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Computational Design and Biosecurity Assessment of a Dual-Function *E. coli* Circuit for *S. aureus* Sensing and Targeted Lysostaphin Therapy

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Abstract — Engineered therapeutic microbes represent a promising strategy to combat antibiotic-resistant pathogens such as *Staphylococcus aureus*; however, the absence of predictive biosafety frameworks remains a major barrier to their clinical translation. Here, we present an *in silico* design of a dual-function *Escherichia coli* genetic circuit integrating pathogen sensing via the AgrC/AgrA system, targeted lysostaphin-mediated killing, and a tightly regulated MazE/MazF kill switch for biocontainment. A hybrid computational framework was developed to systematically evaluate functional performance and biosafety risks, combining ordinary differential equations (ODEs) for deterministic dynamics with Gillespie-based stochastic simulations to capture intrinsic molecular noise. The model predicts robust and responsive bacteriolytic activity upon detection of *S. aureus* signaling molecules, while maintaining controlled system behavior under varying conditions. Additionally, critical thresholds for kill-switch leakiness were identified, and evolutionary stability was assessed through failure-time analysis. Collectively, this study provides a quantitative framework for integrating circuit design with biosafety evaluation, offering practical guidance for the development and regulatory assessment of engineered therapeutic microbes. However, these computational predictions require future experimental validation to confirm circuit efficacy and safety.

Keywords—synthetic biology; *in silico* risk assessment; dual-function genetic circuit; *staphylococcus aureus*; biosecurity

I. INTRODUCTION

The rise of antibiotic-resistant pathogens has driven a paradigm shift in therapeutic strategies, with engineered microbial therapeutics emerging as a promising alternative to conventional antibiotics. Engineered bacteria can sense disease-associated signals and respond by producing therapeutic agents; this synthetic biology approach enables targeted and dynamic intervention in disease processes. This

approach exploits the inherent advantages of microbial systems, such as rapid growth, amenability to genetic manipulation, and the capacity to colonize specific niches within the host, offering precision and sustained therapeutic effects that conventional drugs often lack [1, 2].

Synthetic biology enables the construction of complex genetic circuits that integrate sensor modules and regulatory elements, allowing microbes to detect biomarkers and actuate

therapeutic responses in a controlled manner. These engineered systems have demonstrated potential in treating gastrointestinal disorders, metabolic diseases, infections, and cancer, leveraging biosensors and dynamic control elements to maximize efficacy while minimizing off-target effects [1, 3]. However, the application of engineered microbes in clinical or environmental settings raises significant safety concerns. Unintended release, horizontal gene transfer, and uncontrolled proliferation of genetically modified organisms could pose ecological and health risks; these concerns underscore the importance of robust biocontainment strategies and proactive risk assessment frameworks. Recent advances in kill switches, toxin-antitoxin systems, and containment designs reflect efforts to mitigate these risks and align microbial therapeutics with rigorous biosafety standards [4].

The aim of this study is twofold: (i) to design a dual-function genetic circuit in *Escherichia coli* for targeted therapeutic activity and controlled biocontainment, and (ii) to develop an *in silico* framework for the proactive assessment of functional, toxic, stochastic, and evolutionary risks, providing predictive safety guidelines before laboratory implementation.

II. METHODS

A. Circuit design

The proposed system is engineered to operate within an *E. coli* host. It consists of three integrated modules: (i) a sensing module based on quorum sensing components (AgrC/AgrA) for detection of *Staphylococcus aureus* [5], (ii) a therapeutic module encoding lysostaphin for targeted antibacterial activity [6], [7], and (iii) a kill switch module based on the MazE/MazF toxin-antitoxin system under the control of an arabinose-inducible promoter (pBAD) [8]. The circuit is designed to enable conditional activation of therapy and externally controlled termination.

B. Mathematical modeling

A system of coupled ordinary differential equations (ODEs) was used to model gene expression, regulation, and degradation dynamics across the circuit [9], [10], [11] (Table

1). Table 1: State variables used in the mathematical modeling of the genetic circuit.

Table I. State variables

Symbol	Description
A	Active AgrA
L	lysostaphin concentration
M	MazF toxin
E	MazE antitoxin
S	External signal
Ara	Arabinose inducer
B	<i>S. aureus</i> population

C. Core equations

Sensing module:

$$\frac{dA}{dt} = \alpha_A \cdot \frac{s^n}{k_s^n + s^n} - \delta_A \cdot A$$

Therapeutic module:

$$\frac{dL}{dt} = \alpha_L \cdot \frac{A^n}{K_A^n + A^n} - \delta_L \cdot L$$

Kill switch:

$$\frac{dM}{dt} = a_M \cdot \frac{Ara^n}{k_{Ara}^n + Ara^n} - \delta_M \cdot M$$

$$\frac{dE}{dt} = a_E - \delta_E \cdot E$$

Bacterial dynamics:

$$\frac{dB}{dt} = r \cdot B \left(1 - \frac{B}{k}\right) - \gamma \cdot L \cdot B$$

In order to guarantee reproducibility, Table 2 lists important model parameters together with their matching values and initial conditions. Representative kinetic rates were chosen for the parameter values. To conduct a qualitative proof-of-concept evaluation in cases where direct empirical data were not available, values were presumed to be within physiologically plausible ranges.

Table II. Summary of key parameter values and initial conditions.

Parameter(s)	Description	Value(s)	Unit	Justification
k₁, k₂	AgrA activation and deactivation rates	1.0, 0.5	min ⁻¹	Assumed*
α_L, α_M, γ_E	Max transcription/production rates (Lysostaphin, MazF, MazE)	2.0, 3.0, 1.0	nM·min ⁻¹	Assumed*
β_L, β_M	Translation rates (Lysostaphin, MazF)	2.0, 2.0	min ⁻¹	Assumed*
δ_L, δ_F, δ_E	Degradation rates (Lysostaphin, MazF, MazE)	0.3, 0.3, 0.2	min ⁻¹	Assumed*
K_L, k_{bind}	Half-activation constant, Complex binding rate	0.5, 1.0	nM, nM ⁻¹ min ⁻¹	Assumed*
μ, growth_m	Mutation rate, Mutant relative growth rate	10 ⁻³ , 1.2	gen ⁻¹ , N/A	Literature
A₀, L₀, M₀, F₀	Initial states (Sensing, Therapy, Toxin)	0	nM	Baseline
E₀	Initial state (Antitoxin MazE)	1.0	nM	Basal state

*Values were assumed within biologically plausible ranges to perform a qualitative proof of concept assessment, in the absence of direct empirical data for this specific circuit.

D. Stochastic simulation

To account for intrinsic molecular noise, stochastic simulations were performed using the Gillespie algorithm [12], [13], [14]. A total of 200 independent Monte Carlo simulation replicates ($n = 200$) were run to capture intrinsic variability and robustly assess the probability of unintentional kill switch activation.

E. Sensitivity analysis

A global sensitivity analysis was conducted by varying key parameters, particularly MazF production rate (α_M) and lysostaphin expression rate (α_L). Results were visualized using response curves and heatmaps [15].

F. Leakiness analysis

Basal expression of MazF was simulated under non-induced conditions ($Ara = 0$). The maximum allowable leakiness was determined by identifying the threshold at which MazF exceeds the toxicity limit [16].

G. Evolutionary stability modeling

A simplified population model was used to simulate competition between functional and mutant cells. Circuit failure time was defined as the point at which mutant cells outcompete functional cells [17, 18].

H. Failure criteria definition

The specific failure conditions and their corresponding definitions are summarized in Table III.

Table III. Summary of the predefined failure criteria and their corresponding definitions used to evaluate the genetic circuit.

Failure Type	Definition
Toxicity failure	$M > M_{\text{threshold}}$
Leakiness failure	MazF activation without induction
Stochastic failure	Noise-induced MazF activation
Evolutionary failure	Mutants dominate population
Functional failure	$L < L_{\text{eff}}$ or insufficient bacterial killing

III. RESULTS

A. Circuit dynamics

Time-course simulations (Figure 1) demonstrated that the sensing module effectively activates downstream components in response to *S. aureus*-associated signals. Upon activation, lysostaphin production increased significantly, leading to a strong therapeutic response. In contrast, the kill switch

remained inactive under normal conditions and was only triggered upon external induction.

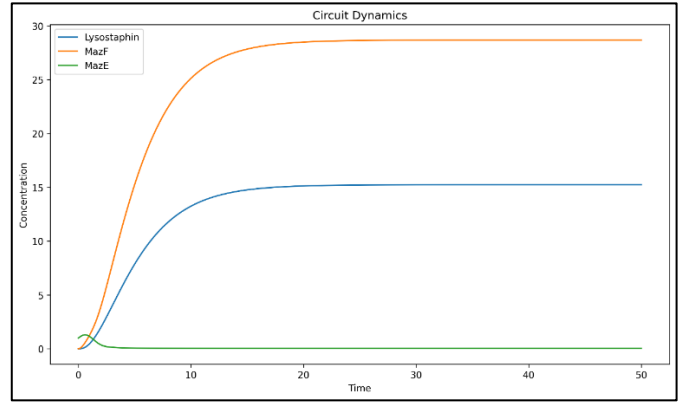


Figure 1. Time-course simulation of the genetic circuit dynamics. The plot illustrates the expression levels of lysostaphin, MazF, and MazE over time following system activation.

B. Sensitivity analysis

Sensitivity analysis (Figure 2) revealed that the MazF production rate (α_M) is a critical parameter governing system behavior. Small increases in (α_M) led to rapid activation of the kill switch, while lower values maintained system stability. This highlights the importance of precise tuning of toxin expression.

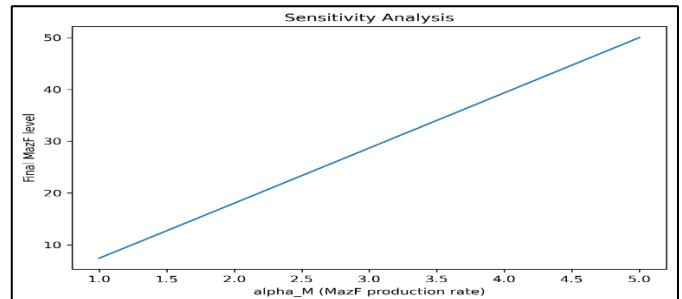


Figure 2. Sensitivity analysis demonstrating the effect of the MazF production rate (α_M) on the final MazF level. The linear increase highlights the critical threshold for kill switch activation.

C. Operational safety and stability

Heatmap analysis (Figure 3) identified distinct regions of safe and unsafe operation. Stable regions were characterized by low MazF expression and sustained therapeutic activity, whereas unstable regions exhibited premature activation of the kill switch.

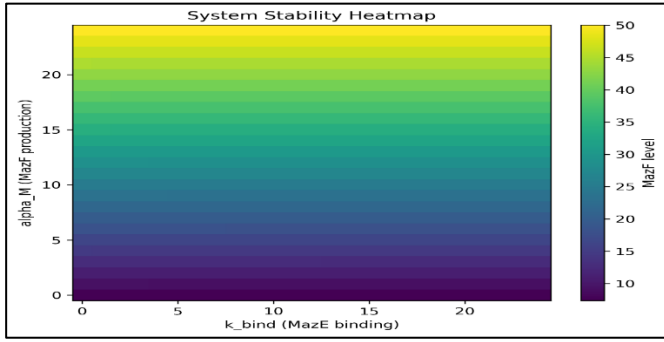


Figure 3. System stability heatmap illustrating safe and unsafe operational regions. The gradient displays MazF levels across varying rates of toxin production (α_m) and antitoxin binding (k_{bind}).

D. Stochastic robustness and failure probability

To evaluate the robustness of the kill switch under intrinsic molecular noise, multiple stochastic simulations were performed using the Gillespie algorithm. A failure event was defined as spontaneous activation of MazF exceeding a predefined toxicity threshold in the absence of induction. The distribution of final MazF levels (Figure 4) shows variability across stochastic trajectories. The calculated failure probability indicates the likelihood of unintended system collapse due to intrinsic noise.

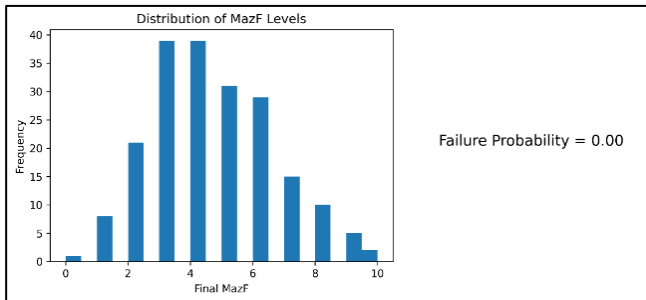


Figure 4. Distribution of MazF levels across stochastic simulations and calculated probability of failure under leakiness conditions.

E. Leakiness threshold

To assess the impact of basal expression on system safety, leakiness analysis was conducted by varying the MazF production rate under non-induced conditions. As shown in Figure 5, increasing leakiness leads to a gradual rise in MazF levels.

A critical threshold was identified beyond which MazF accumulation exceeds the toxicity limit, resulting in unintended system collapse. This threshold defines the maximum allowable basal expression for safe circuit operation.

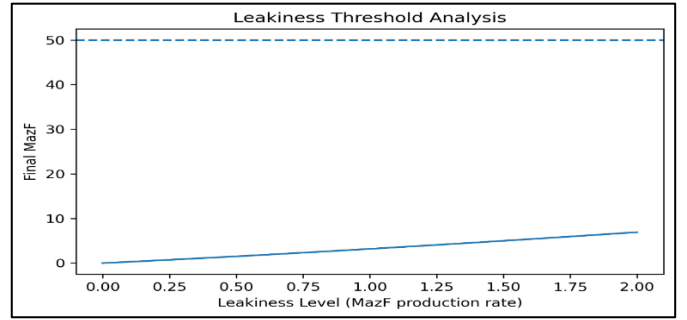


Figure 5. Leakiness threshold analysis showing the relationship between basal MazF production and final toxin levels. The dashed line represents the toxicity threshold.

F. Evolutionary stability

Evolutionary simulations revealed that mutant cells gradually accumulate and eventually dominate the population due to their growth advantage. As shown in Figure 6, the population of mutant cells surpasses functional cells after a finite number of generations, defining the circuit failure time. The estimated failure time depends on mutation rate and growth advantage, highlighting the importance of minimizing metabolic burden and genetic instability in circuit design.

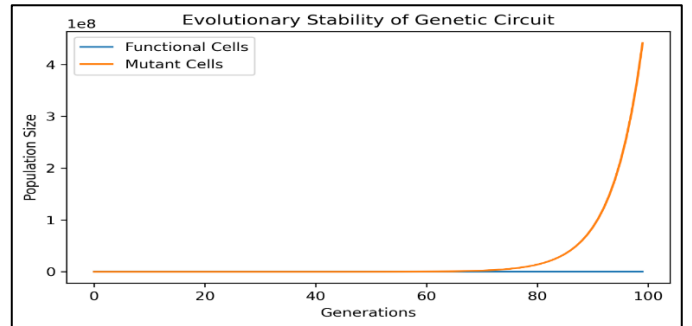


Figure 6. Population dynamics of functional and mutant cells showing evolutionary takeover and circuit failure point.

IV. DISCUSSION

This study presents a computationally guided design of a dual-function synthetic genetic circuit that integrates pathogen sensing, targeted antimicrobial activity, and biosafety control within a single framework. By combining quorum sensing-based detection of *Staphylococcus aureus* with lysostaphin-mediated bacteriolysis and an inducible MazE/MazF kill switch, the proposed system addresses both therapeutic performance and containment considerations, which are essential for the safe application of engineered microbes.

The results indicate a consistent coupling between the sensing and response modules. Activation of the Agr quorum sensing system leads to density-dependent gene expression and downstream activation of target genes, enabling condition-dependent functional responses in engineered systems. This behavior is consistent with recent studies demonstrating successful integration of the Agr quorum sensing system into heterologous hosts, where increased signal accumulation results in activation of gene expression and functional output [19, 20].

From a biosafety perspective, the integration of the MazE/MazF toxin-antitoxin module provides a controllable mechanism for terminating the system when required. Incorporating the kill switch within the same circuit architecture, rather than as a separate safeguard, allows coordinated regulation between therapeutic activity and containment. This design approach aligns with current strategies emphasizing built-in biosafety systems in engineered microbes [21].

Stochastic simulations further showed that the circuit maintains a relatively low probability of unintended activation under non-induced conditions. This suggests that the system operates within a parameter regime where intrinsic molecular noise does not significantly compromise stability. Such robustness is important given that stochastic fluctuations in gene expression can influence system behavior, particularly in low-copy-number contexts [22, 23, 24].

Sensitivity analysis identified the MazF production rate (α_M) as a key parameter influencing system stability. The observed threshold-like transition between stable operation and toxicity highlights the importance of precise regulatory tuning. This finding is consistent with systems biology principles demonstrating nonlinear responses in gene regulatory networks [25].

Leakiness analysis demonstrated that basal expression of MazF, even at low levels, may adversely affect host viability. This observation underscores the importance of minimizing unintended expression in inducible systems, as even low-level toxin expression can disrupt cellular function [25]. This concept is supported by recent stochastic modeling studies showing that small changes in regulatory parameters can lead to nonlinear responses and stability shifts in computationally simulated biological systems [26].

In terms of long-term behavior, evolutionary modeling suggests that the circuit may be susceptible to gradual functional loss due to the accumulation of mutations that confer selective advantages to non-functional variants. This is consistent with prior studies showing that synthetic genetic circuits are often subject to evolutionary pressures that reduce their stability over time [27].

In terms of biology, this circuit reduces harm to the commensal microbiome by providing tailored, density-dependent pathogen clearance. However, host immune clearance, microbial competition, biofilm barriers, and metabolic burden on the designed chassis are some of the major *in vivo* obstacles to therapeutic translation. According to recent research, overcoming these translational constraints and successfully combating resistance requires combining rationally designed new antimicrobial medicines [28] with precisely tailored bacteria [29].

An important contribution of this study is the incorporation of multiple failure criteria, including functional, toxicity, stochastic, and evolutionary risks, within a unified modeling framework. This multi-dimensional assessment allows for a more comprehensive evaluation of system performance and provides quantitative thresholds that can guide safer experimental implementation [30].

Despite these findings, several limitations should be considered. The model assumes a well-mixed intracellular environment and does not account for spatial heterogeneity, which may influence system behavior in structured environments such as biofilms [31]. Additionally, metabolic burden associated with gene expression was not explicitly modeled, although it is known to affect host fitness and circuit stability [32]. Furthermore, as the study is based on computational simulations, experimental validation is necessary to confirm the predicted dynamics.

Future work should focus on validating the proposed circuit experimentally and extending the modeling framework to include spatial effects and resource allocation constraints. Further optimization of regulatory elements may also improve system stability and reduce unintended activation, supporting the development of more reliable and safe synthetic biological systems.

A. Limitations

While this study provides a comprehensive *in silico* framework for the design and evaluation of a dual-function genetic circuit, several limitations should be acknowledged.

First, the modeling approach is primarily based on deterministic ordinary differential equations, which assume homogeneous intracellular conditions and do not fully capture stochastic fluctuations at low molecule numbers. Although stochastic simulations were incorporated using the Gillespie algorithm, spatial heterogeneity and cell-to-cell variability were not explicitly modeled.

Second, the system does not account for interactions with the host environment, including immune responses, microbiome competition, or nutrient limitations, all of which may significantly influence circuit performance *in vivo*.

Third, metabolic burden was simplified and not fully integrated into a genome-scale or resource-allocation framework. As a result, the impact of resource competition on long-term circuit stability may be underestimated.

Fourth, the evolutionary stability model assumes simplified mutation dynamics and constant mutation rates, which may differ under real biological conditions where selective pressures and stress responses can alter mutation frequencies. Finally, the study lacks experimental validation. While the computational results provide strong predictive insights, experimental implementation and validation in a suitable host organism are required to confirm the feasibility and robustness of the proposed circuit.

B. Risk Modeling and Biosecurity Assessment

To complement the *in silico* evaluation of the dual-function genetic circuit, a systematic risk modeling approach was implemented to assess potential biosecurity and operational

hazards associated with the designed system. The risk framework integrates four main dimensions:

Functional risk:

Evaluates the probability of insufficient therapeutic activity due to suboptimal lysostaphin expression or sensor failure. Simulations showed that maintaining lysostaphin concentration above the effective threshold ($L > L_{\text{eff}}$) ensures robust bacterial suppression, highlighting regions of functional stability in the operational parameter space [33].

Toxicity and leakiness risk:

Basal expression of MazF under non-induced conditions poses a potential threat to host viability. Leakiness analysis defined a critical threshold beyond which MazF accumulation could unintentionally activate the kill switch, causing premature cell death. This threshold informs safe promoter and ribosome-binding site design, ensuring controlled induction while minimizing accidental toxicity [34].

Stochastic risk:

Intrinsic molecular noise may trigger unintended MazF activation. Gillespie-based stochastic simulations quantified the probability distribution of MazF levels under non-induced conditions. The calculated probability of spontaneous kill-switch activation provides a quantitative metric for system robustness against intrinsic fluctuations [35].

Evolutionary risk:

Long-term stability of the circuit may be compromised by the accumulation of mutant cells that evade circuit control. Simplified population modeling indicates that evolutionary takeover can occur depending on mutation rates and fitness advantages. This highlights the need for minimizing metabolic burden and incorporating genetic safeguards to reduce the likelihood of functional loss over time. By integrating these risk dimensions, the proposed framework provides a quantitative and systematic assessment of biosecurity hazards. This approach not only guides safe circuit design but also establishes thresholds for controlled laboratory implementation, supporting the development of synthetic systems with minimized biosafety risks [36, 37].

C. Biological feasibility

The proposed genetic circuit is designed using well-characterized biological components commonly employed in synthetic biology, supporting its potential feasibility for experimental implementation.

The sensing module is based on quorum sensing elements derived from *Staphylococcus aureus* (AgrC/AgrA system), which have been previously studied and adapted for heterologous expression. The therapeutic module utilizes lysostaphin, a well-established antimicrobial enzyme with high specificity against *S. aureus*, making it a suitable candidate for targeted bacterial killing [38, 39].

The kill switch design incorporates the MazE/MazF toxin-antitoxin system [40], which has been widely used in synthetic biology for biocontainment applications due to its rapid and effective induction of cell death. The use of the arabinose-inducible promoter (pBAD) provides a controllable and externally regulated mechanism for activating the kill switch.

The well-characterized and biosafety level 1 (BSL-1) strain *Escherichia coli* K-12 was selected as the designated chassis for this genetic circuit. The choice of *E. coli* provides significant structural advantages for the system's viability. Primarily, as a Gram-negative bacterium, *E. coli* naturally exhibits resistance to lysostaphin, ensuring that the host acts as a stable production factory without being subjected to autolysis during the therapeutic phase.

Furthermore, since the MazE/MazF toxin-antitoxin module is native to the *E. coli* K12 chromosome, its synthetic overexpression via the pBAD promoter can efficiently leverage the endogenous degradation machinery. However, translating the Gram-positive AgrC/AgrA quorum sensing module into the Gram-negative *E. coli* chassis will necessitate precise codon optimization and targeted membrane localization strategies to ensure functional receptor integration [41]. Additionally, the expression of lysostaphin and MazF may impose a metabolic burden, potentially affecting host cell growth and stability.

Furthermore, precise tuning of promoter strength and ribosome binding sites is critical to balance toxin and antitoxin expression, minimizing leakiness while ensuring effective activation when required. These considerations highlight the importance of iterative design and experimental validation to translate the proposed *in silico* system into a functional biological construct.

V. CONCLUSION

The dual-function genetic circuit was evaluated using the predictive *in silico* framework, quantifying toxicity thresholds and failure risks prior to *in vitro* or *in vivo* trials. By integrating circuit design with computational analysis, evidence-based Standard Operating Procedures (SOPs) can be established, ensuring that engineered microbes meet international biocontainment standards while minimizing operational and environmental hazards. Ultimately, laboratory experiments are necessary to validate these computational predictions.

ETHICS AND CONSENT

This study involved only *in silico* modeling, with no human or animal subjects. All design and analyses prioritize biosafety, incorporating a tightly regulated kill switch and adhering to BSL-1 guidelines and international synthetic biology standards, ensuring responsible and controlled future applications.

DATA ACCESSIBILITY

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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DECLARATION OF AI-ASSISTED TOOLS USAGE

AI-assisted tools were used solely for language editing to improve grammar, clarity, and readability. An AI-detection assessment reported a score below 15%. The authors have critically reviewed and verified all revisions and accept full responsibility for the accuracy, originality, integrity, and entire content of the manuscript.

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