Development of PLGA-Based Nanoparticles for a novel therapeutic peptide in Cardiovascular Diseases

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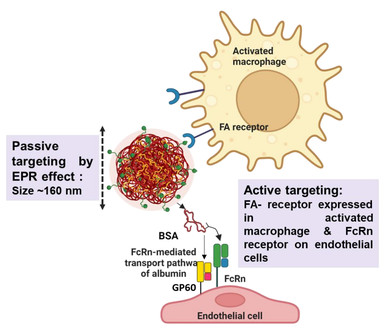
Introduction

Peptide-based therapeutics are increasingly vital in managing cardiometabolic diseases, but their clinical application is hindered by low bioavailability due to limited membrane permeability and in vivo stability. Encapsulation of therapeutic peptides in nanoplatforms, such as biodegradable poly(lactic-co-glycolic acid) (PLGA), offers a promising solution. However, variability in formulation protocols limits the development of new therapies, highlighting the need for optimization tailored to therapeutic peptides, which are distinct from commonly studied structural protein models like albumin

Method

This study employs a novel therapeutic peptide (NTP) with anti-inflammatory, anti-atherosclerotic, and anti-diabetic effects to address these gaps. By systematically varying preparation techniques, sonication conditions (duration and power), organic solvents (water-miscible and immiscible), polymer types (varying molecular weights), PEGylation, stabilizers (type and concentration), and excipient —this research aims to provide a comprehensive understanding of PLGA nanoparticle development for delivering NTP (NTP-NPs). The selected formulation was further evaluated for their ability to enhance the delivery of NTP to target cells involved in atherosclerosis, including Monocyte-Derived Macrophages (HMDMs), RAW 264.7 murine macrophages, and Human Aortic Endothelial Cells (HAECs)).



Scheme 1. *Design of NTP-NPs to target pro-inflammatory phenotype of macrophage and endothelial cells*

Results

NP name	Method preparation	Feed ratio of NTP (%	d.nm	DPI	Zeta potential	% EE	%LC%
NP1	2-step nanoprecipitation	10	169.3 ± 4.9	0.08 ± 0.01	-27.5 ± 3.7	19.3 ± 3.7	1.9 ± 0.3
NP2	Double emulsion evaporation (DE)	10	184 ± 7	0.18 ± 0.04	-29.8 ± 0.9	31.2 ± 7.1	3.2 ± 0.9

Table 1. *Effect of preparation methods on NTP-NPs*

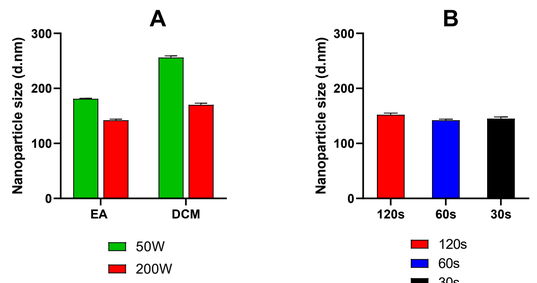
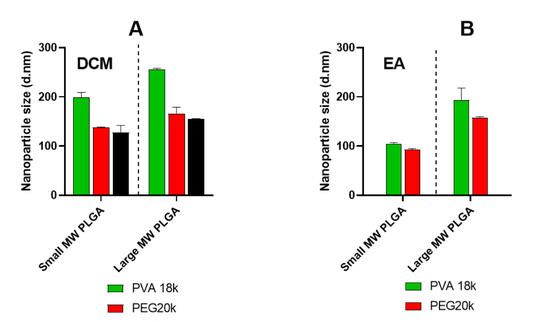


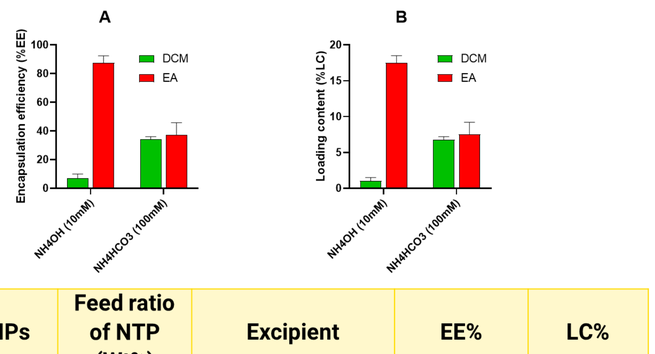
Figure 1. *Effect of sonication conditions on NP size (d.nm). (A) Effect of sonication power (50W vs 200W) and organic solvent (EA vs DCM) on particle size. (B) Effect of sonication time on particle size (EA as organic solvent) with sonication power of 200W*



Group		Name of NPs	Oil phase	Stabilizer	Feed ratio of D6PV (%)	Size	PDI
Non-Pegylated PLGA (NP)	Small Molecular weight	NP3	DCM	PVA20k	20	199 ± 10	0.14 ± 0.04
		NP9	EA	PVA20k	20	105 ± 1	0.08 ± 0.02
		NP6	DCM	PEG20k	20	138 ± 1	0.13 ± 0.04
		NP10	EA	PEG20k	20	93 ± 1	0.14 ±0.02
	Large (L) Molecular weight	NP_L1	DCM	PVA20k	20	256 ± 2	0.1 ± 0.01
		NP_L4	EA	PVA20k	20	194 ± 24	0.13 ± 0.04
		NP_L2	DCM	PEG20k	20	166 ± 13	0.12 ± 0.06
		NP_L5	EA	PEG20k	20	158 ± 1	0.06 ± 0.01

Figure 2. Effect of organic solvent, MW of PLGA and stabilizer on NPs size. (A) nanoparticles prepared using dichloromethane (DCM) as an oil phase, (B) nanoparticles prepared using ethyl acetate (EA) as an oil phase

Figure 3. *Effect of stabiliser and PEGylation on encapsulation efficiency and loading content in small MW PLGA NPs. The no-stabiliser refer No exogenous stabiliser*



Group	NPs	Feed ratio of NTP (Wt%)	Excipient	EE%	LC%
Non-PEGylated PLGA (NP)/Large MW PLGA	NP_L4	20	100 mM NH4HCO3	37.4 ± 8.4	7.5 ± 1.7
	NP_L7	20	10mM NH4OH	87.5 ± 4.9	17.50 ± 1
	NP_L9	10	10mM NH4OH	97.1 ± 2.2	9.7 ± 0.2
	NP_L10	30	10mM NH4OH	80.9 ± 11.7	24.3 ± 3.5
	NP_L11	40	10mM NH4OH	62.3 ± 13.1	24.9 ± 5.2

Figure 4. *Effect of organic solvent and solubility enhancers on encapsulation efficiency and loading capacity*

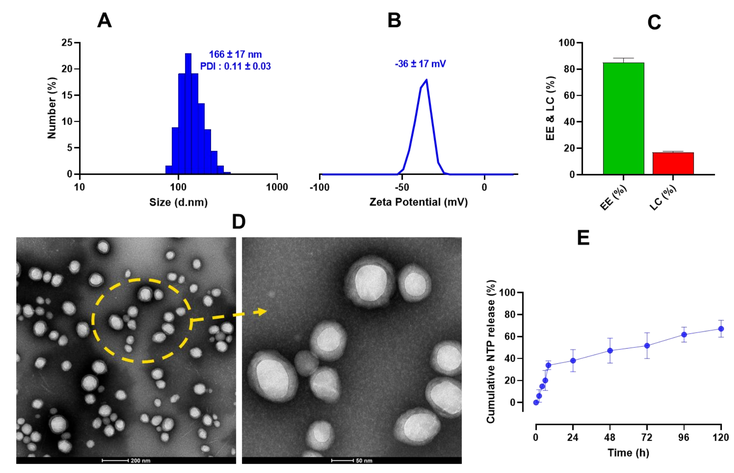


Figure 5. *Characteristics of representative NTP-NPs for in vitro study. (A) Histograms of particle size (d.nm), (B) zeta potential (mV), (C) NTP encapsulation: Encapsulation efficiency (EE) and Loading capacity (LC) represented in percentages (%); (D) Morphology of NTP-NPs by Transmission Electron Microscopy (TEM); and (E) NTP release profile from nanoparticles*

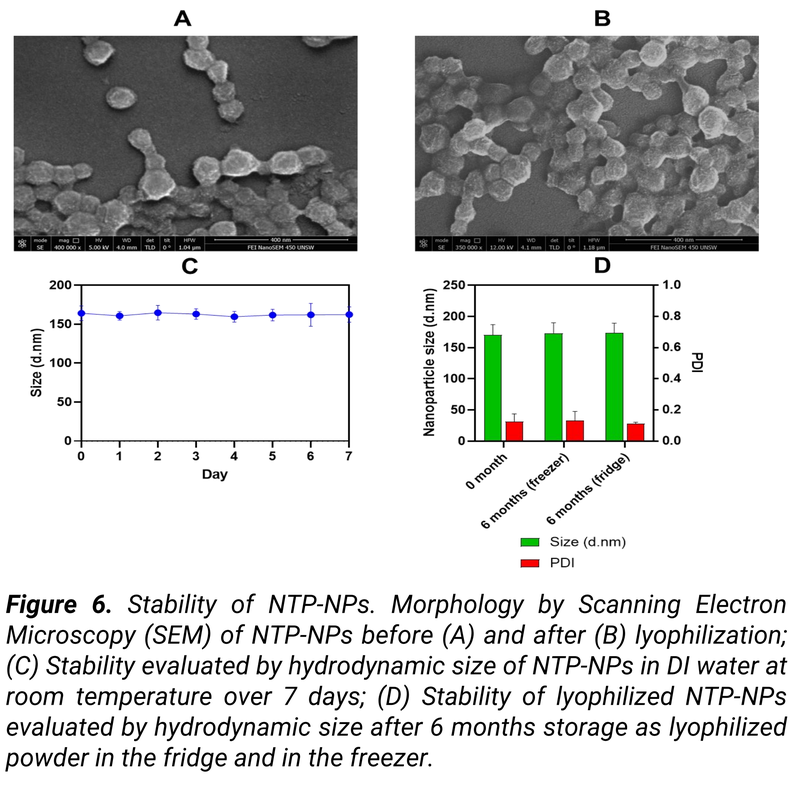


Figure 6. *Stability of NTP-NPs. Morphology by Scanning Electron Microscopy (SEM) of NTP-NPs before (A) and after (B) lyophilization; (C) Stability evaluated by hydrodynamic size of NTP-NPs in DI water at room temperature over 7 days; (D) Stability of lyophilized NTP-NPs evaluated by hydrodynamic size after 6 months storage as lyophilized powder in the fridge and in the freezer.*

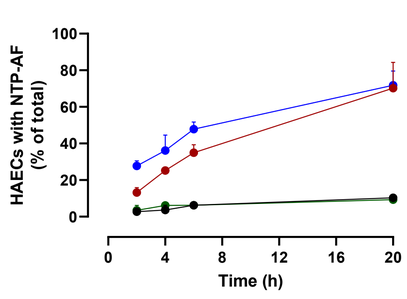


Figure 7. *Time-dependent uptake of NTP-AF and NTP-AF–loaded nanoparticles by resting vs activated human aortic endothelial cells (HAECs). HAECs under resting (−) and pro-inflammatory activated (+) conditions were incubated with either free Alexa Fluor 488–labeled NTP (NTP-AF) or nanoparticles encapsulating NTP-AF (NTP-AF NPs). The percentage of HAECs positive for NTP-AF was quantified over time using flow cytometry (BD LSRFortessa SORP).)*

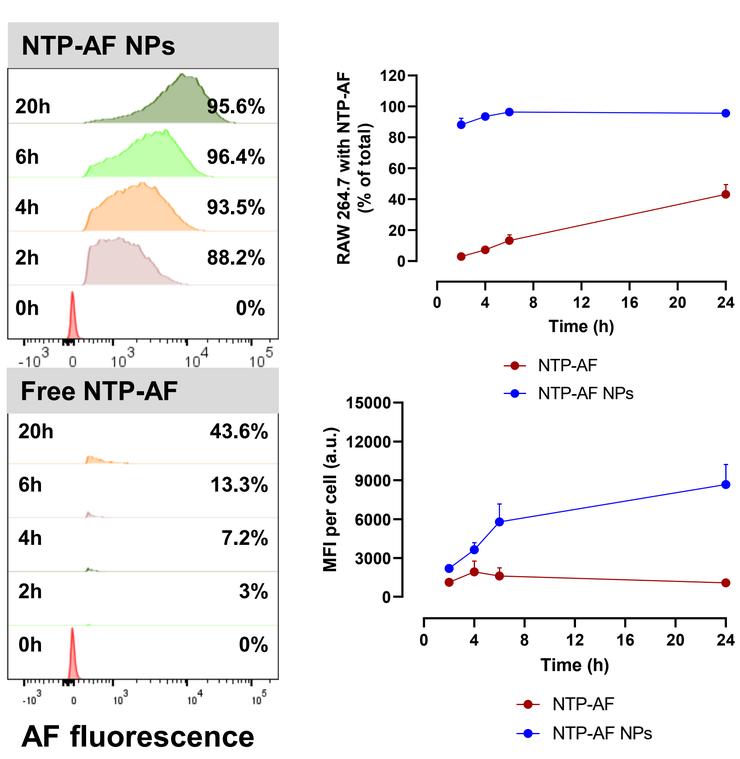


Figure 8. *Time-dependent cellular uptake of free Alexa Fluor 488–labeled NTP (NTP-AF) and NTP-AF–loaded nanoparticles (NTP-AF NPs) by RAW 264.7 analyzed by flow cytometry (BD LSRFortessa SORP instrument)*

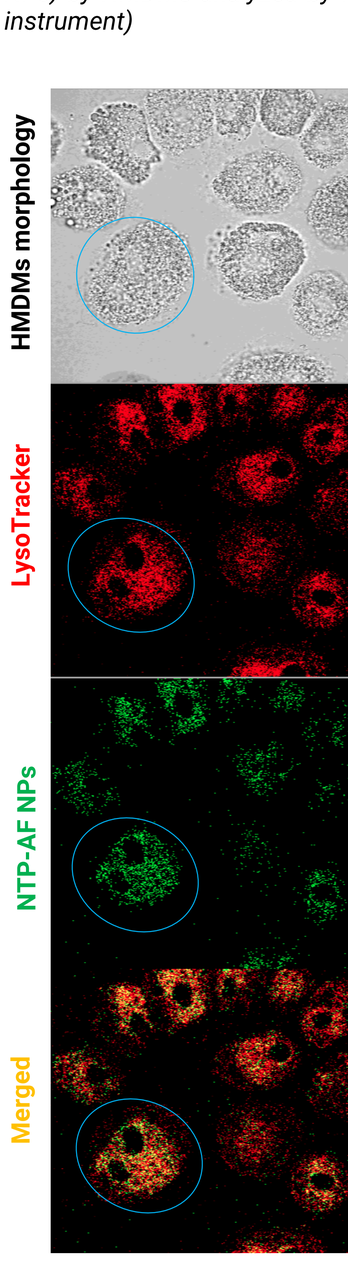


Figure 9. *Time-dependent cellular uptake of free Alexa Fluor 488–labeled NTP (NTP-AF) and NTP-AF–loaded nanoparticles (NTP-AF NPs) by HMDMs analyzed by flow cytometry (BD LSRFortessa SORP instrument)*

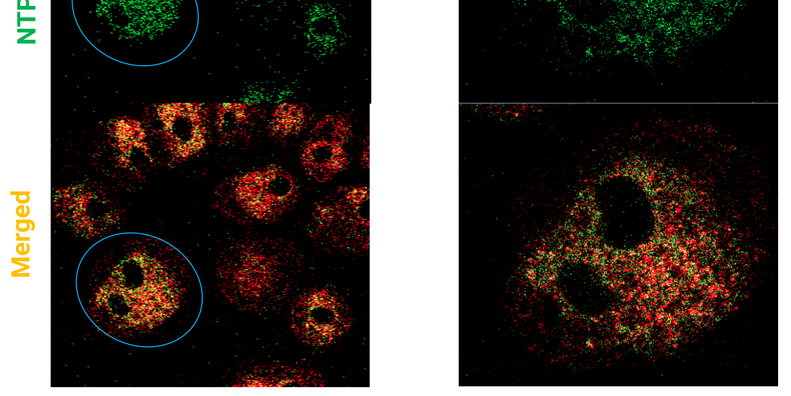


Figure 10. *Intracellular uptake of NTP-AF–loaded nanoparticles by human monocyte-derived macrophages (HMDMs). Live-cell confocal imaging was performed using a Zeiss LSM980 microscope. NTP was pre-labeled with Alexa Fluor 488 (green) and encapsulated into nanoparticles (NTP-AF NPs). Lysosomes were stained with LysoTracker (red).*

Conclusion

- A library of 32 NTP-loaded PLGA nanoparticles (NTP-NPs) was systematically engineered with multi-parameter variations and thoroughly characterized to provide a comprehensive understanding of PLGA nanoparticle development for targeted therapeutic peptide delivery.
- Notably, through this optimization process, we successfully achieved a high peptide loading content of up to 25% under optimal formulation conditions.
- The selected formulation was further evaluated for cellular uptake. While all tested cell types exhibited limited uptake of free NTP, NTP-NPs demonstrated a significantly enhanced rate and extent of internalization.
- These findings offer valuable insights into the design of peptide-encapsulated, biodegradable PLGA nanoparticles and support their potential for therapeutic applications.

Acknowledgement

National health and Medical research Council of Australia; University of New South Wales (UNSW), NSW Cardiovascular Research Network (CVRN) 2025 Professional Development Awards, the National Heart Foundation of Australia