

Revolutionising Periodontal Healing: An In-vitro Study on Antibacterial and Antioxidant Synergy in Graphene Oxide and Resveratrol Nano-enhanced Suture Coatings

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ABSTRACT

Introduction: Periodontitis is a chronic inflammatory disease commonly treated by Periodontal Flap Surgery (PFS), where sutures are essential for wound closure. However, suture-related bacterial colonisation and oxidative stress may impair healing and increase postoperative complications. Nano-enhanced suture coatings with antibacterial and antioxidant properties may improve periodontal wound healing outcomes.

Aim: To evaluate the antibacterial and antioxidant efficacy of reduced Graphene Oxide (rGO) and Resveratrol (RV) nano-enhanced silk suture coatings for periodontal surgical applications.

Materials and Methods: This in-vitro experimental study was conducted at Department of Periodontology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India, from January 2025 to April 2025. Silk sutures were divided into four groups: uncoated (control), 0.05% rGO-coated, 0.1% rGO-coated and 0.1% RV-loaded rGO-coated (RV-rGO). Fourier Transform Infrared Spectroscopy (FTIR) was used to confirm chemical integration and Scanning Electron Microscopy (SEM)

was performed to assess surface morphology. Antibacterial activity against *Streptococcus mutans* and *Escherichia coli* was evaluated using the zone of inhibition assay and antioxidant activity was assessed using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay. All data are presented as mean±Standard Deviation (SD).

Results: Data were collected as zone of inhibition values (mm) and absorbance readings and presented as mean±standard deviation. FTIR spectra demonstrated characteristic Amide I and II bands of silk fibroin and additional peaks confirming the successful integration of rGO and RV. SEM analysis revealed a roughened suture surface with uniformly distributed nanoparticles in the RV-rGO group. The RV-rGO-coated sutures showed the highest antibacterial activity (SM: 25±1 mm; EC: 23±1 mm) and superior antioxidant activity (72% DPPH scavenging).

Conclusion: The RV-rGO nano-enhanced silk sutures exhibit synergistic antibacterial and antioxidant effects, supporting their potential to enhance periodontal wound healing and reduce suture-related complications.

Keywords: Antimicrobial agents, Disease, Infectious disease, Nano-coatings, Periodontitis, Wound closure

INTRODUCTION

Periodontitis, a chronic inflammatory condition impacting the supporting structures of the teeth, is a major global health issue. It is marked by the gradual breakdown of the periodontal ligament, alveolar bone and gingival tissues, which can eventually result in tooth loss if not properly managed [1]. Periodontal therapies aim to eliminate the causative pathogens, control inflammation and regenerate the lost or damaged periodontal tissues [2]. A cornerstone of periodontal treatment is the surgical intervention known as PFS. Following the flap surgery, meticulous wound closure is crucial to promote healing and minimise postoperative complications. This wound closure is commonly achieved using sutures, which approximate the surgical margins, provide tissue stability and maintain the integrity of the healing wound [3].

While sutures are essential for achieving successful outcomes in PFS, they also introduce a potential risk for postoperative complications, particularly suture-related infections [4]. Sutures can create a favourable environment for bacterial colonisation and biofilm formation, as the suture material can act as a nidus for bacterial adhesion and proliferation. Furthermore, the suture material can elicit a foreign body reaction from the host immune system, leading to chronic inflammation and delayed wound healing [5]. The risk of suture-related infections is further amplified in the context of periodontal surgery, as the surgical site is inherently contaminated

with a diverse array of microorganisms, including periodontopathic bacteria. These factors contribute to the overall Surgical Site Infection (SSI) rate, which remains a significant concern in various surgical disciplines, including periodontal surgery [6].

The prevalence of SSI following periodontal surgery is reported to range from 1.5% to 20%, with the specific rate influenced by several factors, including patient-related variables (e.g., systemic health status, smoking habits), surgical site characteristics (e.g., surgical complexity, degree of contamination) and suture material properties (e.g., biocompatibility, surface texture) [7]. Periodontitis involves complex host immune responses and underlying molecular pathways [8]. Various periodontal pathogens, including gram-negative anaerobes, have been implicated in periodontal suture-related infections are gram-negative anaerobes, which are known to be key contributors to periodontal disease pathogenesis [9]. These pathogens can establish biofilms on suture surfaces, protected from host defenses and antimicrobial agents. Consequently, these infections can lead to prolonged healing, persistent inflammation, pain and even complications such as wound dehiscence or fistula formation. In severe cases, the infection can spread systemically, resulting in potentially life-threatening conditions.

The use of sutures in periodontal therapy is not without its challenges. The need for innovative strategies to address the challenges associated with suture-related infections in periodontal

surgery has fuelled research into the development of novel suture materials and coatings. Ideally, these advanced materials should exhibit enhanced biocompatibility, reduced bacterial adhesion and the ability to promote wound healing and tissue regeneration. Various approaches have been explored to achieve these objectives, including the incorporation of antimicrobial agents within the suture material, the development of bioabsorbable sutures and the application of bioactive coatings [10,11].

Among the bioactive molecules with potential applications in periodontal wound healing, RV stands out as a promising candidate. RV, a naturally occurring polyphenol found in grapes, berries and peanuts, has gained significant attention for its diverse pharmacological properties, including antioxidant, anti-inflammatory and antimicrobial effects [12]. The antioxidant properties of RV stem from its ability to scavenge Reactive Oxygen Species (ROS), which are detrimental to tissue health and contribute to the inflammatory process. Its anti-inflammatory effects are mediated through the modulation of various inflammatory pathways, including the inhibition of proinflammatory cytokines and chemokines [13]. Additionally, RV has demonstrated antimicrobial activity against a range of bacteria, including periodontopathic organisms. These combined properties make RV an attractive candidate for incorporation into suture coatings aimed at reducing bacterial colonisation, mitigating inflammation and accelerating wound healing.

The rGO nanoparticles, on the other hand, represent a promising nanomaterial with unique properties that render them suitable for applications in wound healing and tissue engineering [14]. The rGO, a derivative of graphene, possesses a high surface area, excellent biocompatibility and a remarkable ability to load and deliver various bioactive molecules [15]. Its high surface area enables efficient drug loading and sustained release, while its biocompatibility minimises the risk of adverse immune responses. Furthermore, rGO has been shown to promote cell proliferation and differentiation, potentially accelerating tissue regeneration [16]. The combination of RV's therapeutic potential and rGO's unique properties offers a novel strategy to develop advanced suture coatings aimed at improving the outcomes of PFS.

In the present study, the authors aimed to investigate the potential of rGO nanoparticles loaded with RV as a novel silk suture coating for periodontal applications. The authors hypothesised that the RV-loaded rGO (RV-rGO) coating will effectively reduce bacterial adhesion and biofilm formation on the suture surface, mitigate the inflammatory response and promote wound healing.

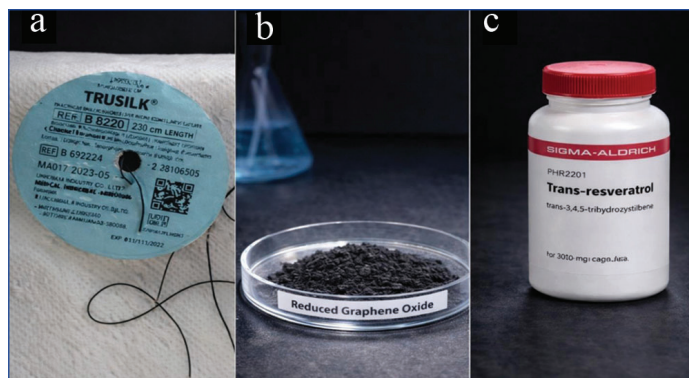
MATERIALS AND METHODS

The present study was an in-vitro experimental study conducted at the Department of Periodontology, Saveetha Dental College and Hospitals, SIMATS, Chennai, Tamil Nadu, India, from January 2025 to April 2025. The study protocol was reviewed and approved by the Scientific Review Board (SRB) of Saveetha Dental College and Hospitals (SRB/SDC/PERIO-2302/24/296).

Study Procedure

Materials: Silk suture (4-0) was procured (Trusilk USP 4-0, 3/8 Circle Cutting SN 5001). rGO nanoparticles were synthesised using the modified Hummers method, wherein graphite powder underwent controlled oxidative exfoliation using concentrated sulfuric acid and potassium permanganate, followed by chemical reduction to obtain rGO nanoparticles with high surface area and oxygen-containing functional groups [17]. The trans-3,4',5-trihydroxystilbene (RV) was purchased from Sigma-Aldrich® US, Merck (PHR2201-200MG-Transresveratrol) [Table/Fig-1]. Ascorbic acid, Mueller Hinton Broth (MHB), Brain Heart Infusion (BHI) broth, Tryptic Soy Agar (TSA), Dimethyl Sulfoxide (DMSO) and other chemicals used were of analytical grade.

Preparation of silk suture coatings: Four distinct groups of silk suture were prepared: 1) uncoated silk suture (control group); 2) silk suture coated with 0.05% rGO nanoparticles (0.05% rGO group); 3)

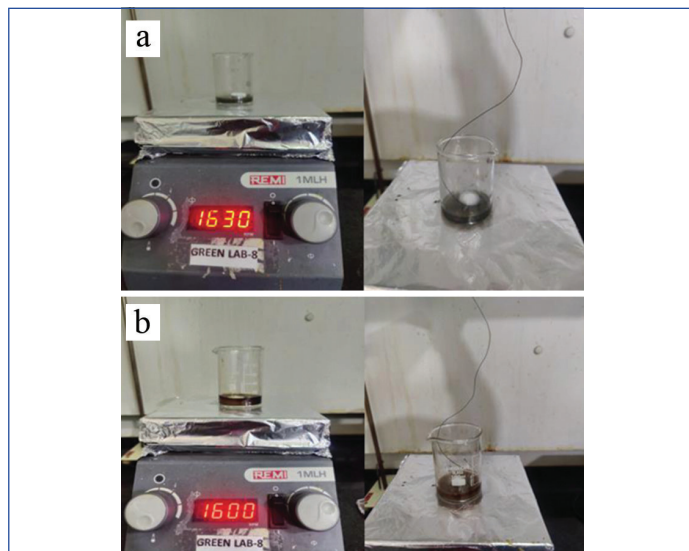


[Table/Fig-1]: Materials used in the fabrication of reduced Graphene Oxide (rGO)-Resveratrol (RV)-loaded sutures: (a) Black braided non absorbable surgical suture (Trusilk® USP 4-0); (b) reduced Graphene Oxide (rGO) synthesised by the modified Hummers' method; (c) Trans-resveratrol (RV) (PHR2201; Sigma-Aldrich, Merck, USA), incorporated into the rGO-coated sutures.

silk suture coated with 0.1% rGO nanoparticles (0.1% rGO group); and 4) silk suture coated with 0.1% rGO nanoparticles loaded with RV (0.1% RV-rGO group). The coatings were applied using a dip-coating technique, with minor modifications [18].

Dip-coating procedure: Prior to coating, the silk sutures were sterilised by autoclaving at 121°C for 15 minutes. A 0.05% and 0.1% concentrations of rGO were dispersed in 10 mL of water and kept in a magnetic stirrer for three hours. The sterilised silk sutures were then dipped into the rGO dispersion for 30 seconds, ensuring complete immersion. Subsequently, the coated sutures were carefully withdrawn and allowed to air-dry at room temperature for 24 hours.

For the RV-rGO group, RV was incorporated into the rGO dispersion. A 500 mg RV capsule was dispersed in water and then added to 0.1% of rGO. This mixture was sonicated for 30 minutes to ensure homogenous dispersion of RV within the rGO nanoparticles. The sterilised silk suture was then dipped into the RV-rGO dispersion using the same protocol as described for the rGO group [Table/Fig-2].



[Table/Fig-2]: Preparation of silk suture coating: a) Magnetic stirring and dip-coating procedure for coating sterilised silk sutures with reduced Graphene Oxide (rGO); b) Magnetic stirring/sonication and dip-coating procedure for coating sterilised silk sutures with Resveratrol (RV)-loaded reduced Graphene Oxide (RV-rGO).

For each experimental group, three silk sutures (n=3) were prepared and subjected to physicochemical characterisation and biological evaluation. After coating, all suture groups were stored in a sterile, airtight container until further analysis.

Antibacterial assay: The antibacterial activity of the coated silk sutures was evaluated against *Streptococcus mutans* (ATCC 25175) and *Escherichia coli* (ATCC 25922), obtained from the Department of Microbiology, Saveetha Dental College and Hospitals, Chennai, India. The strains were revived from stock cultures and grown overnight in their respective growth media BHI broth for S.

mutans and MHB for *E. coli*) at 37°C. The coated sutures were then placed in a sterile Petri dish containing TSA inoculated with the respective bacterial strain at 37°C for 24 hours. The zone of inhibition surrounding the sutures of all four groups was measured to determine the antibacterial efficacy of the coatings against amoxicillin (positive control) and distilled water (negative control). All experiments were carried out in triplicate (n=3) and data were obtained from three independent experimental runs and expressed as mean±standard deviation.

Fourier Transform Infrared (FTIR) spectroscopy: The FTIR spectroscopy was performed on uncoated silk suture, coated silk suture with 0.1% rGO nanoparticles and silk suture coated with 0.1% rGO nanoparticles loaded with resveratrol (RV-rGO) to characterise the chemical changes resulting from the coating process. The spectra were recorded in the range of 4000-400 cm⁻¹ using Bruker's Alpha II FTIR Spectrometer (Thermo Scientific Nicolet 6700). The obtained spectra were analyzed to identify any functional group changes indicative of the successful loading of rGO and RV onto the silk sutures.

DPPH radical scavenging assay: The antioxidant activity among the four groups was evaluated using the 2,2- DPPH radical scavenging assay. Briefly, a DPPH solution (0.1 mM in methanol) was prepared. The 0.1% RV-rGO extract, 0.1% rGO extract and uncoated extract were prepared by extracting the coating from the suture using a suitable solvent (DMSO). The prepared extracts and ascorbic acid solution were combined with the DPPH solution and incubated in darkness for 30 minutes. The absorbance of the resulting mixture was then measured at 517 nm using a spectrophotometer. The percentage inhibition of DPPH radicals was calculated and the results were compared with the standard ascorbic acid [19].

Scanning Electron Microscopy (SEM) analysis: The surface morphology of the 0.1% rGO coated and 0.1% RV-rGO coated sutures was examined using SEM. The samples were sputter coated with gold and then observed under a Scanning Electron Microscope (SEM) (ZEISS Gemini SEM) at an accelerating voltage of 1 kV. The obtained images were analysed to visualise any morphological changes induced by the coating process.

STATISTICAL ANALYSIS

The collected data were compiled and analysed using the Statistical Package for the Social Sciences IBM SPSS software, version 23.0. All data are presented as mean±SD.

RESULTS

Antibacterial Assay

The antibacterial efficacy of the four suture groups (uncoated, 0.05% GO-coated, 0.1% rGO-coated, 0.1% RV-rGO-coated) against *S. mutans* and *E. coli* was assessed and compared to the standard antibiotic, amoxicillin. Notably, the 0.1% RV-rGO-coated sutures displayed the most pronounced antibacterial effect, achieving a higher zone of inhibition of 25±1 mm for *S. mutans* and 23±1 mm for *E. coli* than the 0.1% rGO-coated sutures, which showed a zone of inhibition of 22±1 mm for *S. mutans* and 20±1 mm for *E. coli* [Table/Fig-3].

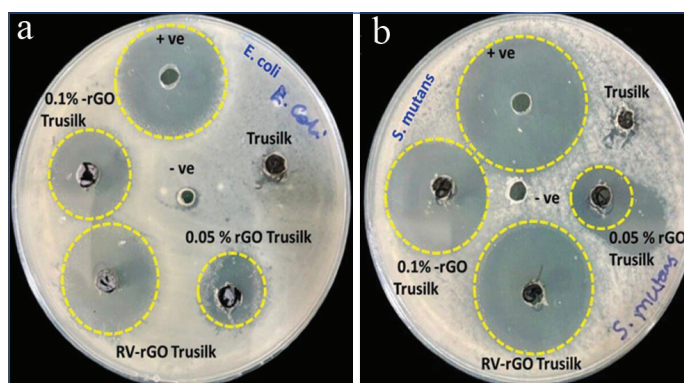
This finding suggests that the incorporation of RV into the rGO coating synergistically enhanced the antibacterial properties of the material. Furthermore, the antibacterial activity of the RV-rGO-coated sutures was almost equivalent to that of the standard amoxicillin treatment, indicating its potential as a promising antimicrobial agent for surgical sutures [Table/Fig-4].

FTIR Analysis

The FTIR analysis revealed the presence of Amide I (around 1600 cm⁻¹) and Amide II (around 1500 cm⁻¹) bands, confirming the proteinaceous nature of silk fibroin. The presence of the 1030 cm⁻¹

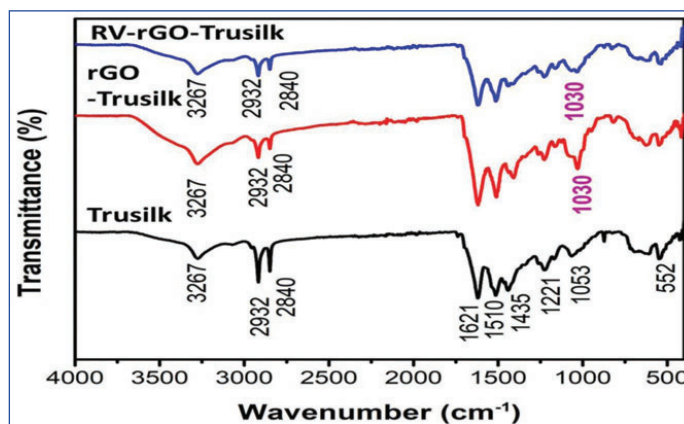
S. No.	Sample	Zone of inhibition (mm)	
		<i>S. mutans</i>	<i>E. coli</i>
1	Amoxicillin	26±1	21±1
2	Distilled water	0	0
3	Uncoated	0	0
4	0.05% rGO coated	20±1	18±1
5	0.1% rGO coated	22±1	20±1
6	RV-0.1% rGO coated	25±1	23±1

[Table/Fig-3]: Measurement of zone of inhibition against *S. mutans* and *E. coli*.



[Table/Fig-4]: Petri plate showing zone of inhibition of all four groups.

peak indicates C-O stretching from residual oxygen groups in rGO. FTIR spectrum provides detailed structural information, confirming the integration of GO onto the silk suture. Furthermore, in the RV-rGO-coated sutures, the presence of RV was confirmed by the appearance of new peaks corresponding to its functional groups, including phenolic hydroxyl and aromatic ring vibrations. These results confirmed the successful integration of both rGO and RV onto the silk sutures [Table/Fig-5].



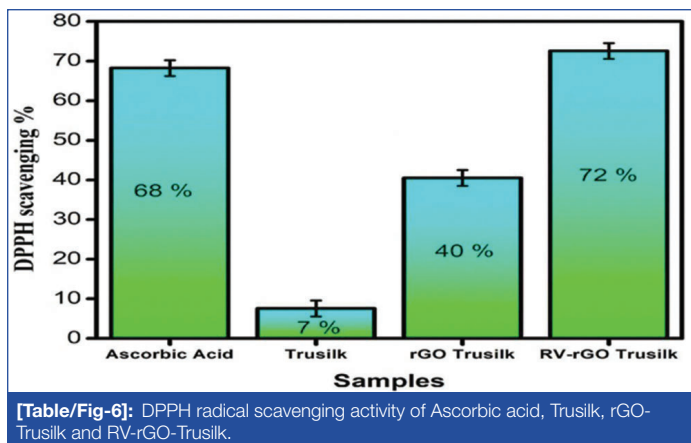
[Table/Fig-5]: FTIR spectra of Trusilk, rGO-Trusilk and RV-rGO-Trusilk.

DPPH Radical Scavenging Assay

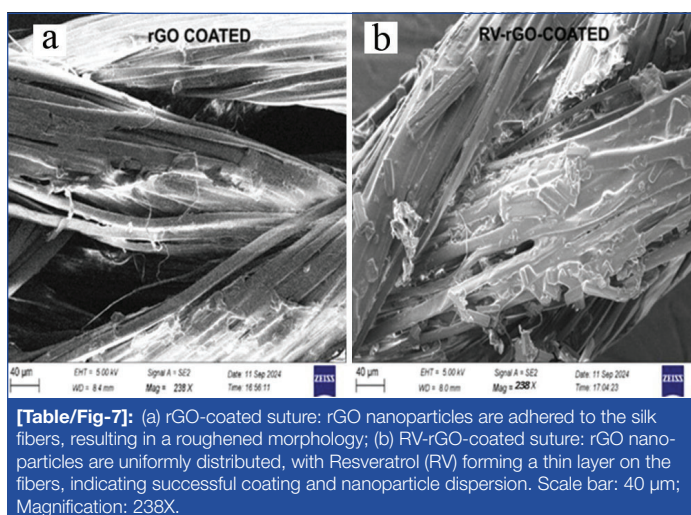
The antioxidant potential of the RV-rGO-coated sutures was evaluated using the DPPH radical scavenging assay. The percentage of DPPH radical scavenging by the RV-rGO-coated suture (72%) was higher than that of the standard antioxidant, ascorbic acid (68%), indicating its potent antioxidant properties. The addition of rGO to Trusilk enhances its antioxidant activity (40%) since it has properties that can contribute to free radical scavenging. RV is a potent antioxidant and its combination with rGO synergistically enhances the overall antioxidant potential up to 72%. The results demonstrated that the RV-rGO-coated suture exhibited a significant increase in DPPH radical scavenging activity compared to both the uncoated and rGO-coated sutures [Table/Fig-6].

SEM Analysis

SEM analysis was employed to visualise the surface morphology of the two suture groups (0.1% rGO coated and 0.1% RV-rGO



coated sutures). The rGO-coated sutures displayed a rougher surface with the presence of rGO nanoparticles adhered to the silk fibers. The RV-rGO-coated sutures displayed a similar morphology to the rGO-coated sutures, with the rGO nanoparticles appearing more uniformly distributed across the silk fibers [Table/Fig-7]. These observations confirmed the successful coating of rGO and RV-rGO onto the silk suture surface and provided visual evidence of the nanoparticle dispersion on the suture material.



DISCUSSION

The present in-vitro study aimed to investigate the efficacy of a novel silk suture coating composed of rGO and RV by evaluating the antibacterial efficacy of different suture groups (uncoated, 0.05% rGO-coated, 0.1% rGO-coated and 0.1% RV-rGO-coated) against *S. mutans* and *E. coli* and was observed that the 0.1% RV-rGO-coated sutures demonstrating the most pronounced antibacterial activity, achieving inhibition zones comparable to the standard antibiotic, amoxicillin. Also, FTIR analysis confirmed the successful integration of rGO and RV onto silk sutures, while SEM revealed uniform distribution of rGO and RV-rGO nanoparticles on the suture surface. The antioxidant activity of the RV-rGO-coated sutures, measured by DPPH scavenging assay, was significant, reaching 72%, indicating enhanced antioxidant potential compared to uncoated and rGO-coated sutures. These findings highlight the promising antibacterial and antioxidant properties of RV-rGO-coated sutures for surgical applications, thus accepting the study hypothesis.

The GO Nanoparticles (GONPs) have garnered significant attention for their potential in combating bacterial infections due to their unique physicochemical properties. Literature consistently demonstrates the antibacterial efficacy of GONPs against a wide range of bacterial species, including *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Sengupta I et al., reported that GO inhibited the growth of *S. aureus* and *P. aeruginosa* by 93.7% and

48.6%, respectively, primarily through chemical interactions leading to cell membrane damage, whereas rGO exhibited growth inhibition of 67.7% for *S. aureus* and 93.3% for *P. aeruginosa* by inducing mechanical stress and membrane-piercing effects [20].

The antibacterial activity of graphene-based materials is primarily attributed to their direct physicochemical interactions with bacterial cells, leading to membrane damage, disruption of essential cellular components and interference with nucleic acid function, as well as indirect mechanisms involving oxidative stress mediated by ROS generation [21]. The sharp edges and large surface area of GONPs contribute to their ability to disrupt bacterial cell membranes, leading to leakage of intracellular components and ultimately cell death. However, the precise mechanisms and the influence of GONPs' size, functionalisation and concentration on their antibacterial activity remain areas of ongoing research. Understanding these complexities is crucial for developing safe and effective GONP-based antibacterial therapies, while also addressing potential concerns regarding toxicity and environmental impact [22].

Regarding the antibacterial efficacy of RV, Elshimy R et al. and Cui W et al., have reported significant inhibitory activity of RV against both Gram-positive and Gram-negative pathogens [23,24]. In-vitro studies demonstrated that RV exhibited a Minimum Inhibitory Concentration (MIC) of 256 µg/mL and a Minimum Bactericidal Concentration (MBC) of 512 µg/mL against *Pseudomonas aeruginosa*. Additionally, RV at sub-inhibitory concentrations was shown to suppress *Staphylococcus aureus* virulence by inhibiting quorum sensing, biofilm formation and toxin production, while also enhancing bacterial susceptibility to selected antibiotics through efflux pump inhibition. Donadio G et al., elucidated the mechanisms underlying this activity through their study, which included the disruption of bacterial cell wall synthesis, interference with bacterial membrane integrity and inhibition of bacterial Deoxyribonucleic Acid (DNA) replication and protein synthesis [25].

Inflammation and oxidative stress are deeply interlinked, each fuelling the other in a symbiotic cycle. Oxidative stress typically arises from an overproduction of ROS, which is considered a key factor in impairing wound healing. RV has been identified as a scavenger of various free radicals, although its direct radical-scavenging activity is relatively modest. It reduces ROS production by inhibiting nicotinamide adenine dinucleotide phosphate oxidase activity and downregulating its expression. Additionally, RV enhances the expression of several antioxidant enzymes, contributing to its overall antioxidant properties [26]. RV also reduces JNK and COX-2 expression in keratinocytes [27].

The present novel RV-rGO-coating offers several advantages. Firstly, it is biocompatible and biodegradable, reducing the risk of adverse reactions and foreign body response. Secondly, a sustained release of RV from the GO scaffold would ensure a prolonged therapeutic effect, promoting optimal wound healing. Lastly, the ease of fabrication and potential application of this coating would make it a promising candidate for clinical translation.

The results of the present study are particularly relevant in the context of surgical wound healing, where the use of sutures is ubiquitous. Optimal wound healing is dependent on a complex interplay of factors, including inflammation, cell proliferation and tissue remodelling. The present findings suggest that the RV-rGO-coating can positively influence these processes, leading to accelerated wound healing and reduced complications.

Limitation(s)

The present in-vitro study does not replicate the complex biological environment of periodontal wounds, limiting direct clinical extrapolation. Further investigations, including in-vivo studies and clinical trials, are warranted to validate these findings and translate this promising technology into clinical practice.

CONCLUSION(S)

The RV-loaded rGO nano-coated silk sutures exhibited synergistic antibacterial and antioxidant activity. These findings underscore their potential to minimise suture-associated complications and enhance periodontal wound healing outcomes.

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