Modified Glucagon-like Peptide-1 Receptor Agonist Producing Bacteriophages for the Treatment of Type 2 Diabetes Mellitus

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Background

Type 2 Diabetes Mellitus (T2DM) is a common metabolic disorder, affecting over 38 million Americans, with 90–95% of cases classified as type 2 [1]. Primarily driven by insulin resistance, T2DM leads to complications like cardiovascular and kidney disease, which in 2019 accounted for approximately 1.5 million deaths globally and an additional 460,000 deaths from related complications [2]. While GLP-1 receptor agonists (GLP-1RAs) enhance insulin secretion and beta-cell survival, their application is limited by side effects, including gastrointestinal disturbances, acute kidney injury, and immunogenic reactions [3].

To address these limitations, we propose a single-infection phage system capable of delivering and sustaining GLP-1 production directly in the gut. This approach promises reduced risk of adverse effects, minimal dependency on patient compliance, and long-term glucose regulation. Our solution enables effective, localized production within the gut, avoiding industrial production complications and discomfort associated with injectables. To prevent uncontrolled phage proliferation, our engineered phage allows only a single infection per phage, ensuring safe and controlled GLP-1RA release.

Mission Statement

Our mission is to improve Type 2 diabetes care and patient outcomes through the development of advanced biological technologies through innovation, teamwork, and continuous learning.

Device Concept and Design

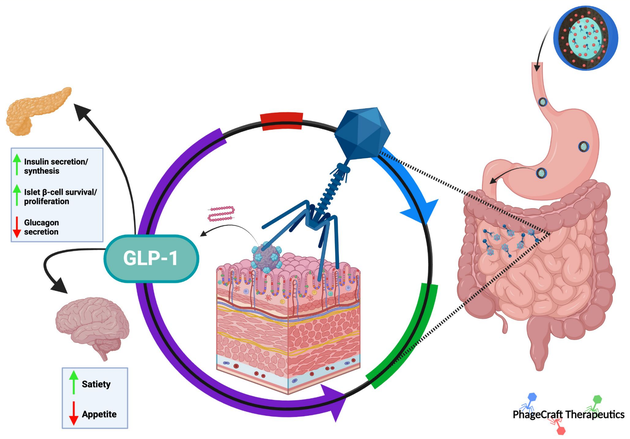

Figure 1. Schematic representation of enteric-coated capsules carrying phages to the distal small intestine, inducing GLP-1RA production in targeted microbe, inducing downstream effects on glucose metabolism

Final Product Specifications

Specification	Target Value
Treatment Effectiveness	Decrease of 2.5% in A1C levels
Ease of Use (5 pt. scale)	5
Treatment Dosage	0.5 mg
Patient Compliance	80% Regimen Adherence after 12 months
Cost of Production	$15 per dose
Risk of Abuse	< 2% incidence
Immunogenicity	< 5% of patients show immune response
Product Half-Life	> 2 days

Final Technical Model

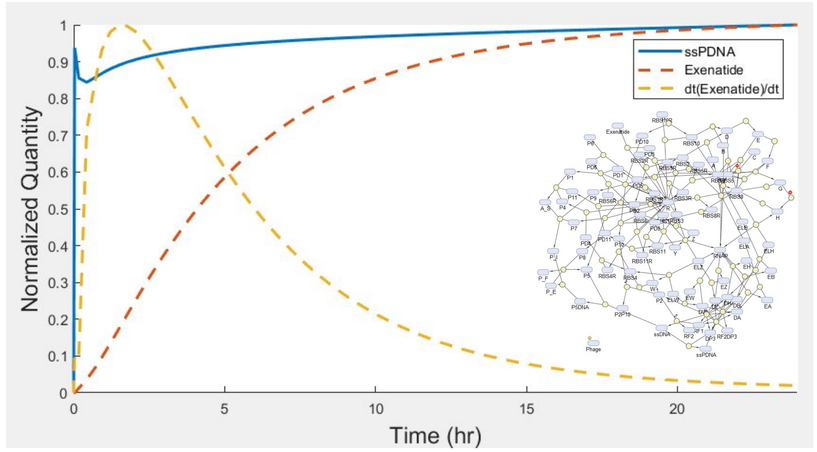

Figure 2. Model of M13 life cycle showing normalized quantity of DNA polymerase III-bound ssDNA and exenatide within single E. *coli* bacterium and normalized rate of exenatide change. 64 kinetic parameters and 81 differential equations were used to calculate the concentration of different nucleic acid and protein species, modeling processes such as DNA replication, mRNA transcription, protein translation and particle assembly [4].

Prototype

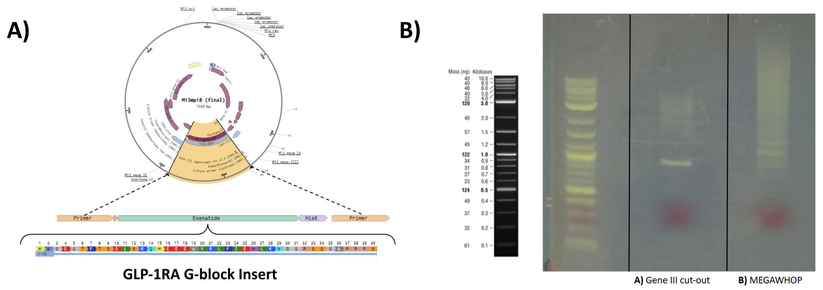

Figure 3. **(A)** Schematic representation of MEGAWHOP with custom gene block (G-block) insertion and peptide sequence for exenatide, the selected GLP-1RA sequence. **(B)** Agarose gel of constructed plasmid prototypes consisting of a A) Gene III cut-out and B) MEGAWHOP Gene III substitution

Verification Results

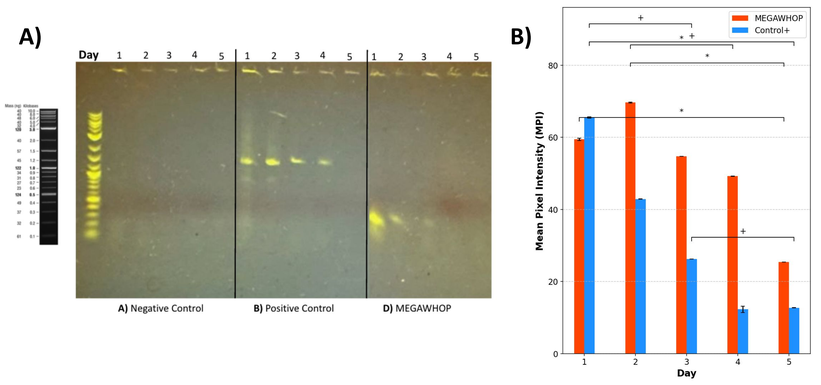

Figure 4. **(A)** Agarose gel representing stability performance across five days between negative control, MEGAWHOP, and positive control groups. **(B)** Accompanying quantitation via ImageJ with statistical significance determined by one-way ANOVA with Tukey's HSD post-hoc test ($p < 0.05$).

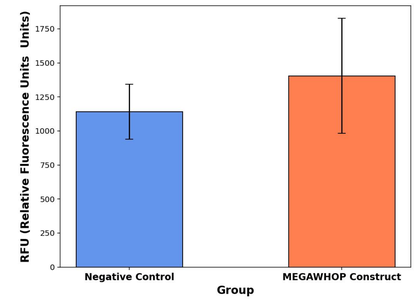

Figure 5. ELISA (enzyme-linked immunosorbent assay) results (reported in RFU) for His-6 specific assay across negative controls group (unmodified m13mp18 template transformed) and MEGAWHOP modified group (gene III substitution with exenatide). With three samples each day across five days (n = 15 samples per group), the mean RFU was compared with a two-sample t-test suggesting a possible statistical significance ($p < 0.15$)

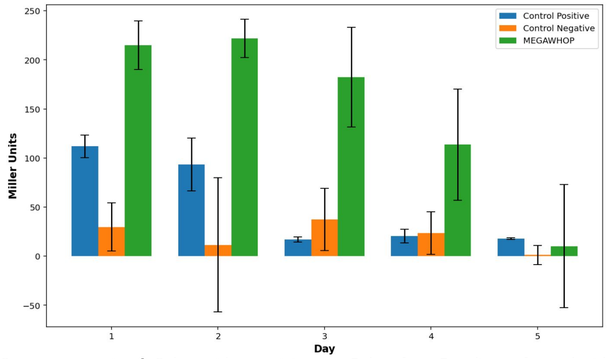

Figure 6. ONPG assay measuring β-Galactosidase activity over 5 days from E. *coli* transformed with A) Nothing B) Unmodified M13mp18 RF DNA C) M13mp18 MEGAWHOP Gene III substitution. One-way ANOVA analysis showed significant variation across days for positive control and MEGAWHOP treatment groups ($p < 0.05$)

Design Status and Future Work

As we complete the prototyping and validation process our team was able to successfully create a modified m13 bacteriophage construct with a gene III deletion and GLP-1RA insertion. This was verified via gel electrophoresis. Subsequently, we conducted a PCR-based stability tests as well as an ONPG assay to verify expression and its decrease over time. Additionally, an ELISA was conducted to verify the specificity of the expression. Moving forward, the team hopes to further develop the enteric encapsulation system and begin designing studies for in-vivo models to assess the systemic effects and evaluate therapeutic efficacy. In order to reach market, we will perform bench testing and animal testing for IND (Investigation New Drug) approval to begin clinical trials. This would be followed by a BLA (Biologics License Application) submission for final FDA approval to market our product.

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