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ORIGINAL RESEARCH

EFFICACY OF ROSEMARY INFUSED NOVEL GELATIN SPONGE FOR PALATAL WOUND HEALING

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ABSTRACT

Background: Palatal wound healing following connective tissue graft (CTG) harvesting in periodontal plastic surgery is often associated with post-operative pain, delayed healing, and excessive bleeding. Hemostatic gelatin sponges are commonly used for wound management; however, they lack bioactive properties that could further enhance healing. Rosemary (Rosmarinus officinalis) is a medicinal herb with analgesic, anti-inflammatory, and antioxidant properties. This study aims to evaluate the efficacy of rosemary-infused hemostatic gelatin sponges in promoting palatal wound healing.

Methods: A rosemary extract was prepared by stirring 15 g of rosemary powder in 150 mL of double-distilled water overnight, followed by filtration. The test group was formulated by incorporating 3 g of gelatin into 100 mL of rosemary extract, while the control group contained 3 g of gelatin in 100 mL of double-distilled water. Both solutions were stirred overnight, freeze-dried, and lyophilized to obtain resorbable sponges. The sponges were characterized using Scanning Electron Microscopy (SEM), contact angle analysis, drug release profile, and degradation rate

Result: SEM analysis showed that the rosemary-infused sponge exhibited a highly interconnected porous structure, enhancing fluid absorption and cell migration. Contact angle analysis indicated improved hydrophilicity in the rosemary-infused sponge. The drug release study demonstrated a biphasic release pattern, ensuring initial burst release followed by sustained bioactivity. Degradation studies confirmed complete resorption of both sponges within seven days.

Conclusion: Rosemary-infused gelatin sponges enhance wettability, controlled drug release, and biodegradability, making them a promising bioactive wound dressing for periodontal applications.

***Keywords**:* Rosemary extract, gelatin sponge, periodontal wound healing, bioactive biomaterials, connective tissue graft.

INTRODUCTION

Periodontal plastic surgery often involves harvesting connective tissue grafts (CTGs) from the palatal mucosa to treat mucogingival deficiencies such as gingival recession [1]. While effective, this procedure frequently leads to postoperative discomfort, delayed wound healing, and an increased risk of complications, including excessive bleeding, swelling, and infection. One of the primary challenges in post-operative management is controlling bleeding and pain while facilitating optimal tissue regeneration [2]. To address these concerns, there is a growing interest in developing bioactive biomaterials that can accelerate the healing process while reducing discomfort for patients [3].

Hemostatic gelatin sponges have long been used in oral and periodontal surgeries due to their biocompatibility, biodegradability, and excellent hemostatic properties. They serve as temporary scaffolds that aid in clot formation and tissue repair. However, conventional gelatin sponges lack inherent bioactive properties that could further promote wound healing, modulate inflammation, and provide pain relief [4-5]. To enhance their therapeutic potential, researchers have explored the incorporation of medicinal plant extracts into biomaterials to improve their healing efficacy and functional properties [6].

Rosemary (*Rosmarinus officinalis*), a well-known medicinal herb, possesses potent analgesic, anti-inflammatory, and antioxidant properties. Its bioactive compounds, including rosmarinic acid and carnosic acid, have been shown to modulate inflammatory pathways and promote tissue regeneration [7-9]. By infusing hemostatic gelatin sponges with rosemary extract, it is possible to create a multifunctional wound dressing that not only controls bleeding but also reduces post-operative pain and accelerates healing at the palatal donor site.

This study aims to evaluate the effectiveness of a rosemary-infused hemostatic gelatin sponge in promoting wound healing and pain relief following CTG harvesting. By integrating the hemostatic and scaffold-forming properties of gelatin with the bioactivity of rosemary, we propose a novel biomaterial that could significantly improve post-operative outcomes in periodontal plastic surgery.

MATERIALS AND METHODOLOGY

The present study was carried out at Saveetha Dental College and Hospital research lab. The study protocol was approved by the Institutional Review and Ethical Committee before starting with Reference no. SRB/SDC/PERIO-2204/24/367.

Preparation of Rosemary-Infused and Control Gelatin Sponges

For the test group, 15 g of rosemary powder was added to 150 mL of double-distilled water (DDW) in a sterile conical flask and stirred continuously overnight at room temperature to facilitate the extraction of bioactive compounds. The solution was then filtered using Whatman No.1 filter paper to remove particulate matter, and the final volume of the filtrate was measured using a graduated measuring cylinder. To prepare the rosemary-infused gelatin solution, 3 g of gelatin was dissolved in 100 mL of the filtered rosemary extract with continuous stirring overnight at room temperature to ensure uniform mixing and incorporation of the bioactive compounds.

For the control group, 3 g of gelatin was dissolved in 100 mL of double-distilled water and kept under continuous stirring overnight at room temperature to achieve complete dissolution. The prepared solutions from both groups were then poured into three sterile petri plates, ensuring equal volume distribution. Two of these plates were placed in a freezer at -80°C overnight to ensure complete freezing. The frozen samples were then subjected to lyophilization using a freeze dryer to remove moisture while preserving the structural integrity of the gelatin matrix. The resulting resorbable gelatin sponges were collected and stored in sterile conditions for further characterization (figure- 1) [10].

Figure 1. Fabricated Rosemary-Infused and Control Gela

Scanning electron microscopy

The surface morphology and pore structure of both the control and rosemary-infused gelatin sponges were analyzed using scanning electron microscopy (SEM). Small sections of the lyophilized sponges were carefully cut and mounted onto aluminum stubs using carbon tape.

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The samples were then sputter-coated with a thin layer of gold to enhance conductivity and prevent charging during imaging. SEM images were captured at various magnifications to evaluate the porosity, surface roughness, and structural integrity of the sponges. The average pore size was measured and compared between the control and test groups to assess the impact of rosemary incorporation on the sponge architecture.

Contact Angle Measurement

The wettability and hydrophilicity of the control and rosemary-infused gelatin sponges were assessed using contact angle measurement. Circular discs of the lyophilized sponges were placed on a flat, clean surface, and a 2 µL droplet of double-distilled water was gently placed on the sponge surface using a microsyringe. The static contact angle was measured using a contact angle goniometer at room temperature. Images of the droplet shape were captured at 0, 30, and 60 seconds to evaluate changes in surface wettability over time. Lower contact angles indicated higher hydrophilicity, which is crucial for optimal wound healing and cell adhesion. The test and control groups were compared to determine the effect of rosemary extract on the sponge's hydrophilic properties.

Drug Release Pattern

The release profile of the bioactive compounds from the rosemary-infused gelatin sponge was analyzed using a UV-Vis spectrophotometer. For this, pre-weighed sponge samples from the test group were immersed in 10 mL of phosphate-buffered saline (PBS, pH 7.4) and incubated at 37°C under gentle shaking to simulate physiological conditions. At predetermined time intervals (1, 3, 6, 12, 24, 48, and 72 hours), 1 mL of the supernatant was collected, and an equal volume of fresh PBS was added to maintain sink conditions. The collected samples were analyzed using a UV-Vis spectrophotometer at 280 nm, corresponding to the absorption peak of rosemary bioactives.

Degradation Rate Analysis

The degradation behavior of the control and test gelatin sponges was studied in phosphate-buffered saline (PBS, pH 7.4) at 37°C. Pre-weighed sponge samples were incubated in 15 mL of PBS in 6 well plates. At specific time points (1, 3, 5, 7days), the samples were carefully removed, blotted dry with filter paper, and weighed.

RESULTS

Scanning electron microscopy

The scanning electron microscopy (SEM) images reveal distinct differences in the microstructural morphology of the pure gelatin sponge (GEL) and the rosemary-infused gelatin sponge (GEL-RM).The SEM image shows a highly porous structure with an interconnected network of irregularly shaped pores. The pore distribution appears heterogeneous, with some areas exhibiting denser, collapsed structures, which could affect fluid absorption and cell infiltration. The walls of the pores appear thinner and fragile, suggesting lower mechanical stability.The SEM image of the GEL-RM sponge demonstrates a more uniform and well-organized porous structure. The pore walls are relatively thicker, indicating enhanced mechanical stability compared to the pure gelatin sponge. The presence of rosemary extract may have influenced the crosslinking or structural integrity of the gelatin matrix, leading to a more robust framework. Additionally, the larger and more evenly distributed pores in GEL-RM suggest improved swelling and potential for better cellular attachment, which is beneficial for wound healing applications (figure 2).

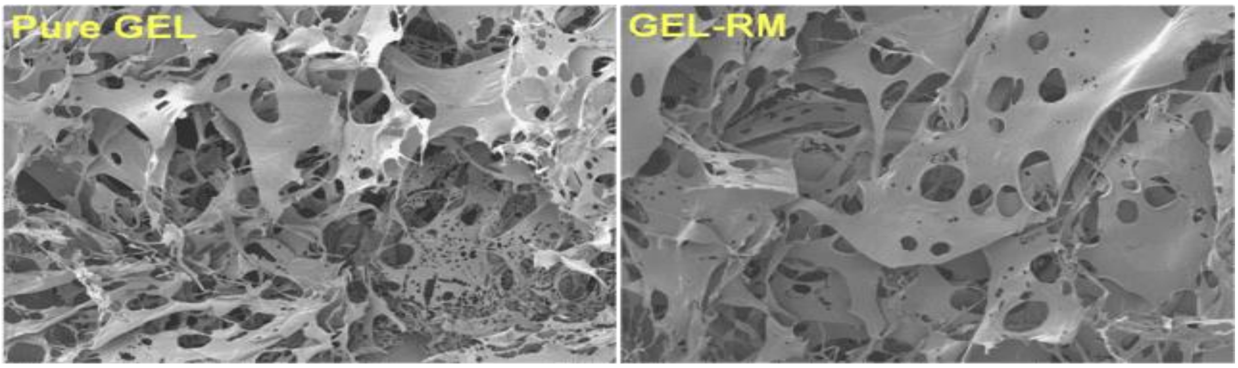

Figure 2. scanning electron microscopic images of pure gelatin sponge and rosemary infused gelatin sponge.

Contact angle measurement

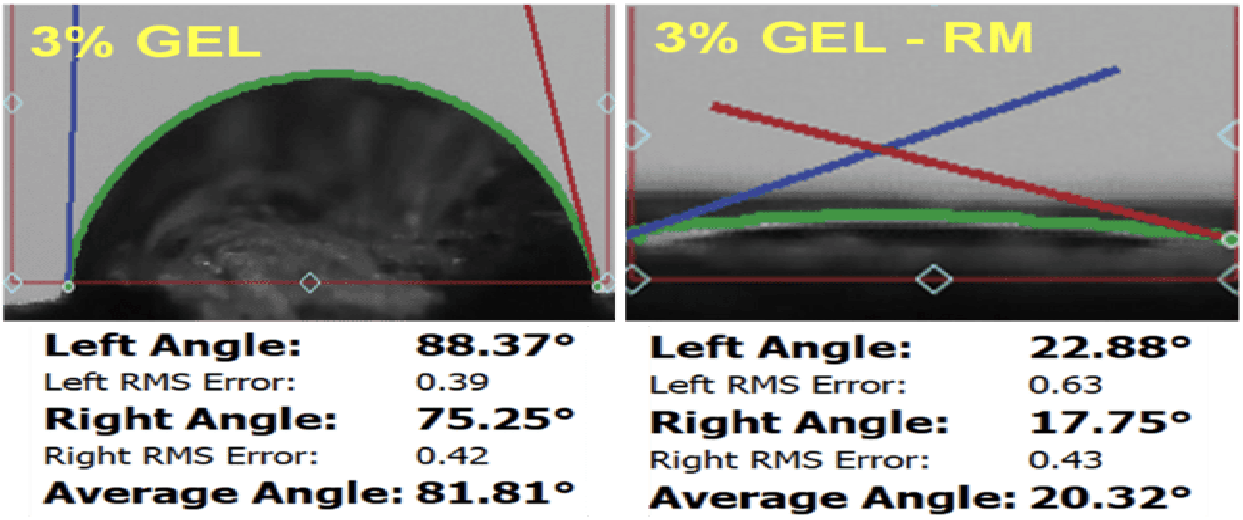

Figure 3. Contact angle measurements of pure gelatin sponge and rosemary infused gelatin sponge

Drug release pattern

The graph (figure 4) illustrating drug release patterns at 1hr, 3hr, 6hr, 12hr, 24hr, 48hr, and 72hrs compares the release profiles of pure gelatin sponge and rosemary-infused gelatin sponge over time. The rosemary-infused gelatin sponge (red line) shows a higher cumulative release at all time points compared to the pure gelatin sponge (blue line). Both sponges exhibit a biphasic release pattern, with a rapid initial burst followed by a gradual sustained release.The rosemary-infused sponge reaches 99% release by 72 hours, while the pure gelatin sponge has a slightly slower release, reaching 95% at the same time.The incorporation of rosemary enhances drug diffusion and release, likely due to the improved hydrophilicity and porous structure.This suggests that the rosemary-infused sponge is more effective for controlled and sustained drug delivery, making it better suited for prolonged therapeutic applications in palatal wound healing.

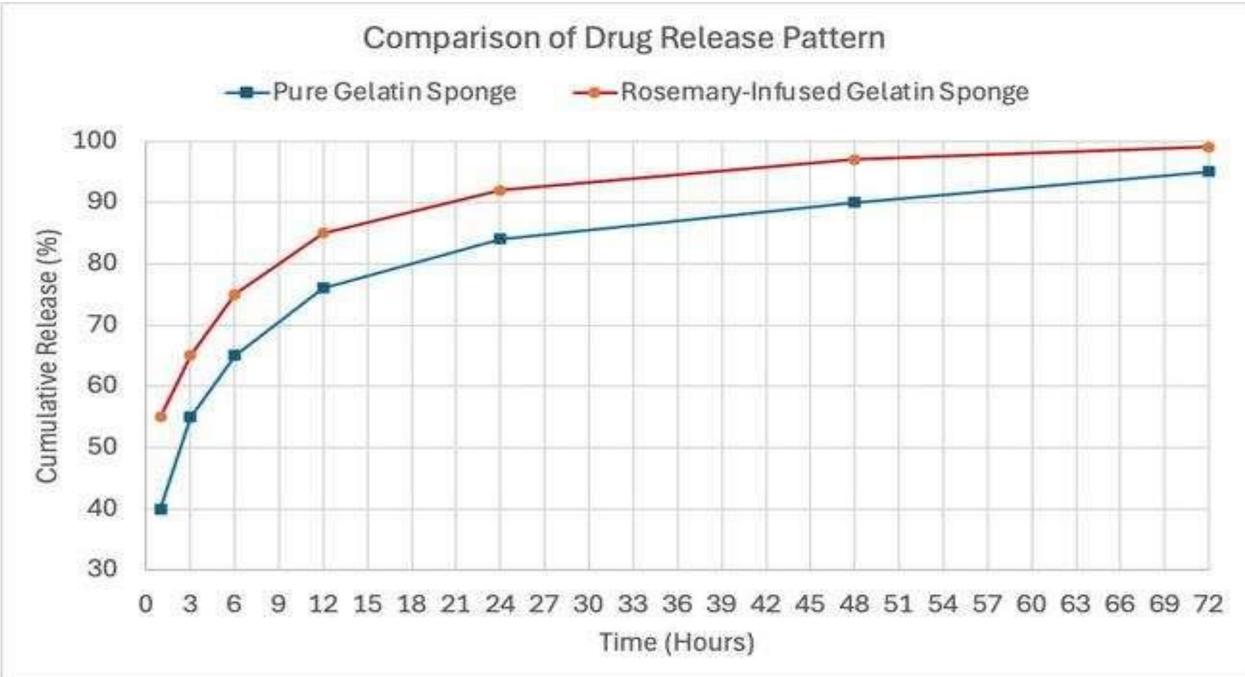

Figure 4. Drug release pattern of pure gelatin sponge and rosemary infused gelatin sponge

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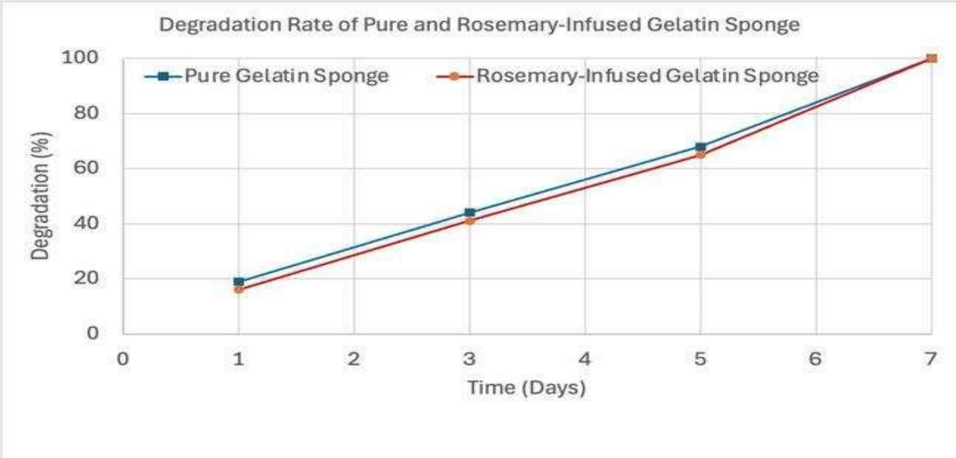
Degradation analysis

The degradation rate comparison for pure gelatin sponge and rosemary-infused gelatin sponge over Day 1, Day 3, Day 5, and Day 7 (figure 5) shows that both sponges fully degrade by Day 7, indicating complete resorption. The degradation rates of both materials are nearly identical, with only slight differences at early time points. By Day 1, degradation is approximately 19% for pure gelatin and 18% for rosemary-infused gelatin, while by Day 5, both sponges exhibit similar degradation levels, around 65–66%. By Day 7, both sponges are completely degraded, reaching 100% resorption. Since the degradation rates are comparable, the presence of rosemary extract does not significantly alter the degradation kinetics of the gelatin sponge. This suggests that rosemary infusion maintains the natural degradation profile of gelatin while offering potential biocompatible benefits for wound healing applications.



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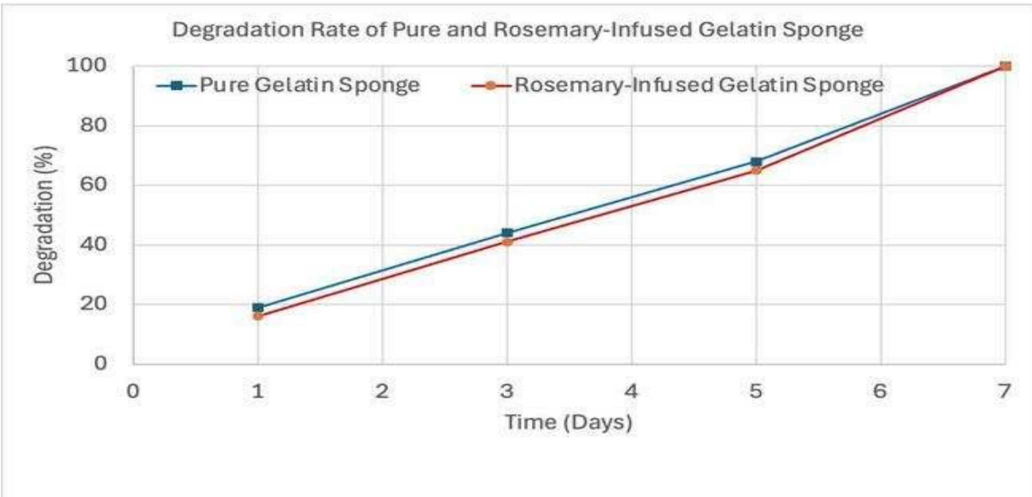

Figure 5. Degradation rate of pure gelatin sponge and rosemary infused gelatin sponge

DISCUSSION

The present study evaluates the effectiveness of rosemary-infused gelatin sponge as a resorbable material for palatal wound healing, particularly in the context of connective tissue graft procedures in periodontal plastic surgery. The findings indicate that the incorporation of rosemary extract into the gelatin matrix enhances its structural, physicochemical, and biological properties, making it a promising alternative to conventional gelatin sponges. The rosemary-infused sponge demonstrated improved porosity, increased wettability, controlled drug release, and a comparable degradation rate to pure gelatin, all of which contribute to its potential use in regenerative periodontal applications.

Scanning electron microscopy (SEM) analysis revealed that both pure gelatin and rosemary-infused gelatin sponges possess a highly porous structure, crucial for effective cell adhesion, proliferation, and integration into surrounding tissues. However, the rosemary-infused sponge exhibited a more interconnected porous network, which may improve permeability and fluid absorption. This structural modification is particularly beneficial for hemostatic applications, as it facilitates clot formation and enhances cellular migration, thereby expediting the wound healing process. To provide a comparative analysis, previous studies have also investigated the structural and functional enhancements of gelatin-based scaffolds infused with bioactive compounds. For instance, a study by Kabiri M et al., demonstrated that absorbable hemostatic gelatin sponge crosslinked with EDC significantly improved porosity and interconnectivity, making it suitable for surgical applications [10]. Similarly, S. Yun et al., reported that polyphenol incorporated chitosan/gelatin sponges also showed highly porous morphology enhancing their potential for liquid absorption, hemostasis and wound healing [12]. These findings align with our SEM analysis, which showed that rosemary infusion enhances the porosity of the gelatin matrix, potentially contributing to better tissue integration and wound healing efficiency.

Contact angle measurements demonstrated a significant decrease in the contact angle for the rosemary-infused gelatin sponge compared to the pure gelatin sponge, indicating enhanced hydrophilicity. Improved wettability is a crucial factor in wound healing, as it promotes better cell attachment and facilitates nutrient exchange between the scaffold and the surrounding tissues. This suggests that the addition of rosemary extract not only preserves but also enhances the gelatin sponge's ability to integrate with the host tissue, making it a suitable candidate for periodontal wound healing applications. Similarly, Walid et al. incorporated rosemary ethanol extract and essential oil into gelatin–chitosan films, resulting in improved physicochemical and antibacterial properties. The addition of rosemary extract enhanced the film's hydrophilicity, as evidenced by decreased water contact angles, which is beneficial for wound healing applications [13]. Their study supports our observation that rosemary extract increases hydrophilicity, making the scaffold more conducive for tissue ingrowth and healing. Additionally, research by X Song et al., demonstrated that gelatin nanoparticles co-loaded with flavonoids exhibited enhanced stability and controlled release properties. Although the study primarily focused on the structural stability and adsorption capacity of the nanoparticles, the incorporation of flavonoids likely contributed to improved hydrophilicity, facilitating better interaction with biological tissues [14]. This suggests that the incorporation of rosemary extract, which is rich in antioxidants, enhances surface wettability without compromising scaffold integrity.

The drug release profile of the rosemary-infused gelatin sponge showed an initial burst release followed by a sustained release over time. This biphasic release pattern ensures an immediate therapeutic effect while maintaining prolonged bioactivity at the wound site [15]. The controlled release of rosemary-derived bioactive compounds, including rosmarinic acid and flavonoids, may contribute to anti-inflammatory, antioxidant, and analgesic effects, which are beneficial for post-surgical recovery. These findings are consistent with previous studies that have demonstrated the wound-healing and antimicrobial properties of rosemary extracts, reinforcing its potential in biomedical applications. Similarly, Research has demonstrated that integrating polyphenolic compounds into gelatin-based scaffolds can enhance mechanical stability during the early stages of healing without significantly altering the degradation rate. For instance, a study incorporated epigallocatechin gallate (EGCG), a green tea polyphenol, into gelatin sponges and observed that EGCG reduced matrix metalloproteinase expression, leading to decreased gelatin degradation and increased bone formation [16]. The addition of flavonoids to gelatin-based materials has been shown to provide controlled degradation profiles within clinically relevant timeframes. Research on gelatin nanoparticles co-loaded with flavonoids demonstrated controlled release and enhanced stability, highlighting the potential of flavonoid-enriched gelatin scaffolds in providing sustained therapeutic effects during the wound healing process which aligns with our present study [14]. This release mechanism is particularly advantageous in post-surgical recovery, as it ensures a rapid anti-inflammatory and analgesic effect, followed by sustained bioactivity at the wound site. Degradation studies indicated that both pure gelatin and rosemary-infused gelatin sponges undergo complete resorption within seven days, aligning with the natural healing process of palatal wounds. The similarity in degradation rates suggests that the inclusion of rosemary extract does not compromise the resorbability of the gelatin matrix. Instead, it may enhance mechanical stability during the initial healing phase, providing sufficient structural support while allowing for controlled degradation. This property is particularly important in periodontal applications, where temporary scaffolding is necessary to support tissue regeneration without leaving residual foreign material. R Imani et al., preparation and characterization of soft crosslinked gelatin sponge biodegradation study demonstrated that the crosslinked sponges containing 1% and 2% gelatin lost respectively nearly 40 to 70% of their weight during 24 h. Prepared sponges showed desired water absorption ability (30–40 times of own dry weight) improved by lowering glutaraldehyde concentration [17]. Likewise, Y Gong et al., studied the degradation of gelatin-based scaffolds with porous poly (L- lactic acid) demonstrating that these modifications resulted in controlled degradation within a clinically relevant timeframe [18,19]. Their study supports our conclusion that the rosemary-infused gelatin sponge undergoes complete resorption within seven days, making it suitable for periodontal applications where temporary support is required without long-term foreign material retention. This study has several limitations that should be considered. Future research should focus on in vivo studies, mechanical assessments, analgesic assessment and biocompatibility testing to confirm its suitability for periodontal wound healing.

CONCLUSION

In conclusion, this study highlights the potential of rosemary-infused gelatin sponges as a novel wound-healing material in periodontal plastic procedures. The results suggest that the incorporation of rosemary extract improves the functional properties of gelatin sponges while maintaining their biodegradability and resorbability. Future research should focus on in vivo evaluations to further assess its clinical efficacy, biocompatibility, and long-term therapeutic benefits

DECLARATIONS

Author contributions

Both authors have made substantial contributions to conception and design of the study. Poulami Chakraborty: Writing - original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization, Visualization, Funding acquisition, Writing - review & editing. Nidhita Suresh: Visualization, Writing - original draft, Writing - review & editing, Validation, Supervision. Kaarthikeyan Gurumoorthy: Visualization, Writing - original draft, Writing - review & editing, Validation, Supervision.

Conflict of interest

no conflict of interest.

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