A scaffold-based platform for nanoparticle-mediated mRNA delivery in breast cancer therapy

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INTRODUCTION

mRNA therapeutics show strong potential for cancer treatment but are limited by instability and poor cellular uptake [1]. Nanoparticle systems, such as lipid nanoparticles (MessengerMAX, MM) and cell-penetrating peptides (mGET), can enhance delivery [2], though precise localisation and sustained release remain challenging.

Embedding mRNA nanoparticles within biomaterial scaffolds offers a strategy for localised and controlled release [3]. After tumour resection, these scaffolds can be implanted into the surgical cavity to reduce local recurrence.

TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) selectively induces tumour cell apoptosis via death receptor signalling [4]. This study explores a scaffold-based mRNA delivery system for localised TRAIL therapy to prevent post-surgical breast cancer recurrence.

Hypothesis

Localised TRAIL mRNA delivery via an implantable collagen scaffold will reduce triple-negative breast cancer (TNBC) recurrence following surgical resection by providing sustained, tumour-targeted pro-apoptotic signalling within the resection cavity.

AIM

To optimise and evaluate mGET as a therapeutic TRAIL mRNA delivery platform by comparing its transfection efficiency, cytocompatibility, and anti-tumour efficacy with MessengerMAX in both 2D cultures and 3D collagen/hyaluronic acid scaffold models.

MATERIALS AND METHODS

- mRNA delivery vector formulation *(MessengerMAX, mGET)*
- 2D and 3D scaffold screening *(collagen, hyaluronic acid)*
- Therapeutic and mechanistic evaluation

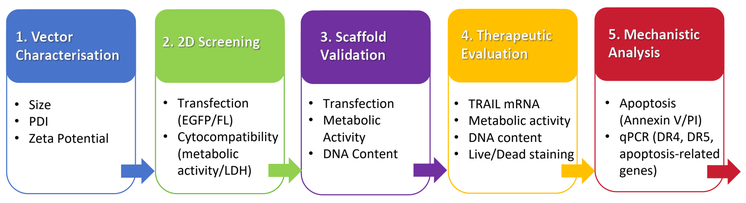


Figure 1. Experimental workflow and analysis strategy.

RESULTS

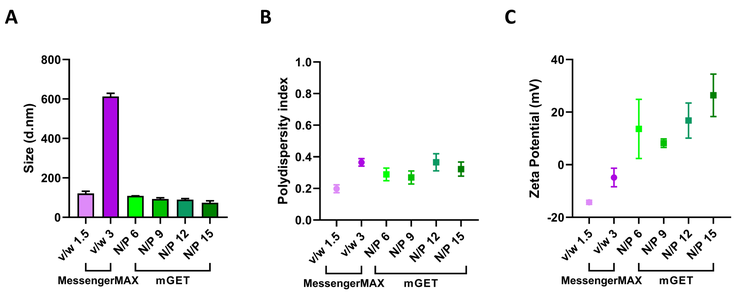


Figure 2. Physicochemical characterisation of mRNA nanoparticles. (A) Particle size. (B) Polydispersity index (PDI). (C) Zeta potential.

Outcome: MessengerMAX (v/w 3) produced larger nanoparticles with negative surface charge, whereas mGET formed smaller particles with N/P-dependent cationic properties. All formulations demonstrated acceptable polydispersity.

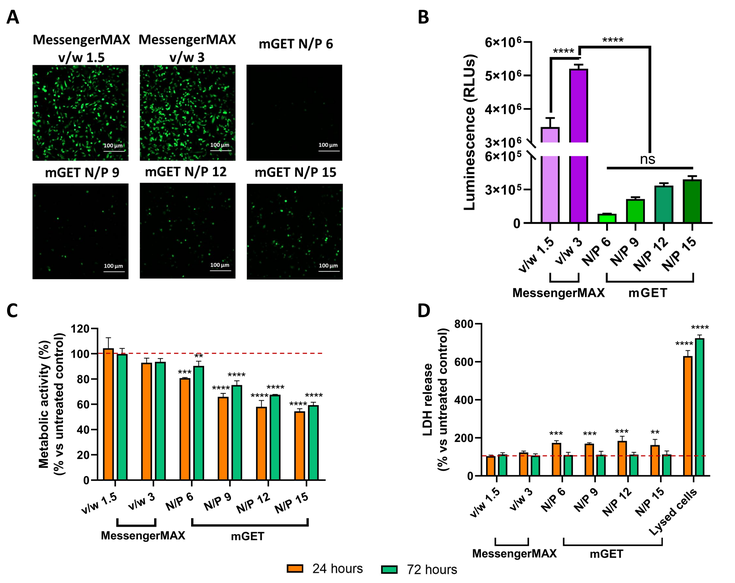


Figure 3. mRNA transfection efficiency and cytocompatibility in MDA-MB-231 cells. (A) EGFP fluorescence.(B) Luciferase expression.(C) Metabolic activity.(D) LDH release.

Outcome: MessengerMAX enabled robust mRNA expression with low cytotoxicity, while mGET displayed reduced efficiency and dose-dependent toxicity at higher N/P ratios.

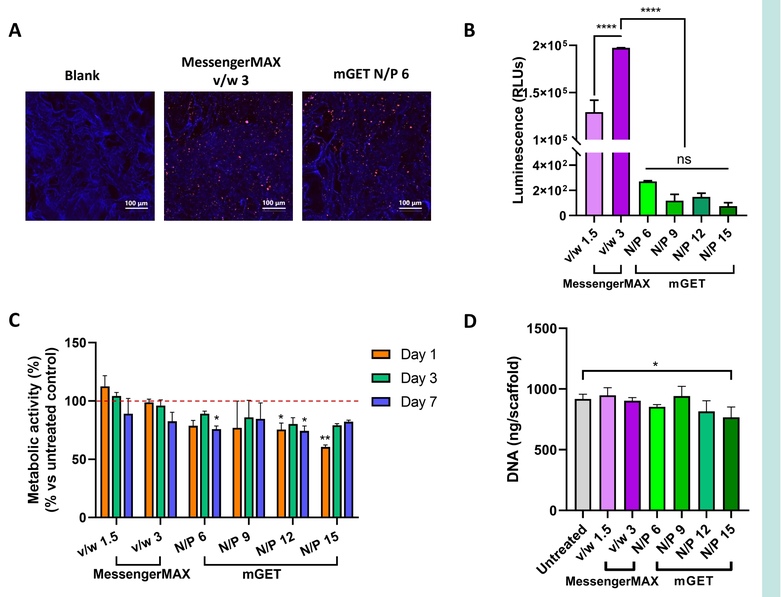


Figure 4. Evaluation of mRNA delivery efficiency and cytocompatibility in 3D collagen/hyaluronic acid scaffolds. (A) Distribution of Cy-5 labelled mRNA nanoparticles within scaffolds. (B) Luciferase expression. (C) Metabolic activity over time. (D) DNA content at day 7.

Outcome: MessengerMAX achieved superior transfection in 3D scaffolds. While higher N/P ratios induced transient metabolic reduction, recovery over time and stable DNA content confirmed preserved cell viability and overall cytocompatibility.

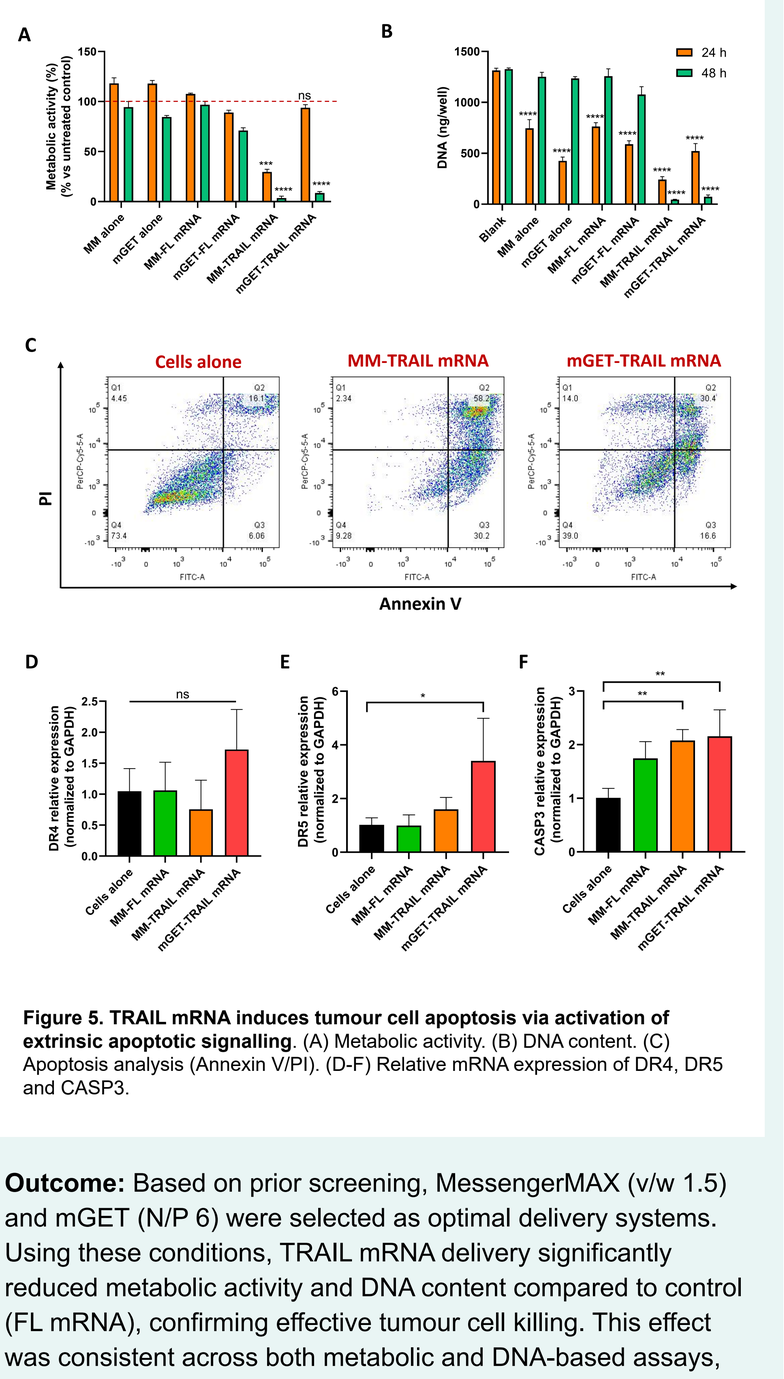


Figure 5. TRAIL mRNA induces tumour cell apoptosis via activation of extrinsic apoptotic signalling. (A) Metabolic activity. (B) DNA content. (C) Apoptosis analysis (Annexin V/PI). (D-F) Relative mRNA expression of DR4, DR5 and CASP3.

Outcome: Based on prior screening, MessengerMAX (v/w 1.5) and mGET (N/P 6) were selected as optimal delivery systems. Using these conditions, TRAIL mRNA delivery significantly reduced metabolic activity and DNA content compared to control (FL mRNA), confirming effective tumour cell killing. This effect was consistent across both metabolic and DNA-based assays, indicating a robust therapeutic response. While MessengerMAX achieved the highest efficacy, mGET also induced measurable therapeutic effects, demonstrating its potential as an alternative delivery platform. Increased apoptosis further supported these findings, while upregulation of DR4, DR5 and CASP3 confirmed activation of the extrinsic apoptotic pathway at the molecular level.

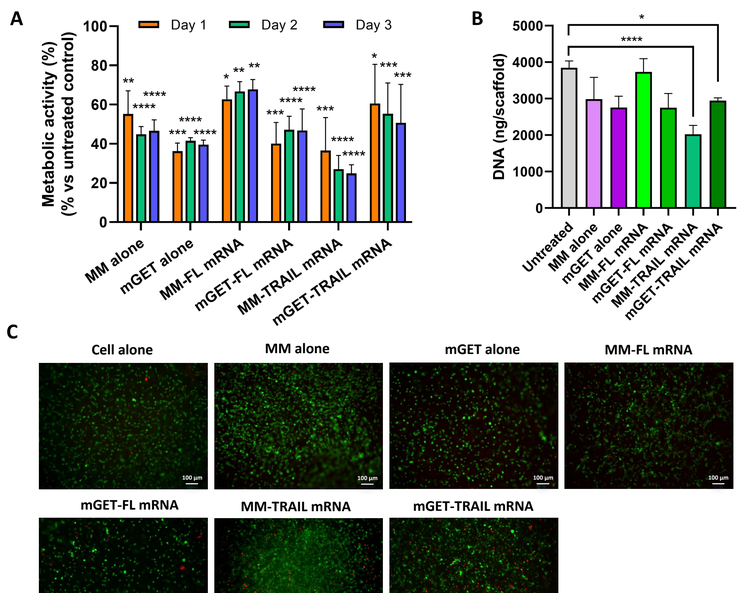


Figure 6. TRAIL mRNA therapeutic effects in 3D scaffolds. (A) Metabolic activity over time. (B) DNA content. (C) Live/Dead staining. (D-F) Relative mRNA expression of DR4, DR5 and CASP3.

Outcome: TRAIL mRNA delivery reduced metabolic activity to below 25% in the MM-TRAIL group by day 3 within 3D scaffolds. MessengerMAX-mediated delivery induced reduced DNA content at day 3, while mRNA delivery also decreased death signals in the scaffolds, as indicated by reduced live/dead staining.

Upregulation of DR4 and DR5 confirmed activation of death receptor signalling. TRAIL mRNA delivery reversed thie BCL2 effect, restoring BCL2 expression to baseline levels. This suggests that TRAIL counteracts anti-apoptotic signalling, promoting apoptotic cell death.

DISCUSSION & CONCLUSIONS

1. Transfection performance:
MessengerMAX showed higher efficiency, while mGET enabled consistent mRNA delivery in both 2D and 3D models.

2. Cytocompatibility:
mGET exhibited good cytocompatibility, with transient metabolic reduction and recovery over time.

3. Therapeutic activity:
mGET-mediated TRAIL mRNA induced tumour cell death and increased DR4/DR5 expression, confirming apoptotic signalling.

mGET is a promising and tunable platform for therapeutic mRNA delivery with balanced efficiency and cytocompatibility.

FUTURE WORK

Future studies will evaluate scaffold-based mGET-mediated TRAIL mRNA delivery in an in vivo post-surgical breast cancer model to assess its potential for preventing local tumour recurrence.

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