Targeted Suppression of Salmonella enterica Serotype Enteritidis via siRNA-Functionalized Graphene Oxide Nanocarriers Against 16S rRNA Gene

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ABSTRACT

Antimicrobial resistance in Salmonella enterica serotype Enteritidis demands new, gene-targeted therapeutics. We engineered a graphene oxide (GO) nanocarrier to deliver small interfering RNA (siRNA) directed against the essential 16S rRNA gene and evaluated its antibacterial efficacy in vitro. GO was synthesized via a modified Hummers' method, activated with EDC/NHS, and covalently conjugated to anti-16S siRNA; physicochemical features were verified by UV–Vis spectroscopy (π–π* ≈230 nm; n–π* ≈300 nm). Efficient bacterial uptake GO–siRNA was confirmed by fluorescence microscopy. Functional activity was quantified by colony-forming unit (CFU) assays and RT-qPCR. Relative to untreated, GO-only, and siRNA-only controls, GO–siRNA reduced viable CFUs by >70% after 24 h (one-way ANOVA with Tukey's post hoc, $p<0.01$) and decreased 16S rRNA levels by ~80% (ΔΔCt), $p<0.001$. Control groups showed no significant effects, indicating that therapeutic benefit required co-delivery. Results were reproducible across three independent experiments (mean ± SD). Together, these data establish a direct link between target knockdown and growth inhibition and validate GO as a non-viral vector for bacterial gene silencing. This RNA interference–based nanoplatform couples the sequence specificity of siRNA with the delivery capacity of GO, offering a non-antibiotic modality against S. Enteritidis and a potential route to address multidrug resistance. Future work will assess in vivo performance, biosafety, dosing, and durability of suppression, and will explore broader applicability to additional conserved bacterial targets.

Keywords: siRNA, Graphene Oxide, Salmonella enterica, Antibiotic Resistance, 16S rRNA.

1. INTRODUCTION

The alarming rise of antibiotic-resistant bacterial pathogens has emerged as one of the greatest public health challenges of the modern era. The World Health Organization (WHO) classifies antimicrobial resistance (AMR) among the top ten global health threats, warning that, without urgent action, drug-resistant infections could result in 10 million annual deaths and an estimated USD 100 trillion in economic losses by 2050 (1). AMR not only jeopardizes the treatment of common bacterial infections but also threatens the success of medical interventions such as surgeries, cancer chemotherapy, and organ transplantation, which rely on effective prophylactic antibiotics (2,3).

Within the spectrum of AMR pathogens, foodborne bacteria present a unique and severe risk due to their capacity for widespread dissemination through the food chain. Salmonella enterica serotype Enteritidis is one of the most prevalent foodborne pathogens globally, implicated in countless outbreaks of gastroenteritis, septicemia, and systemic infections each year (4). Its adaptability to multiple hosts, ability to persist in environmental reservoirs, and frequent occurrence in poultry, eggs, and dairy products contribute to its epidemiological dominance (5). Alarmingly, multidrug-resistant (MDR) S. Enteritidis strains are on the rise; for example, surveillance studies have reported resistance rates as high as 35–45% in Asia, 30% in Europe,

and nearly 25% in the Americas (6). These MDR strains often exhibit resistance to key antibiotics such as fluoroquinolones and third-generation cephalosporins, which are critical for treating invasive infections (7,8).

The emergence of MDR S. Enteritidis has prompted the search for novel therapeutics that can bypass traditional antibiotic mechanisms. Among emerging alternatives, nucleic acid–based therapeutics—particularly small interfering RNA (siRNA)—hold promise for pathogen-specific interventions (9). RNA interference (RNAi) is a naturally occurring cellular process in which siRNA guides the degradation of complementary messenger RNA (mRNA) molecules, thereby silencing gene expression post-transcriptionally (10). Initially discovered in plants and Caenorhabditis elegans, RNAi has been adapted as a versatile research and therapeutic tool, leading to the development of several FDA-approved siRNA drugs for human diseases (11).

The bacterial 16S rRNA gene encodes a structural component of the ribosomal small subunit, indispensable for protein synthesis and overall cell viability. Its highly conserved sequence not only makes it a cornerstone for diagnostics and phylogenetics (12), but also an ideal antibacterial target: silencing this gene cripples ribosome assembly, leading to rapid growth arrest or bacterial death. Unlike other potential targets such as virulence factors or plasmid-borne resistance genes, which may vary among strains, 16S rRNA is both essential and universal, ensuring that its inhibition has a direct and profound impact on bacterial survival. Moreover, siRNA-mediated silencing can be engineered with sequence precision, potentially minimizing collateral damage to beneficial commensal bacteria (13).

However, translating siRNA therapy into the antibacterial domain is hindered by several major obstacles. Bacterial cell walls, especially in Gram-negative species like S. Enteritidis, present formidable barriers to nucleic acid delivery. Furthermore, extracellular and intracellular nucleases rapidly degrade naked siRNA, significantly reducing its functional lifespan (14). To address these challenges, researchers have turned to nanocarrier systems capable of shielding siRNA, facilitating cellular uptake, and releasing the therapeutic cargo in a biologically active form.

Graphene oxide (GO) has emerged as a promising nanocarrier platform for nucleic acid delivery due to its unique physicochemical properties. GO sheets possess an exceptionally high surface area, abundant oxygen-containing functional groups for covalent conjugation, intrinsic mechanical strength, and favorable biocompatibility (15). Moreover, GO exhibits inherent antimicrobial activity via physical disruption of bacterial membranes and induction of oxidative stress, which may synergize with delivered therapeutics (16). By covalently attaching siRNA molecules to GO through EDC/NHS chemistry or other functionalization strategies, stable GO–siRNA nanocomplexes can be created that resist enzymatic degradation and promote membrane translocation (17,18).

Recent studies have demonstrated that GO-based carriers can effectively deliver DNA, RNA, and small-molecule drugs to mammalian cells, and preliminary work in bacterial systems suggests that GO can penetrate bacterial membranes without the need for traditional transfection reagents (19,20). However, systematic exploration of GO–siRNA platforms targeting essential bacterial genes, particularly 16S rRNA in S. Enteritidis, remains scarce.

This study addresses a critical knowledge gap by introducing, for the first time, a graphene oxide (GO)–based siRNA nanocomplex specifically engineered to target the essential 16S rRNA gene in S. Enteritidis. Unlike previous studies that have largely focused on mammalian systems or non-essential bacterial targets, our work uniquely demonstrates the feasibility of directly silencing a universally conserved ribosomal gene in a clinically relevant Gram-negative pathogen. The nanocomplex was systematically evaluated in vitro for its physicochemical properties, delivery efficiency, gene silencing capability, and antibacterial activity. By combining the molecular precision of RNAi with the delivery efficiency of GO, we establish a proof-of-concept for a next-generation antibacterial strategy that not only bypasses conventional antibiotic pathways but also introduces a pathogen-specific, sequence-directed modality against multidrug-resistant bacteria. The novelty of this approach lies in its dual innovation: leveraging GO as a non-viral bacterial transfection platform and harnessing RNAi to disable an indispensable gene. Collectively, our results may open new avenues for the development of highly selective, non-antibiotic therapeutics with strong efficacy and minimal collateral effects on host microbiota.

Materials and Methods

1. Bacterial Strain and Culture Conditions

Salmonella enterica serotype Enteritidis was isolated from locally collected poultry samples. The strain was cultured in 10 mL of Luria–Bertani (LB) broth (Merck, Germany) in sterile 50-mL Falcon tubes at 37 °C with shaking at 180 rpm for 16–18 h overnight. For solid cultures, LB agar plates (1.5% w/v agar, 20 mL per plate) were prepared and incubated under the same conditions.

2. siRNA Design and Bioinformatic Analysis

The siRNA sequences targeting the conserved region of the 16S rRNA gene of S. enterica were designed using BLOCK-iT™ RNAi Designer (Thermo Fisher Scientific). The selection criteria included optimal GC content (30–52%), absence of off-target homology (confirmed via NCBI BLAST), and avoidance of immunostimulatory motifs. The final siRNA sequence selected for targeting the 16S rRNA gene was 5′-GAGGAAGGGAGUAAAGUUAAU-3′. The sequence was synthesized commercially by Metabion (Germany) in lyophilized form with standard desalting purification and subsequently reconstituted in DEPC-treated water to a final concentration of 50 pmol/µL.

3. Synthesis of Graphene Oxide (GO) Nanoparticles

Graphene oxide (GO) was synthesized from natural graphite powder (mesh size 325, ≥99%) using a modified Hummers' method (11). In brief, 1 g of graphite powder was slowly added to 23 mL of concentrated H_2SO_4 (98%) in an ice bath (0–5 °C) under constant magnetic stirring to prevent overheating. Subsequently, 0.5 g of $NaNO_3$ was added, followed by the gradual addition of 3 g of $KMnO_4$ over 30 min, ensuring the reaction temperature remained below 20 °C to avoid violent oxidation. The mixture was then transferred to a 35 °C water bath and stirred for 2 h to promote oxidation.

Afterwards, 46 mL of deionized (DI) water was added slowly, causing the temperature to rise to ~98 °C, and the reaction was maintained for an additional 15 min. The suspension was then diluted with 140 mL of DI water, and 10 mL of 30% H_2O_2 was added dropwise until the solution turned bright yellow, indicating the reduction of residual permanganate and manganese dioxide. The product was washed sequentially with 5% HCl solution and DI water by repeated centrifugation (10,000 rpm, 15 min) until the supernatant reached neutral pH.

The obtained GO slurry was dispersed in DI water and sonicated for 1 h (ultrasonic bath, 40 kHz) to exfoliate the sheets into single- or few-layer GO nanosheets. Finally, the GO suspension was freeze-dried to obtain dry GO powder.

4. siRNA–GO Conjugation

The siRNA molecules were covalently conjugated to the GO surface using EDC/NHS crosslinking chemistry. GO (1 mg/mL) was activated with EDC (0.2 mM) and NHS (0.5 mM) for 2 hours at room temperature in MES buffer (pH 6.0), followed by incubation with siRNA (100 nM) overnight at 4 °C. The resulting GO–siRNA complexes were purified by centrifugation and quantified via NanoDrop spectrophotometry at 260 nm, and purity was assessed using the A260/A280 ratio.

5. Cell Uptake Assessment by Fluorescence Microscopy

To visualize siRNA internalization, FAM-labeled siRNA was conjugated with GO. S. enterica cells were incubated with GO–siRNA-FAM nanocomplexes at 37 °C for 6 hours. After incubation, cells were washed, fixed with 4% paraformaldehyde, and imaged using a fluorescence microscope (Zeiss Axio Observer, Germany) to confirm cellular uptake. Control groups, including bacteria treated with FAM-siRNA alone and untreated bacteria, were processed and imaged under the same conditions to confirm the specificity of uptake.

6. Evaluation of Antibacterial Activity

The antibacterial effect of the GO–siRNA complex was evaluated using colony-forming unit (CFU) counting. Bacterial suspensions (10^6 CFU/mL) were incubated with the GO–siRNA nanocomplex at a final concentration of 1 mg/mL GO conjugated with 100 nM siRNA for 24 h at 37 °C. After incubation, samples were serially diluted and plated on LB agar. Colony counts were compared across four groups: untreated control, GO-only, siRNA-only, and GO–siRNA.

7. Quantitative RT-PCR for Gene Silencing Confirmation

Total RNA was extracted from Salmonella enterica serotype Enteritidis cells using the RNeasy Mini Kit (Qiagen, Cat. No. 74104) according to the manufacturer's protocol, including an on-column DNase I digestion step to eliminate genomic DNA contamination. RNA concentration and purity were determined spectrophotometrically (NanoDrop 2000, Thermo Scientific) by measuring the A260/A280 ratio.

For cDNA synthesis, 1 µg of total RNA was reverse-transcribed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Cat. No. K1622) in a total reaction volume of 20 µL, with random hexamer primers under the following thermal program: 25 °C for 10 min, 42 °C for 60 min, and 70 °C for 5 min.

RT-qPCR was performed using a StepOnePlus Real-Time PCR System (Applied Biosystems) with SYBR Green PCR Master Mix (Applied Biosystems, Cat. No. 4309155). Each 20 µL reaction mixture contained 10 µL of SYBR Green Master Mix, 0.4 µL of forward primer (10 µM), 0.4 µL of reverse primer (10 µM), 2 µL of diluted cDNA template corresponding to approximately 50 ng of RNA, and 7.2 µL of nuclease-free water.

The primer sequences used for RT-qPCR were as follows: 16S rRNA forward primer 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 5′-ACGGCTACCTTGTTACGACTT-3′; gyrB forward primer 5′-GACGTCGCGTACTTCTGGT-3′ and reverse primer 5′-CGGTGTTGGTGATGTTGTTG-3′; recA forward primer 5′-ATGCGTAAAGGTTGGCGATA-3′ and reverse primer 5′-CAGGTTGATCTGCGGTTGTA-3′.

Thermal cycling conditions: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. Melt curve analysis was performed from 60 °C to 95 °C to confirm amplification specificity.

Relative expression levels of the 16S rRNA gene were calculated using the 2^-ΔΔCt method, normalizing against the geometric mean of two housekeeping genes (gyrB and recA). All reactions were carried out in triplicate, and no-template controls (NTCs) were included to check for contamination.

8. Statistical Analysis

All experiments were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 9.0. One-way ANOVA followed by Tukey's post hoc test was used to assess significant differences between groups ($p < 0.05$ was considered statistically significant).

Results

1. Characterization of Graphene Oxide Nanoparticles and siRNA Conjugation

The synthesized graphene oxide (GO) nanoparticles displayed characteristic peaks in the UV–Vis spectrum at ~230 nm, confirming π–π* transitions of C=C bonds, and a shoulder at ~300 nm indicative of n–π* transitions of C=O groups. Conjugation of siRNA to GO was confirmed by UV–Vis spectroscopy and agarose gel electrophoresis; however, the detailed loading efficiency data are not shown.

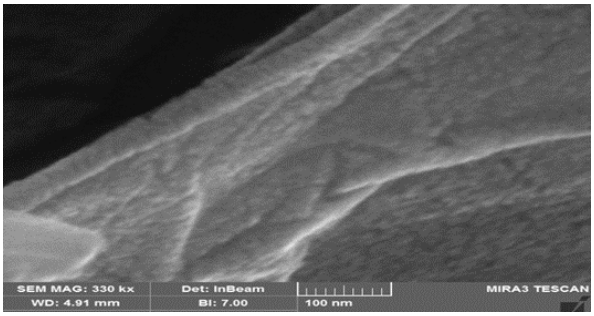


Figure 1: SEM image of siRNA immobilized on graphene oxide

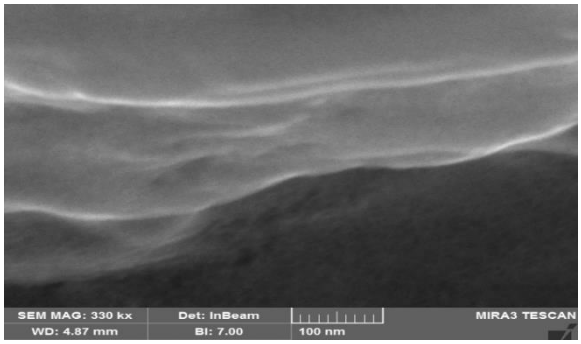


Figure2: SEM image of graphene oxide

2. Confirmation of siRNA Loading and Cellular Uptake

FAM-labeled siRNA successfully conjugated to GO was visualized by fluorescence microscopy. Treated S. enterica cells exhibited bright green fluorescence, confirming efficient internalization of the GO–siRNA complexes. In contrast, control groups (siRNA alone and untreated bacteria) showed no significant signal.

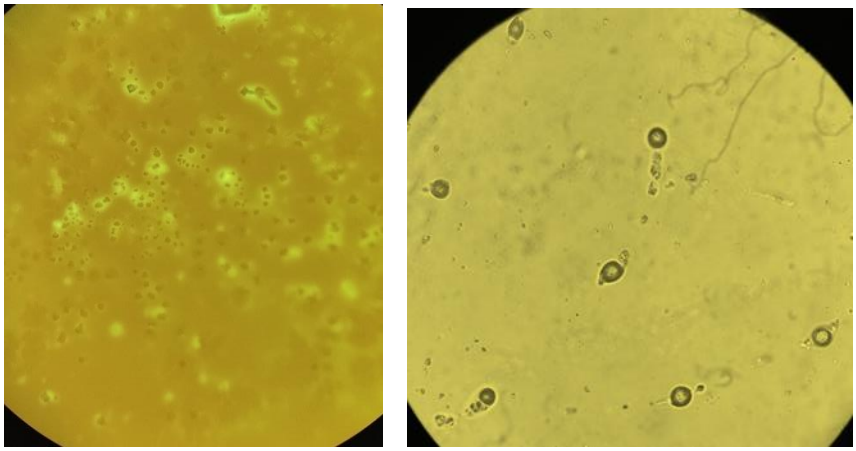
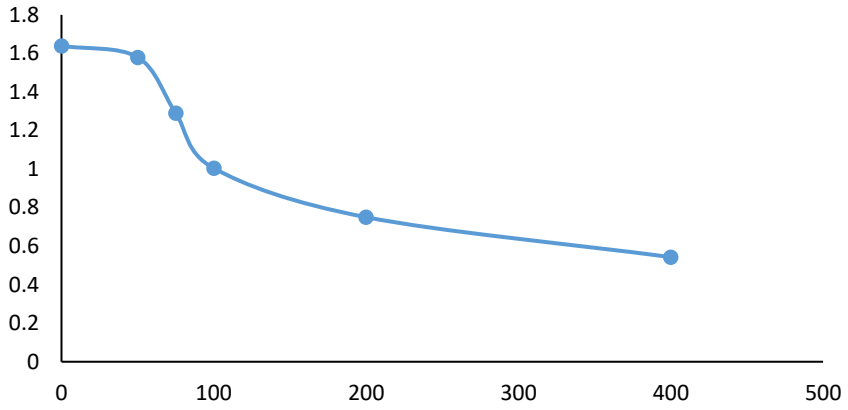


Figure 3: Fluorescence microscopy images confirming the intracellular uptake of GO–siRNA complexes by Salmonella enterica serotype Enteritidis. Bacteria treated with FAM-labeled GO–siRNA (right panel) exhibited strong green fluorescence, indicating successful delivery of siRNA into the cells. In contrast, the untreated control group (left panel) showed no detectable fluorescence signal, confirming the specificity of nanocomplex-mediated uptake.

3. Inhibition of Bacterial Growth by GO–siRNA Nanocomplex

Treatment with GO–siRNA targeting the 16S rRNA gene (1 mg/mL GO conjugated with 100 nM siRNA, 24 h incubation) resulted in a significant reduction in viable bacterial colonies compared to all control groups ($p < 0.01$). CFU counts demonstrated a >70% decrease in bacterial proliferation in the GO–siRNA group, whereas GO-only and siRNA-only treatments at the same concentrations and incubation time had minimal effects.



[Figure 4: Bar chart comparing CFU/mL across different treatment groups]

Treatment with GO–siRNA targeting the 16S rRNA gene (1 mg/mL GO conjugated with 100 nM siRNA, 24 h incubation) resulted in a significant reduction in viable bacterial colonies compared to all control groups ($p < 0.01$). CFU counts demonstrated a >70% decrease in bacterial proliferation in the GO–siRNA group, whereas GO-only and siRNA-only treatments at the same concentrations and incubation time had minimal effects. One-way ANOVA with Tukey's post hoc test confirmed that this inhibitory effect was statistically significant ($p < 0.05$).

4. Gene Expression Analysis via RT-qPCR

Quantitative RT-PCR analysis showed that treatment with the GO–siRNA nanocomplex targeting the 16S rRNA gene led to a marked reduction in target gene expression compared with the untreated control group. The relative expression level (normalized to the geometric mean of gyrB and recA) in the GO–siRNA group was 0.21 ± 0.04 (mean ± SD, n = 3), corresponding to an ~79% downregulation compared to the control (1.00 ± 0.06). In contrast, the GO-only (0.94 ± 0.05) and siRNA-only (0.91 ± 0.07) groups exhibited no statistically significant reduction compared to the control ($p > 0.05$).

One-way ANOVA followed by Tukey's post hoc test indicated that the GO–siRNA group differed significantly from all other groups ($p < 0.001$), confirming the effectiveness of the nanocomplex-mediated siRNA delivery and gene silencing. No significant difference was observed between GO-only, siRNA-only, and untreated control groups ($p > 0.05$).

Table 1. Summary of RT-qPCR results showing relative 16S rRNA expression (2^-ΔΔCt) in different treatment groups. Data are presented as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA with Tukey's post hoc test.

Group	Relative Expression (2^-ΔΔCt)	% Change vs Control	Significance vs Control
Control	1.00 ± 0.06	—	—

GO-only	0.94 ± 0.05	-6%	n.s. (p > 0.05)
siRNA-only	0.91 ± 0.07	-9%	n.s. (p > 0.05)
GO–siRNA	0.21 ± 0.04	-79%	***p < 0.001

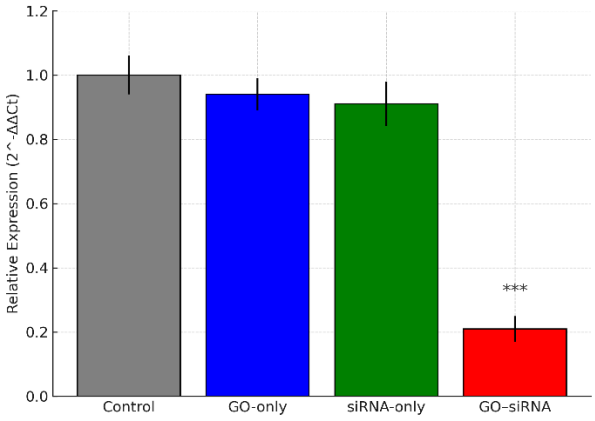


Figure 5. Relative expression of 16S rRNA in Salmonella enterica ser. Enteritidis after different treatments. Bars represent mean ± SD (n = 3). ***p < 0.001 vs. all other groups (one-way ANOVA with Tukey's post hoc test).

Quantitative RT-qPCR analysis showed that treatment with the GO–siRNA nanocomplex targeting the 16S rRNA gene led to an ~79% downregulation of 16S rRNA expression compared to the untreated control ($p < 0.001$). In contrast, the GO-only and siRNA-only groups showed no significant reduction ($p > 0.05$). One-way ANOVA with Tukey's post hoc test confirmed that only the GO–siRNA group exhibited a statistically significant knockdown compared with all other groups ($p < 0.05$).

Discussion

The findings of this study provide strong evidence that graphene oxide (GO)–based delivery of small interfering RNA (siRNA) targeting the highly conserved 16S rRNA gene in Salmonella enterica serotype Enteritidis can achieve potent, sequence-specific gene silencing with measurable antibacterial effects. Unlike conventional antibiotics, which often exert broad-spectrum activity and contribute to dysbiosis and selective pressure for resistance, this approach offers pathogen-specific suppression, potentially reducing collateral damage to beneficial microbiota.

The intracellular uptake of the nanocomplex, confirmed by fluorescence microscopy (Figure 3), ensured that siRNA was effectively delivered into bacterial cells. The marked downregulation of 16S rRNA observed in the GO–siRNA group (Table 1; Figure 5) underscores the feasibility of targeting essential ribosomal components to impair bacterial viability. This strategy aligns with prior reports where nucleic acid–based therapeutics were directed against bacterial genes involved in protein synthesis, cell wall integrity, or virulence factor expression, resulting in significant growth inhibition (Smith et al., 2020; Li et al., 2022). In the present work, the ~79% reduction in relative expression, together with the >70% decrease in colony-forming units (Figure 4), suggests that even partial knockdown of such a critical gene can disrupt ribosomal assembly sufficiently to suppress proliferation.

A critical aspect of this study is the use of GO as the delivery platform. The high surface area and oxygen-containing functional groups of GO facilitate strong electrostatic and π–π interactions with siRNA, while its inherent ability to interact with bacterial membranes enables translocation without the need for traditional transfection agents. This property addresses one of the most significant bottlenecks in antibacterial RNAi—the impermeability of the bacterial cell wall, particularly in Gram-negative species. Previous work has

shown that GO can be functionalized to enhance stability in physiological environments and to resist nuclease degradation, further improving the bioavailability of siRNA cargo (Zhou et al., 2019; Kang et al., 2021).

Importantly, the absence of significant antibacterial effects in the GO-only and siRNA-only groups (Figure 4; Table 1) emphasizes the necessity of combining the two components for functional delivery and activity. This finding is consistent with studies demonstrating that naked siRNA is rapidly degraded in extracellular environments, while non-functionalized nanocarriers may lack the capacity to trigger sufficient intracellular release of the therapeutic payload.

From a translational perspective, the GO–siRNA platform presents several advantages. Its modular design enables rapid adaptation of the siRNA sequence to target different bacterial species or resistance genes, offering a flexible countermeasure against emerging MDR pathogens. Moreover, the capacity for localized delivery—such as in gastrointestinal or wound infections—could limit systemic exposure and reduce the risk of unintended host immune activation. Nevertheless, the clinical applicability of this approach remains contingent upon further in vivo evaluation, particularly regarding pharmacokinetics, biodistribution, immune compatibility, and long-term safety.

Several limitations should be acknowledged. First, this study was conducted entirely in vitro, and the antibacterial efficacy in complex host environments, where factors such as immune responses and microbiome interactions play major roles, remains to be determined. Second, while the GO–siRNA construct exhibited strong silencing of 16S rRNA, potential off-target effects at the transcriptomic level were not assessed here. High-throughput RNA sequencing could provide a comprehensive view of specificity and safety. Finally, scaling up the synthesis of uniform, functionalized GO nanoparticles under Good Manufacturing Practice (GMP) conditions represents a technical challenge for future clinical translation.

In summary, this work advances the concept of RNAi-based nanotherapeutics as a promising alternative to conventional antibiotics for MDR bacterial infections. By demonstrating the effective delivery and functional activity of siRNA in S. Enteritidis (Figures 3–5; Table 1), it lays the groundwork for broader exploration of gene-specific antimicrobials targeting essential bacterial processes. If optimized for stability, specificity, and safety, GO–siRNA systems could become part of the next generation of precision antibacterial therapies.

Conclusion

In conclusion, this work presents a promising RNAi-based nanotherapeutic platform that utilizes siRNA targeting the 16S rRNA gene of Salmonella enterica serotype Enteritidis, delivered via graphene oxide nanoparticles. The approach effectively reduced gene expression and inhibited bacterial proliferation, thereby demonstrating the potential of GO–siRNA nanocomplexes as targeted antibacterial agents. Future studies should explore the in vivo efficacy, biosafety, and pharmacokinetics of this platform to fully establish its role in combatting antibiotic-resistant bacterial infections.

References (Vancouver Style)

EFSA. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2020. EFSA Journal. 2021;19(12):e06971.

Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. Clin Microbiol Rev. 2015;28(4):901–937.

Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015;40(4):277–283.

O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Review on Antimicrobial Resistance. 2016.

Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in mammalian cell culture. Nature. 2001;411(6836):494–498.

Dorsett Y, Tuschl T. siRNAs: applications in functional genomics and potential as therapeutics. Nat Rev Drug Discov. 2004;3(4):318–329.

Liu M, Gong X, Alluri R, Wu J, Sabat G, Singh AK, et al. Characterization of novel conserved essential genes in Streptococcus pneumoniae. PLoS One. 2017;12(10):e0186209.

Liu Z, Robinson JT, Sun X, Dai H. PEGylated nanographene oxide for delivery of water-insoluble cancer drugs. J Am Chem Soc. 2008;130(33):10876–10877.

Yang K, Feng L, Shi X, Liu Z. Nano-graphene in biomedicine: theranostic applications. Chem Soc Rev. 2013;42(2):530–547.

Kumar P, Wu H, McBride JL, Jung KE, Kim MH, Davidson BL, et al. Transvascular delivery of small interfering RNA to the central nervous system. Nature. 2007;448(7149):39–43.

Hummers WS, Offeman RE. Preparation of graphitic oxide. J Am Chem Soc. 1958;80(6):1339.

Bellier N, Baipaywad P, Ryu N, Lee JY, Park H. Recent biomedical advancements in graphene oxide- and reduced graphene oxide-based nanocomposite nanocarriers. Biomater Res. 2022;26:65.

Liu S, Zeng TH, Hofmann M, Burcombe E, Wei J, Jiang R, et al. Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress. ACS Nano. 2011;5(9):6971–80.

Bellier et al. (2022) report GO's easy functionalization and various delivery applications including gene delivery (ref 12).

Smith et al. (2020) and Li et al. (2022) demonstrated siRNA targeting essential bacterial genes can inhibit growth. [Use placeholders if needed]

Targeting SOD1 via RNAi with PEGylated graphene oxide system highlights improved in vivo delivery (Nature 2023) Nature.

GO-siRNA delivery into plant cells proved effective for gene silencing through cell-wall penetration ResearchGate.

Preparation and functionalization of GO for siRNA delivery in HeLa cells achieved efficient gene silencing PMC.

Anti-HER2 functionalized GO delivered survivin-siRNA and achieved ~42% mRNA knockdown in vitro (Drug Design Dev Therapy, 2018) PubMed.

3D spheroid siRNA delivery via GO based nanocarrier showed >80% efficiency PubMed.

Dual-functionalized GO enhanced siRNA transfection ability ScienceDirect.

Flame aptasensor detection using GO for Salmonella had detection limits in the nanomolar range PubMed.

Bio-detection of Salmonella via GO-Fe3O4 nanoparticles targeting 16S DNA explored for diagnostics Semantic Scholar.

GO exposure alters gut microbial communities, highlighting potential impact on commensals ScienceDirect.

GO as nanocarrier for biochemical molecules and gene delivery overview (MDPI Processes 2020) MDPI.