated (n=2)	80.5 ± 6.5	0.198 ± 0.036	-	-	-	-	-
	PEGylated + BSA-647 (n=3)	116.2 ± 1.1	0.156 ± 0.023	+ 8.7 ± 1.9	136.9 ± 4.6	0.158 ± 0.029	47.33 ± 3.49	1.46 ± 0.11

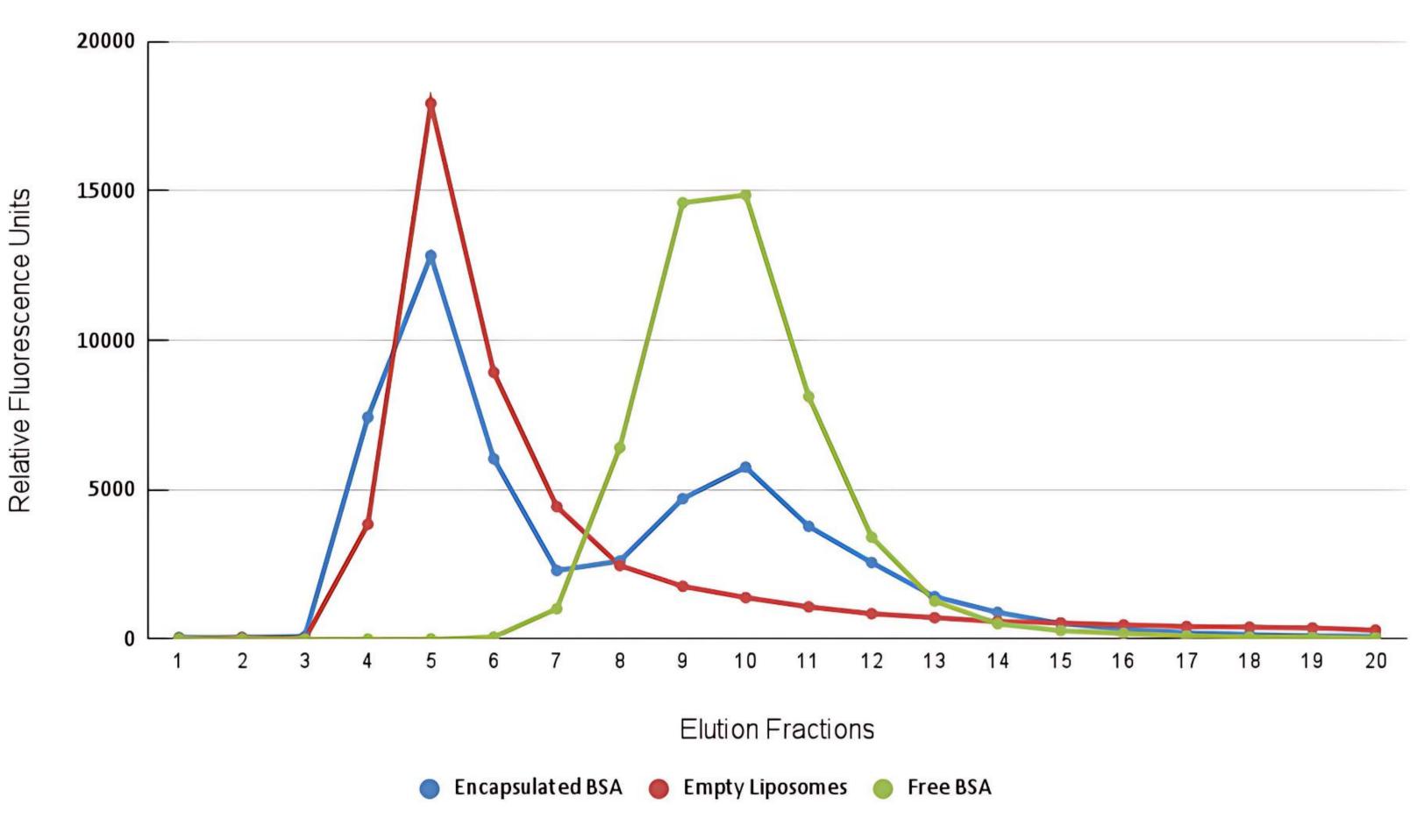Elution Profiles of Empty Liposomes, Free BSA, and Encapsulated BSA

Figure 1. Elution profile of microfluidic-prepared liposomes following size-exclusion chromatography. The relative fluorescence units were measured for each elution fraction to distinguish between liposomal formulations and free protein. Empty liposomes (emission measured at 520 nm – TopFluor™ Cholesterol) are primarily observed in fractions 4, 5, and 6. In contrast, BSA-Alexa Fluor™ 647 (emission measured at 660 nm) begins to elute in fraction 7. For the BSA-Alexa Fluor™ 647- encapsulated liposomes, a portion of the BSA signal is detected in fractions 4, 5, and 6, confirming successful encapsulation, while the unencapsulated, free BSA begins to elute in fraction 7.

CONCLUSION

This work has successfully established a method for producing PEGylated liposomes with high encapsulation efficiency. Currently, the antibody functionalization is being optimized, which will allow us to choose the best formulation to proceed with cell culture experiments. This project presents significant scientific-technological potential to improve the quality of life for patients with Fabry disease, in addition to validating a platform technology capable of being applied in the future to other lysosomal storage diseases.