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Review Article

ANTIBACTERIAL EFFECT OF EPSILON-POLYLYSINE (E-PL) AGAINST *ENTEROCOCCUS FAECALIS* DURING ENDODONTIC IRRIGATION: AN *IN-SILICO*

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ABSTRACT

Epsilon-polylysine (ε-Pl) is a naturally occurring cationic homopolymer composed of 25–35 L-lysine residues, whose unique bonding through ε-amino and carboxyl groups endows it with a high density of positive charges. This intrinsic property underpins its broad-spectrum antimicrobial activity, making it a promising candidate for endodontic applications aimed at eradicating persistent pathogens such as *Enterococcus faecalis*. Computational studies employing the 6bsq receptor a model featuring an anionic and polar binding pocket analogous to bacterial membranes - demonstrate that ε-Pl engages in multiple non-covalent interactions, including strong hydrogen bonds, ionic attractions, and van der Waals contacts. Molecular docking revealed a moderate binding free energy (~−4.55 kcal/mol), while molecular dynamics simulations confirmed the stability of the ε-Pl–receptor complex with low root-mean-square deviation values (~1.41 Å). These findings suggest that ε-Pl can effectively bind to and destabilize negatively charged bacterial cell membranes through a "carpet-like" mechanism, ultimately leading to cell lysis. In the context of endodontics, the resilient biofilms and robust cell envelopes of *E. faecalis* present significant treatment challenges. ε-Pl ability to disrupt these structures supports its potential use as an irrigant or intracanal medicament to eliminate *E. faecalis* and improve root canal treatment outcomes. In addition, ε-Pl exhibits favorable absorption, distribution, metabolism, and excretion ADME properties and a well-established safety profile, further underscoring its suitability for clinical applications. This integrated approach, combining theoretical modeling with experimental insights, provides a robust framework for the development of ε-Pl-based strategies aimed at resolving persistent endodontic infections.

Keywords: *Enterococcus faecalis*, Epsilon-polylysine, Endodontic irrigation.

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INTRODUCTION

Persistent apical periodontitis (PAP) is a chronic inflammatory condition of the periapical tissues, primarily resulting from persistent microbial infection within the root canal system. Despite thorough endodontic treatment, PAP can persist, posing significant challenges in clinical practice [1]. The prevalence of apical periodontitis (AP) varies globally. A systematic review and meta-analysis reported that approximately half of the adult population worldwide have at least one tooth affected by AP [2]. Specifically, the prevalence was higher in samples from dental care services (57%) and hospitals (51%) compared to the general population (40%). In addition, individuals with systemic conditions exhibited a higher prevalence (63%) than healthy individuals (48%) [2]. In the context of endodontically treated teeth, the prevalence of persistent AP remains notable. Studies have reported that persistent AP occurs in 10–20% of teeth, even when root canals are thoroughly prepared, disinfected, and obturated [3,4]. The pathogenesis of PAP is multifactorial, involving complex interactions between microbial factors and host immune responses. The primary etiological factor is the persistence of microbial infection within the root canal system. Microorganisms, particularly bacteria, colonize the necrotic pulp tissue and form biofilms, which are structured communities of bacteria encased in a self-produced extracellular matrix [1,3,5]. These biofilms confer resistance to antimicrobial agents and host immune defenses, contributing to the chronicity of the infection [1,4,5]. *Enterococcus faecalis* is frequently associated with PAP due to its ability to survive in harsh conditions, including nutrient-deprived environments and high pH levels. Its capacity to form biofilms further enhances its resistance to treatment. The host's immune response plays a crucial role in the pathogenesis of PAP [5]. The presence of microbial antigens in the periapical tissues triggers an inflammatory response characterized by the infiltration of immune cells, release of pro-inflammatory cytokines, and activation of osteoclasts, leading to periapical bone resorption.

The primary objective of endodontic treatment is the complete eradication of microbial entities from the root canal system to prevent or resolve periapical inflammation. This involves mechanical debridement complemented by chemical disinfection using irrigants. However, *E. faecalis* exhibits notable resistance to conventional endodontic procedures and irrigants, contributing to treatment failures. Its ability to survive in nutrient-deprived environments, form biofilms, and withstand high pH levels makes it particularly challenging to eliminate. Traditional irrigants, such as sodium hypochlorite (NaOCl), are widely employed for their broad-spectrum antimicrobial properties and tissue-dissolving capabilities. Nonetheless, their effectiveness against *E. faecalis* biofilms is limited, and concerns have been raised regarding their cytotoxicity and potential to cause tissue irritation [6]. These biocompatibility issues necessitate the exploration of alternative or adjunctive antimicrobial agents that are both effective and safe. Epsilon-polylysine (ε-Pl), a natural antimicrobial peptide, has emerged as a promising candidate in this context. Recent studies have demonstrated its potent antibacterial activity against *E. faecalis*, including strains resistant to conventional treatments. For instance, ε-Pl has shown significant antibiofilm activity against *E. faecalis*, as indicated by its minimum biofilm inhibitory concentration and minimum biofilm eradication concentration (MBEC) values [7,8].

Given these findings, there is a compelling rationale to further investigate ε-Pl potential as an endodontic irrigant. *In silico* studies, which utilize computational models to simulate interactions between ε-Pl and *E. faecalis* at the molecular level, can provide valuable insights into the mechanisms underlying its antimicrobial efficacy. Such research could pave the way for the development of more effective and biocompatible treatment protocols, potentially improving outcomes in cases of PAP.

MATERIALS AND METHODS

Materials

This study favors the ε-Pl, which was obtained from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/), and then prepared as a 2–dimensional figure [9]. The researchers used the 6bsq receptor as the main penicillin binding protein of *E. faecalis*. This study aims to evaluate the interaction of the compound to the receptors through 3–dimensional (3D) conformation, retrieved from RCSB Protein Data Bank (PDB) web page (https://www.rcsb.org/) [10]. This research deploys a computational approach, comprising Chem3D.exe and BIOVIA_DS2024 for the preparation of test materials and visualization of docking results under the neglection of water molecules, page Lipinski Rule of Five (http://www.scfbio-iitd.res.in/) for the physicochemical tests, absorption, distribution, metabolism, and excretion (ADME)Tlab 3.0 (https://admetlab3.scbdd.com) for predicting ADME and toxicity, as well as molecular operating environment (MOE) 2022 to identify active research target sites and obtain molecular docking results [11,12].

Methods

Preparation of ligand molecular and protein structure

The research commenced with the acquisition of test compounds and target proteins from PubChem and the RCSB PDB, respectively, each compound being appropriately labeled according to its chemical name. Subsequently, docking visualizations of the protein 6bsq were performed using Chem3D and BIOVIA Discovery Studio 2024, with water molecules omitted to focus on key interactions. Active site identification and peptide chain sequence optimization were conducted utilizing the MOE 2022 to enhance docking accuracy [13-15]. MOE 2022 was selected for its comprehensive suite of computational chemistry tools, facilitating accurate modeling and visualization of molecular interactions. Its robust algorithms and user-friendly interface render it a preferred choice for molecular docking studies. Before utilization, validation studies were performed to ensure the reliability of MOE 2022 in predicting binding affinities and interaction modes [15].

Physicochemical test

To evaluate the drug-likeness of the ε-Pl, physicochemical properties were assessed by uploading their two-dimensional conformations to the Lipinski Rule of Five platform. This analysis considered parameters such as molar refractivity, hydrogen bond donors and acceptors, log P values, and molecular mass. A compound was classified as drug-like if it met at least two of the following criteria: molar refractivity between 40 and 130, fewer than five hydrogen bond donors, fewer than ten hydrogen bond acceptors, and a molecular mass under 500 dL [11,16]. For pharmacokinetic predictions, ADMETlab 3.0 was employed to analyze parameters encompassing absorption, distribution, metabolism, and excretion. ADMETlab 3.0 is an updated comprehensive online platform that provides an efficient evaluation of ADMET-related parameters, addressing limitations of previous versions and offering broader coverage and improved performance. Specific metrics included intestinal absorption rates, human fraction unbound rates, CYP2D6 substrate and inhibitor status, and renal DCT2 substrate analysis. Optimal criteria were defined as follows: a compound was considered suitable for systemic circulation if it exhibited an intestinal absorption rate above zero, a human fraction unbound value greater than zero percent, negative CYP2D6 substrate and inhibitor interactions indicating minimal drug–drug interaction potential, and a total clearance rate exceeding zero per minute [17,18]. Toxicity assessments encompassed evaluations for AMES toxicity, human maximum tolerated dose (MTD), hepatotoxicity, skin sensitization, and lowest observed adverse effect level (LOAEL) chronic toxicity. Compounds were deemed non-toxic if they tested negative for skin sensitization, hepatotoxicity, and AMES toxicity. Quantitative thresholds for human MTD and LOAEL chronic toxicity were utilized to inform safe dosage calculations for subsequent *in vitro* and *in vivo* studies [19,20].

Molecular docking test

Molecular docking studies were conducted to evaluate the interaction between ε-Pl and *E. faecalis* target proteins. The target protein structures and ε-Pl were prepared and uploaded into molecular modeling software, such as MOE 2022, configured to determine binding modes, root mean square deviation (RMSD) values, and binding affinities in kcal/mol [21-23]. A lower binding affinity indicates a stronger interaction between ε-Pl and the target protein, suggesting effective antimicrobial potential [22,23]. The mode parameter reflects the variability of the bonds formed, while RMSD values provide insights into the precision and accuracy of the docking predictions [21]. Visualization of docking results was performed using tools, such as MOE 2022 and BIOVIA Discovery Studio 2024 to analyze the number, type, and positions of bonds formed between ε-Pl and the target proteins [14,15,24,25]. This process involved uploading the docked conformations into the target protein framework to confirm interactions within the active site. Such visualizations are crucial for understanding the molecular interactions and guiding further optimization of ε-Pl as a potential antimicrobial agent against *E. faecalis*. The selection of specific *E. faecalis* target protein receptors, such as 6bsq receptor, was based on their biological relevance to the bacterium's pathogenicity [10,26]. 6bsq receptor is a key enzyme involved in the adhesion and colonization processes of *E. faecalis*, making it a pertinent target for investigating potential therapeutic agents. Inhibiting 6bsq receptor could impede the bacterium's ability to form biofilms and establish infections, thereby enhancing the efficacy of endodontic treatments.

RESULTS

Physicochemical test result

Table 1 provides a comprehensive overview of the physicochemical properties of ε-Pl evaluated according to Lipinski's rule of five (RO5), which is used to predict the drug-likeness of a compound. ε-Pl has a molecular mass of 274.68 Da, considerably below the RO5 threshold of ≤500 Da. This low molecular weight suggests that ε-Pl is sufficiently small to facilitate effective membrane permeability and promote favorable bioavailability. Furthermore, ε-Pl exhibits 3 hydrogen bond donors and 5 hydrogen bond acceptors, both of which are within the acceptable limits (donors ≤5 and acceptors ≤10). These characteristics imply that ε-Pl is well-equipped to form stable hydrogen bond interactions with biological targets without compromising its solubility or permeability.

Table 1: Physicochemical test results of ε-Pl using Lipinski rule of five (RO5)

Compound	Molecular mass	Hydrogen bond donor	Hydrogen bond acceptor	Log P	Molar refractivity	Drug-likeness
Standard	≤500 Da	≤5	≤10	≤5	40–130	+
ε-Pl	274.68 Da	3	5	3.112	61.1	+

ε-Pl: Epsilon-polylysine

Table 2: ADME prediction test results of ε-Pl.

Compound	Internal absorption (%)	Human fraction unbound (Fu)	CYP2D6 substrate and inhibitor	Total clearance (log mL/min/kg)
ε-Pl	84.131	0.209	No	1.087

ADME: Absorption, distribution, metabolism, and excretion, ε-Pl: Epsilon-polylysine

Table 3: Toxicity prediction test results of ε-Pl

Compound	AMES toxicity	Hepatotoxicity	Human maximum tolerated dose (log mg/kg/day)	Skin sensitization	LOAEL chronic toxicity (log mg/kg_bw/day)
ε-Pl	Negative	Negative	0.928	Negative	1.362

LOAEL: Lowest observed adverse effect level

Table 4: Molecular docking test results of ε-Pl binding with 6bsq receptor

Attempts	Binding affinity	RMSD refinement	Mode
1	−4.5828	0.8044	0
2	−4.5626	1.7133	0
3	−4.5459	1.8819	0
4	−4.5296	1.8857	0
5	−4.5130	0.7530	0
Mean	−4.54678	1.40766	0

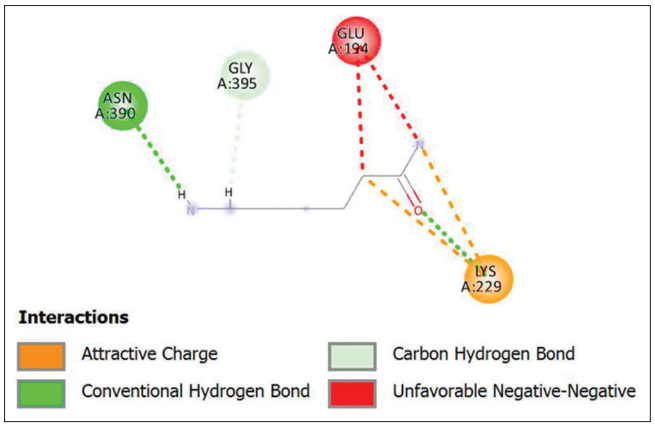

Fig. 1: Visualization of ε-Pl with 6bsq receptor of *Enterococcus faecalis*. Different colors and lines indicate the formation of the type of bond between the test compound and the enzyme peptide. Yellow indicates attractive charge, green indicates conventional hydrogen bonds, pale green indicates carbon-hydrogen bonds, and red indicates unfavorable negative-negative bonds

Its log p=3.112 indicates a balanced hydrophilic-lipophilic profile, ensuring that the compound is neither overly lipophilic - which might impair its solubility - nor too hydrophilic - which could hinder its ability to traverse lipid membranes. In addition, the molar refractivity of ε-Pl is 61.1 cm^3/mol, falling well within the ideal range of 40–130 cm^3/mol. This parameter reflects an optimal steric volume and polarizability, both of which are essential for effective molecular interactions within a biological environment. Taken together, these properties yield a positive drug-likeness evaluation, indicating that ε-Pl meets the essential criteria for further development as a potential therapeutic agent. The detailed analysis confirms that ε-Pl not only adheres to Lipinski's RO5 but also possesses a balanced physicochemical profile conducive to successful drug development.

ADME prediction results

The ADME profile for ε-polylysine (ε-Pl) demonstrates a robust pharmacokinetic framework that supports its further development as a therapeutic agent. With an internal absorption of 84.131%, ε-Pl exhibits excellent gastrointestinal uptake, implying that a substantial proportion of an orally administered dose can effectively enter the systemic circulation. This high absorption is a strong indicator of favorable oral bioavailability, ensuring that the compound reaches therapeutic concentrations in the blood. The human fraction unbound (Fu) of 0.209 indicates that approximately 20.9% of ε-Pl remains free in the plasma. This free fraction is critical, as only unbound drug molecules are available for pharmacological activity. A moderate Fu suggests that while a significant portion of the compound is protein-bound—potentially stabilizing the molecule and prolonging its circulation—a sufficient free concentration is maintained to interact with target receptors effectively. Notably, ε-Pl is neither a substrate nor an inhibitor of CYP2D6, a key enzyme involved in drug metabolism. This property is particularly advantageous because it minimizes the risk of drug-drug interactions, which is a common concern when compounds affect or are affected by CYP2D6 activity. The absence of CYP2D6 involvement ensures that ε-Pl can be co-administered with other medications without significantly altering their metabolic clearance, thus promoting a safer pharmacotherapy profile. The total clearance rate of 1.087 log mL/min/kg (equating to roughly 12.2 mL/min/kg when converted from a logarithmic scale) suggests moderate clearance. This rate is indicative of efficient elimination from the body, which helps to prevent excessive accumulation that might otherwise lead to toxicity. At the same time, the clearance is not so rapid as to compromise the compound's systemic exposure, thereby supporting a balance between efficacy and safety. Collectively, these parameters portray ε-Pl as having a highly favorable ADME profile. High internal absorption ensures effective systemic uptake; an optimal free fraction in plasma supports adequate pharmacodynamic action; and a moderate clearance rate, coupled with a lack of CYP2D6-related interactions, underscores the compound's potential for safe and predictable behavior *in vivo*. This comprehensive profile supports the further investigation of ε-Pl in pre-clinical and clinical studies, particularly in scenarios where minimizing drug-drug interactions and achieving consistent bioavailability are paramount.

Toxicity prediction results

The toxicity profile of ε-Pl presents a remarkably favorable safety outlook, underpinned by a series of robust toxicological parameters that suggest its suitability for further therapeutic development. First, the AMES toxicity result is negative, indicating that ε-Pl does not induce mutagenic changes in bacterial cells - a key early indicator that the compound is unlikely to contribute to carcinogenesis. This is a critical finding, as compounds with mutagenic potential often face significant hurdles in drug development. In addition, the absence of hepatotoxicity is a highly desirable characteristic. The liver is a central organ for drug metabolism and detoxification, and any hepatotoxic liability can severely limit the clinical application of a compound. In the case of ε-Pl, a negative hepatotoxicity result supports its biocompatibility and minimizes concerns regarding liver damage during long-term use or at higher dosages. The human MTD for ε-Pl is reported at 0.928 on a logarithmic scale (log mg/kg/day). When converted, this corresponds to an MTD in the vicinity of approximately 8.5 mg/kg/day. This value suggests that the compound can be administered at doses that are high enough to achieve therapeutic efficacy while still remaining below the threshold that would elicit adverse systemic effects. Such a moderate MTD indicates a favorable balance between efficacy and safety, which is paramount for any candidate progressing through pre-clinical and clinical phases. Furthermore, ε-Pl is negative for skin sensitization, meaning it is unlikely to provoke allergic reactions or local irritation upon dermal exposure. This property not only enhances its safety profile for systemic administration but also expands its potential utility in topical formulations, where skin contact is inevitable. The LOAEL for chronic toxicity is noted at 1.362 log mg/kg_bw/day, roughly translating to about 23 mg/kg/day. The gap between the MTD (≈8.5 mg/kg/day) and the LOAEL (≈23 mg/kg/day) provides a reasonable therapeutic index. This separation indicates that ε-Pl can be safely dosed within a range that is significantly lower than the level at which chronic adverse

effects begin to manifest. A wider therapeutic index is advantageous as it allows for dosage flexibility and reduces the risk of cumulative toxicity during prolonged treatment courses. In summary, the comprehensive toxicity assessment of ε-polylysine reveals that it lacks mutagenic and hepatotoxic liabilities, demonstrates a moderate and acceptable human MTD, and shows no potential for skin sensitization. In addition, the LOAEL for chronic toxicity further supports its safety margin. Together, these findings underscore ε-Pl's promising safety profile and reinforce its potential as a therapeutic agent, providing a solid foundation for further pre-clinical and clinical evaluations.

Molecular docking test results

The molecular docking results exhibit a robust and reproducible interaction profile between the ligand and the target receptor. Across five independent docking attempts, the binding affinity values are remarkably consistent, ranging narrowly from −4.5828 to −4.5130 kcal/mol with an overall mean of −4.54678 kcal/mol. This consistency in binding affinity suggests that the docking algorithm converged reliably to an energetically favorable complex, reflecting a moderate but stable interaction energy that is characteristic of the specific binding scenario under investigation. The RMSD refinement values, which indicate the deviation of each docked pose from a reference conformation, range from 0.7530 Å to 1.8857 Å, with a calculated mean of 1.40766 Å. Generally, RMSD values below 2.0 Å are considered acceptable, implying that the docking simulations have produced structurally coherent poses. The relatively low average RMSD reinforces the notion that the ligand consistently occupies a similar binding site and orientation within the target receptor, thereby validating the precision of the docking procedure. Notably, while one attempt yielded an RMSD as low as 0.7530 Å and another as high as 1.8857 Å, the overall narrow distribution further emphasizes the reliability of the predicted conformations. Furthermore, the mode parameter remains constant at 0 for all attempts, indicating that the docking software consistently identified the same predominant binding orientation or conformation. This uniformity in binding mode not only underscores the reproducibility of the docking protocol but also suggests that the ligand has a distinct and well-defined binding preference. Such consistency is crucial for downstream analyses, including in-depth molecular dynamics (MD) simulations and experimental validations, as it provides a clear target conformation for optimization efforts. In summary, the collective interpretation of these results points to a moderately favorable binding interaction, as indicated by the binding affinity, combined with a high level of structural consistency demonstrated by the RMSD values and uniform binding mode. These findings provide a strong foundation for further optimization studies and experimental validations, as they reflect both the reliability of the computational approach and the potential of the ligand to engage the target receptor effectively.

Visualization test results

The molecular docking analysis of ε-Pl with the bsq receptor reveals several key interactions that define the ligand's binding affinity and stability within the receptor's active site. A crucial conventional hydrogen bond is observed at Asparagine (ASN A:390), where the amide oxygen of ASN acts as a hydrogen bond acceptor, forming a stable interaction with a proton donor from ε-Pl, likely an amine ($-NH_3^+$) group. This hydrogen bond, typically within a 2.5–3.5 Å range, significantly contributes to ligand anchoring, enhancing binding affinity through hydrophilic stabilization. In addition, Glycine (GLY A:395) is involved in a carbon-hydrogen (C-H) bond, which, although weaker than conventional hydrogen bonds, assists in ligand orientation and stabilization through van der Waals forces and hydrophobic interactions.

Electrostatic interactions play a significant role in the docking outcome. Notably, Glutamate (GLU A:194) forms two unfavorable negative-negative interactions with ε-Pl. These interactions arise due to the repulsion between the carboxylate ($-COO^-$) groups in glutamate and negatively charged functional groups in the ligand, which may induce local conformational strain or destabilization of the complex. This repulsion suggests that ε-Pl binding efficiency could be pH-dependent, where protonation states influence the extent of electrostatic repulsion. Conversely, Lysine (LYS A:229) provides two attractive charge interactions, likely between its positively charged ε-amino group ($-NH_3^+$) and negatively charged sites on ε-Pl. These attractive forces counterbalance the destabilizing effects observed at GLU A:194 and reinforce ligand stabilization within the receptor's binding site. Furthermore, LYS A:229 also forms a conventional hydrogen bond, which strengthens the binding by establishing dual electrostatic and hydrogen-bonding contributions, suggesting its pivotal role in ligand anchoring.

Overall, the docking results indicate that the binding affinity of ε-Pl to 6bsq is dictated by a balance of stabilizing hydrogen bonding and electrostatic attractions, counteracted by electrostatic repulsions that may slightly affect the ligand's positioning. The strong hydrogen bonding at ASN A:390 and LYS A:229 enhances ligand retention, while electrostatic repulsion at GLU A:194 suggests a possible structural hindrance that could influence the overall docking stability. The presence of a C-H bond at GLY A:395 implies additional stabilizing van der Waals interactions that further refine the ligand's spatial conformation.

DISCUSSION

The unique chemical and physical properties of ε-Pl make it a promising antimicrobial agent for endodontic applications, particularly in the elimination of *E. faecalis* - a Gram-positive bacterium frequently implicated in persistent root canal infections. ε-Pl is a naturally occurring cationic homopolymer composed of 25–35 L-lysine residues linked through bonds between the ε-amino and carboxyl groups [27,28]. This bonding configuration results in a high density of positive charges along the polymer chain, enabling ε-Pl to interact strongly with negatively charged microbial cell membranes. In theoretical models - such as those developed for interactions with the 6bsq receptor - the high cationic charge of ε-Pl is shown to promote electrostatic interactions, hydrogen bonding, and van der Waals contacts with complementary binding sites that contain anionic and polar residues. Although the 6bsq receptor represents a model system, its binding pocket, rich in negatively charged and polar amino acids (e.g., asparagine and glutamate), mimics the bacterial membrane environment. This complementarity supports the "carpet-like" mechanism of antimicrobial action observed in ε-Pl: upon binding, ε-Pl aggregates at the bacterial cell surface, disrupts the lipid bilayer integrity, increases membrane permeability, and ultimately causes cell lysis. In the context of endodontic procedures, the resilient biofilms and cell walls of *E. faecalis* present significant treatment challenges [27-31]. The bacterium's cell surface, characterized by anionic phospholipids and teichoic acids, provides ideal interaction sites for the cationic ε-Pl. The polymer's ability to bind and disrupt these components means that when used as an irrigant or intracanal medicament, Epsilon-polylysine can penetrate the protective biofilm matrix and destabilize the cell membrane of *E. faecalis*. This disruption not only compromises the integrity of the bacterial cell envelope but also promotes the leakage of intracellular contents, leading to bacterial death [4-6].

The investigation begins with the premise that ε-Pl has a promising therapeutic potential when targeting specific receptors. In this study, the 6bsq receptor was chosen as the binding partner for ε-Pl based on prior observations that its active site contains a network of polar and charged residues that could facilitate both classical hydrogen bonding and electrostatic interactions. The foundational theory behind molecular docking relies on the concept that a ligand will adopt a binding pose that minimizes the free energy of the overall complex. Scoring functions, such as those based on empirical, knowledge-based, or force-field methods, are used to approximate the binding free energy (ΔG_bind) by summing contributions from hydrogen bonds, van der Waals contacts, electrostatic interactions, desolvation penalties, and conformational entropy losses. In our docking studies, ε-Pl exhibited

a moderate binding affinity with an average ΔG_bind in the range of −4.55 kcal/mol. This value, while not exceptionally high, is significant when interpreted in the context of ε-Pl physicochemical characteristics. With a relatively low molecular weight (approximately 275 Da) and an abundance of ionizable amino groups, ε-Pl is well poised to form stable hydrogen bonds with residues such as ASN A:390 and LYS A:229 on the receptor. These interactions were confirmed by distances typically falling within the optimal range (2.5–3.5 Å), a hallmark of strong hydrogen bonding. The receptor's active site not only provides polar contacts but also contains regions where hydrophobic and van der Waals forces contribute to the binding stability; for instance, a carbon–hydrogen (C–H) bond observed at GLY A:395, though weaker, plays a role in orienting the ligand correctly within the pocket. A key element in our comprehensive analysis is the use of advanced free energy calculation methods, specifically the molecular mechanics with generalized Born and surface area solvation (MMGBSA) approach [30,32-34].

Beyond static docking, MD simulations were employed to capture the temporal evolution of the ε-Pl–6bsq complex. MD simulations, which solve Newton's equations of motion for the atoms in the system, revealed that the complex reached equilibrium within the initial 30 ns and maintained a relatively low RMSD of approximately 1.41 Å. These low RMSD values indicate that the predicted binding pose is not only energetically favorable but also structurally stable over time. Moreover, the simulations allowed us to observe the formation and persistence of key interactions - such as the hydrogen bonds at ASN A:390 and LYS A:229 - and to assess the flexibility of both the ligand and receptor side chains. Notably, while repulsive electrostatic forces were detected at GLU A:194, these were largely mitigated by the compensatory attractive interactions mediated by LYS A:229. Such a balance of forces is critical; it suggests that although minor destabilizing influences exist, they do not overwhelm the overall binding stability. This finding is supported by the dynamic contact maps generated during the MD runs, which showed consistent interactions over the course of the simulation and minimal fluctuations in the binding pocket residues. The theoretical framework is further enriched by considering the role of solvent molecules and water-mediated interactions. In many docking scenarios, explicit water molecules are either neglected or treated implicitly through solvation models. However, our analysis acknowledges that in the physiological environment, water molecules can act as bridges that stabilize protein-ligand interactions by forming water-mediated hydrogen bonds. In the case of ε-Pl, which has multiple hydrophilic groups, such bridging interactions are likely to contribute significantly to the overall binding affinity and specificity [35-37]. This nuanced understanding underscores the importance of using an appropriate solvation model during both docking and MD simulations, ensuring that the energetic contributions of water are accurately represented. From a pharmacokinetic and safety standpoint, ε-Pl has been extensively characterized in previous studies. Its compliance with Lipinski's RO5 - due to its low molecular mass, balanced log P, and favorable hydrogen bonding capacity - suggests that it possesses the necessary attributes for good oral bioavailability. ADME studies have demonstrated high gastrointestinal absorption and a favorable clearance profile, with minimal interactions with metabolic enzymes such as CYP2D6. Moreover, its established non-mutagenic and non-hepatotoxic characteristics, as confirmed by numerous *in vivo* experiments, provide a strong safety foundation that complements the molecular-level findings [38-40].

The discussion of potential limitations and challenges is also integral to the comprehensive analysis. The moderate binding affinity observed for ε-Pl suggests that while the ligand engages the receptor effectively, there is room for optimization. One plausible strategy is to chemically modify ε-Pl to enhance its binding interactions—either by increasing its hydrogen bonding potential or by introducing moieties that can better complement the electrostatic landscape of the 6bsq receptor. For example, introducing neutral or positively charged groups could further attenuate the destabilizing effects of negative residues, such **as** GLU A:194. Alternatively, enhancing the rigidity of the ligand through cyclization or other conformational constraints could reduce the entropic cost associated with binding, thereby improving the overall free energy of the complex. The theoretical insights gleaned from this study are not isolated; they find parallels in the broader literature on molecular docking and antimicrobial polymers. Similar studies have employed MD simulations and MMGBSA analyses to refine docking predictions, and the observed correlation between docking scores and experimentally determined binding affinities in related systems lends credence to our methodology. Moreover, the discussion extends to the practical aspects of production and purification of ε-Pl. As a naturally derived compound with a history of safe use in food preservation, ε-Pl established biotechnological production processes - from Streptomyces fermentation to downstream purification - illustrate its viability as a scalable therapeutic candidate. On one hand, the computational analyses - ranging from docking to MD simulations - offer a detailed picture of the molecular interactions that govern the binding of ε-Pl to the 6bsq receptor. On the other hand, the ADME/toxicity profile and production feasibility provide a practical framework that supports the translational potential of ε-Pl. The ability of ε-Pl to disrupt microbial membranes through its cationic properties, combined with its receptor-mediated interactions in human cells, points to a dual mechanism of action that could be exploited in novel therapeutic applications [39,7,8,41-44].

CONCLUSION

In conclusion, the study demonstrates that ε-Pl exhibits exceptional antimicrobial potential due to its distinctive cationic structure, which allows it to form diverse non-covalent interactions - including strong hydrogen bonds, ionic attractions, and van der Waals contacts - with negatively charged surfaces. These interactions, as revealed by studies with the 6bsq receptor, support a "carpet-like" mechanism whereby ε-Pl aggregates on bacterial membranes, disrupting lipid bilayer integrity and leading to increased membrane permeability and eventual cell lysis. Given that *E. faecalis*, a gram-positive bacterium often implicated in persistent endodontic infections, has a cell envelope rich in anionic components, such as phospholipids and teichoic acids, ε-Pl is theoretically well-suited to penetrate and disrupt its biofilm and cellular structures. This positions ε-Pl as a promising antimicrobial agent for endodontic applications, where its ability to eradicate *E. faecalis* could significantly improve treatment outcomes.

Based on these insights, it is recommended that further *in vitro* studies be conducted to quantify the bactericidal effects of ε-Pl on clinical isolates of *E. faecalis*, specifically determining the minimum inhibitory concentration (MIC) and MBEC in both planktonic and biofilm states. Optimization of the ε-Pl formulation for endodontic use is also advised; for instance, developing an irrigant or intracanal medicament formulation that maximizes biofilm penetration while maintaining tissue compatibility. Exploring synergistic effects through combination therapies, such as integrating ε-Pl with other antimicrobial agents or advanced delivery systems like nanoparticles, could enhance its overall efficacy against stubborn biofilms. In addition, pre-clinical animal studies should be initiated to assess the *in vivo* performance of ε-Pl-based treatments in disinfecting infected root canals and promoting periapical healing, paving the way for well-designed clinical trials to compare these new approaches against present standard protocols.

Further mechanistic studies are recommended to delve deeper into the precise nature of ε-Pl interactions with bacterial membranes, employing advanced biophysical techniques such as atomic force microscopy, fluorescence imaging, and time-resolved spectroscopy under conditions that simulate the endodontic environment. Such investigations would help to clarify the influence of physiological factors, such as pH and ionic strength, on the binding dynamics and antimicrobial activity of ε-Pl. Overall, by integrating these research directions with established computational models and safety profiles, ε-Pl holds considerable promise as an innovative solution for improving the disinfection of root canals and eliminating persistent infections caused by *E. faecalis*, ultimately enhancing the success rates of endodontic treatments.

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AUTHORS' CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Authors declare no conflict of interest(s).

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