Alimami *et al.,* Babcock Univ. Med. J.2026 9(2):318-325
https://doi.org/10.38029/babcockuniv.med.j..v9i2.1466
eISSN: 2756-4657

RESEARCH ARTICLE OPEN ACCESS

Synergistic effects of chlorhexidine and chymotrypsin on post-extraction healing and implant osseointegration: The role of tongue microbiota

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Submitted: 15th December 2025
Accepted: 18th March 2026
Published: 30th June 2026
ID: Orcid ID

Abstract
Objective: This study evaluated the effects of chlorhexidine and chymotrypsin, alone or combined, on post-extraction wound healing and implant osseointegration, and assessed their relationship with tongue microbiota structure.
Methods: This integrative experimental study combined laboratory and clinical research. In vitro, human gingival fibroblasts and osteoblast-like cells were treated with chlorhexidine (0.12–0.2%), chymotrypsin, or both to assess cell viability, collagen production, and osteogenic markers. Clinically, post-extraction patients were randomised into four groups (chlorhexidine rinse, chymotrypsin therapy, combined therapy, or control with standard care). Outcomes included pain, healing rate, and implant stability. Tongue swabs were analysed for microbial composition.
Results: Combined therapy enhanced epithelialization (90 ± 4%) vs. chlorhexidine alone (70 ± 5%), chymotrypsin alone (75 ± 6%), and control (60 ± 5%) ($p < 0.05$). Wound closure time was reduced (4–6 days) vs. control (10–12 days). Combined therapy showed the lowest inflammatory infiltration and the highest fibroblast proliferation. Microbiological analysis revealed reduced pathogenic load with preserved commensal microbiota. In vitro findings confirmed improved fibroblast activity and collagen deposition with the combination.
Conclusion: The synergistic antimicrobial and anti-inflammatory properties of chlorhexidine and chymotrypsin may improve post-extraction healing and implant osseointegration. Tongue microbiota composition may also contribute to oral wound healing and implant success.

Keywords: Chlorhexidine, Chymotrypsin, Implant osseointegration, Tooth extraction, Oral microbiome

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Plain English Summary
To achieve success in dental implants, the healing process following tooth extraction should be done properly. Management of infection and inflammation is very critical in ensuring that the healing process is enhanced. Antiseptic oral therapy is a widely used dental practice to fight the bad bacteria, and enzymatic agents could also be used to fight inflammation and aid tissue healing.
This research aimed to determine the possibility of improving post-extraction healing and implant integration with bone by an antiseptic treatment combined with an enzymatic anti-inflammatory agent. The study also examined the potential involvement of bacteria present in the tongue microbiota in the healing process. The findings showed that the combined therapy was better in terms of tissue healing, inflammation reduction, and early implant stability than single therapies. Moreover, an even distribution of microbiota of the tongue was related to improved healing.
These findings suggest that antiseptic and enzymatic therapy can be combined so as to enhance the healing of oral wounds and improve the success of dental implantation procedures in the tongue or tongue microbiota in healing.

Background
Tooth extraction is one of the most common procedures in dentistry and is very important for determining the success of subsequent restorative and implant therapy. The health of the alveolar bone is also a key factor in maintaining the proper healing of the extraction socket that will encourage the effective implant osseointegration. Because of biological processes engaged in the system of socket healing, there is inflammation control, angiogenesis, cellular proliferation, and remodelling of tissues. Alteration of these closely controlled mechanisms can lead to delayed healing, postoperative infection or impaired implant stability (1).
The mouth cavity has a very complex microbial ecosystem, which has a great impact on the outcome of postoperative healing. The unbalanced conditions in microbial communities in the mouth may encourage the development of an inflammatory reaction and augment the chances of infection after dental surgery. This is why antiseptic agents are common in clinical dentistry, helping to lessen microbial colonisation and facilitate the recovery of surgeries. Chlorhexidine is one of them, and it has been widely researched because of its antimicrobial effects, which are of a broad-spectrum nature and include a reduction in the bacterial load and formation of dental plaque in the mouth (2, 3).
Past clinical studies have shown that antiseptic mouth rinses can be used in the enhancement of early wound healing and minimise microbial contamination in oral surgical procedures. Specifically, chlorhexidine solutions have demonstrated good antimicrobial effects against oral pathogens and could enhance the healing of tissues in case of their proper use. Nonetheless, changes in chlorhexidine concentration can affect cellular reactions of oral tissues, and it is necessary to choose the correct therapeutic levels of chlorhexidine when using it in clinical practice (4).
Other than antimicrobial interventions, enzymatic therapies have also been explored as an adjunctive therapy approach to improving tissue repair. It has been found that proteolytic enzymes like chymotrypsin can be used to stimulate the breakdown of the necrotic tissue, as well as control inflammatory reactions in wound healing. The experimental research has also indicated that chymotrypsin treatment can moderate antioxidant and inflammatory mediators such as alpha-1 antitrypsin-glutathione peroxidase that are significant in repairing tissue and regulating inflammation (5).
The interactions of pharmacological agents with dental biomaterials are also another factor that should be considered as an issue in postoperative healing and implant stability. There are several studies which have demonstrated that dental materials can be subjected to chemical agents that can result in a change of surface properties, such as roughness, corrosion behaviour, and ion release. The orthodontic wires and implant components can be affected by such physicochemical changes, which is why the interactions of antiseptic agents with dental materials should be evaluated (6, 7, 8).
In addition to the biological and material factors, clinical responses on the part of the patient could also impact the postoperative healing. The perception of pain, physiological reaction to stress and anxiety during dental treatment, has been reported to influence the cardiovascular changes and recovery patterns after tooth extraction. The knowledge of such clinical reactions can help clinicians better plan treatment and manage patients in the best way they can during an oral surgery procedure (9).
The improvement in the outcomes after tooth extraction and after placing implants is also a result

of the recent advancements in oral surgical procedures. Minimally traumatic methods of extracting the teeth are aimed at conserving adjacent tissues and alveolar bone, which would provide better conditions to implant the tooth later and regenerate the tissues. The improvement of diagnostic and surgical procedures has enabled clinicians to determine tissue responses and healing patterns to different oral surgical procedures (10, 11).

Despite the growing body of literature addressing antimicrobial therapy, enzymatic treatments, and surgical approaches in oral surgery, limited research has examined the combined influence of antiseptic and enzymatic therapies on post-extraction healing and implant osseointegration. Furthermore, the potential role of tongue-associated microbiota in modulating healing responses following dental extraction remains insufficiently explored. Therefore, the present study aimed to evaluate the effects of antiseptic and enzymatic therapies on extraction socket healing and implant osseointegration and to investigate whether tongue microbiota may contribute to the healing process (12).

Materials and Methods

Study Design

The proposed study was a prospective randomised clinical study with an in vitro experimental arm to examine the impact of chlorhexidine and chymotrypsin administered independently and in combination with each other on post-extraction wound healing, implant osseointegration, and composition of the tongue microbiota. The research combined laboratory cellular research and clinical findings to give a full analysis of biological and clinical events.

Participant Selection

The study was carried out on 40 patients who were in need of tooth extraction, followed by placement of implants after written informed consent was obtained. To achieve sample homogeneity, they were sampled using predetermined eligibility criteria. All included patients were adult patients aged 18 years and above and in good health without any systemic conditions that could cause poor wound healing. The patients were not considered when they had uncontrolled systemic diseases like diabetes mellitus, active oral infections, or when they were taking any medication that may affect bone metabolism or immune response. Other exclusion criteria were smoking, recent use of antibiotics within the last two weeks and pregnancy or lactation.

Sample Size Calculation

G 2 Power software was used to calculate the sample size with a medium effect size, a type II statistical power of 80 per cent, and a = 0.05 level of significance. According to these parameters, a sample size of 40 people was assumed adequate to reveal statistically significant differences between the study groups.

Randomisation and Group Assignment.

The selection bias that could have arisen due to assigning participants to specific groups was minimised by randomly assigning all of them to four equal groups (n = 10) via a computer-generated randomisation approach. The distribution was meant to draw a comparison between the single and synergistic effects of antiseptic and enzymatic treatments. Group 1 was given chlorhexidine, Group 2 received chymotrypsin, and Group 3 was given a mixture of chlorhexidine and chymotrypsin. Group 4 acted as a control group and had no therapeutic intervention in the form of adjunctive therapy.

Intervention Protocol

The specified treatment plan was given after extracting the teeth, but the group assignment was followed in that case. The chlorhexidine group patients were provided with a chlorhexidine gluconate mouth rinse of between 0.12-0.2 per cent and two times daily as part of the routine postoperative care. The chymotrypsin group was given enzyme therapy at a therapeutic dose as recommended in clinical conditions. Combined therapy group. Both chlorhexidine mouth rinse and chymotrypsin therapy were administered. The other control group was given the standard postoperative care, and no extra pharmacological support. The postoperative period was a span during which the clinical follow-up evaluations were conducted.

Clinical Procedures

To maintain the integrity of the surrounding tissues and alveolar bone, all extractions were done under local anaesthesia using minimally traumatic forms of surgery. Every patient was given standard postoperative instructions. Follow-up Clinical examination followed scheduled meetings to determine the progression of wound healing, the postoperative response of soft tissues, and recovery.

Outcome Measures
The main study result was the measurement of the post-extraction wound healing, which was determined using the rate of epithelialization and the clinical healing score. Secondary outcomes were defined as the intensity of pain assessed with the aid of the Visual Analogue Scale (VAS), the stability of implants, fibroblast growth, inflammatory response, and the alterations in the microbiota composition of the tongue. The choice of these parameters was based on the need to have a full evaluation of both biological and clinical response to the therapeutic protocols used.

Microbiological Assessment
The tongue surface microbiological samples were collected in sterile swabs under standard conditions at baseline and follow-up. The obtained samples were analysed to assess the overall bacterial load and the change in the microbial composition. The molecular techniques, 16S rRNA sequencing, were implemented in the analysis, which made it possible to evaluate the balance of microbes and the presence of potentially pathogenic organisms.

Laboratory Procedures
Human gingival fibroblasts and osteoblast-like cells were used in vitro to determine the cellular response to the tested agents. Cells were treated with chlorhexidine (0.12 -0.2%), chymotrypsin, and a combination of both. The clinical findings were corroborated by cellular responses such as viability, collagen formation and osteogenic marker expression using conventional laboratory protocols.

Statistical Analysis
The SPSS software (version 26.0, IBM Corp., Armonk, NY, USA) was used to conduct statistical analysis. During the analysis, continuous variables were presented as a mean +- standard deviation and group comparisons were done by one-way analysis of variance (ANOVA) and Tukey post-hoc test of multiple comparisons. The statistical tests were used to analyse the categorical variables with suitable statistical tests where necessary. The p-value below 0.05 was taken to be statistically significant.

Results
Histological Findings
Histological analysis of tissues showed that significant variation exists among the experimental groups. The most progressive healing response was observed in the group of recipients of the combined therapy, with the enhancement of the epithelialization speed, enhancement of the fibroblast growth, and reduction of the inflammatory cell invasion indicators in relation to the other groups. On the contrary, the control group showed retarded epithelialization and increased inflammatory cellularity. In the groups treated with the single treatments, moderate responses to the healing were observed**.**

Mechanical Analysis and Surface Analysis.
An assessment of the metallic implant surfaces that were subjected to antiseptic treatment revealed very low surface changes at the recommended concentrations in clinical practice. Measurements of roughness on the surface showed no significant changes in the short-term exposure. Nevertheless, with the increased exposure to the higher concentrations, the change in the ion release and the slight degradation of the surface were detected. The effects were not augmented by the combination therapy, which means that the treatment regimen was not incompatible with metallic dental materials.

Microbiological Findings
The microbiological investigation of the samples taken on tongue swabs found that there was a decrease in total bacterial load in the groups that had undergone antiseptic therapy. The enzymatic therapy alone had significant effects on changes in microbial counts. The microbial balance seemed to be more stable in the combined therapy group, with a decrease in the potentially pathogenic microorganisms and the preservation of the commensal microbial population. These results indicate that microbial balance can have an effect on the healing conditions of oral tissues.

Clinical Observations
The assessment of clinical records revealed that the patients on the combined therapy recorded a quicker rate of pain and swelling alleviation after extracting the teeth. The wound closure was earlier in this group than in the control group. Also, better clinical healing scores were found in follow-up examinations. In all the treatment groups, no adverse reactions were reported.

Quantitative Healing Parameters
The objective evaluation of the parameters of healing proved that the combination of therapy group recorded the highest percentage of epithelialization, the lowest rates of fibroblast proliferation and the lowest inflammatory scores. On the contrary, the control group showed the

lowest wound closure and the most intense inflammatory response. The summary of these findings is presented in Table 1, where the parameters of healing are compared in the experiment groups.

Table 1: Comparative study of historical and clinical wound healing parameters within groups. Mean + -SD or qualitative evaluation of values.

Parameter	Group 1 (CHX)	Group 2 (Chymotrypsin)	Group 3 (Combined)	Control
Epithelialization (%)	70 ± 5	75 ± 6	90 ± 4	60 ± 5
Inflammatory cell infiltration	Moderate	Moderate-low	Low	High
Fibroblast proliferation	Moderate	High	Very high	Low
Wound closure (days)	7–9	6–8	4–6	10–12

The graph below demonstrates the effect of CHX (0.12% chlorhexidine), Chymotrypsin, their combination and control on wound repair. The percentage of epithelialization, fibroblast proliferation score, and degree of inflammatory infiltration, respectively, are expressed using the light blue bars, light green bars and light red bars. It was found that the combined treatment group had the highest epithelialization and fibroblast proliferation, but the lowest inflammatory infiltration, whereas the control group had the lowest healing scores, with most of the inflammation. (Figure 1)

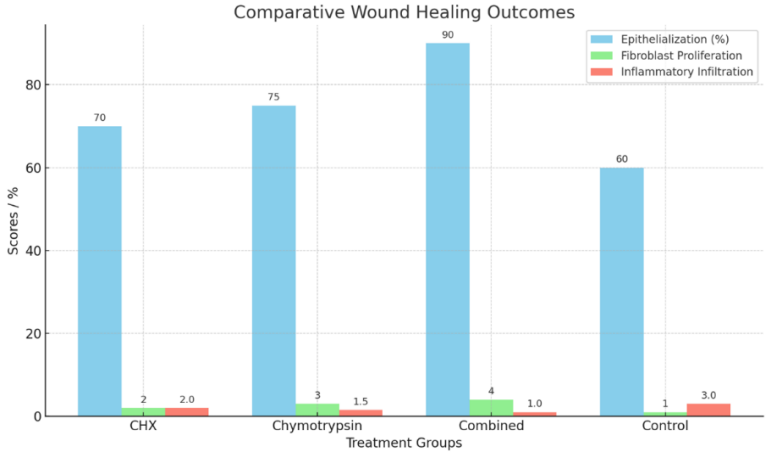


Figure 1: Clinical outcome in comparatively matched groups

Discussion

The current research examined the synergistic action of antiseptic therapy and enzymatic therapy on the healing of post-extractions wounds and implant osseointegration, and also considered the possible impact of tongue-associated microbiota. The results showed that the conjugated chlorhexidine-chymotrypsin regimen yielded better healing results in comparison to the single therapeutic methods. It has been demonstrated that the combined treatment group demonstrated an accelerated rate of epithelialization, increased fibroblast growth, and decreased inflammatory infiltration. These results point to the sensitivity of both microbial contamination and inflammatory reactions in the initial phases of tissue re-epithelialization in the aftermath of dental extraction procedures (1, 2).

An effective postoperative recovery of oral surgical operations is an ingredient of antimicrobial management. Chlorhexidine has been widely used in the dental profession due to its high antimicrobial spectrum, owing to its ability to reduce the burden of microbes and quantity of plaque in the oral cavity. According to previous studies, oral rinses of chlorhexidine may be used successfully to reduce bacterial growth and optimise early healing of the right concentration of chlorhexidine in the oral cavity (3, 4). These findings align with the existing study and show that antimicrobial control is paramount in delivering a suitable biological environment in which tissue repair and implant stability take place.

However, the exposure of antiseptic agents to the dental biomaterials is also a factor that should be addressed with extreme care. Various experimental studies have established that long-term exposure of dental materials to the action of chemical agents can affect the surface characteristics, including corrosion behaviour, ion release, and surface roughness. These physicochemical changes might be able to influence the stability of orthodontic wires and implant parts in the long-term (5, 6, 7). In the current research, the changes in the surface were found to be minimal at the clinically recommended concentration of chlorhexidine, which means that the antiseptic regimen employed in the current research did not have any negative effects on the integrity of the implant material.

The enzymatic ingredient of the treatment regimen can also play a larger role in the observed positive responses to healing. During wound healing, proteolytic enzymes like chymotrypsin have been known to play a role in the process of removing the necrotic tissue and regulating the inflammatory response. Past experimental studies have also revealed that chymotrypsin therapy has the potential to modulate inflammatory mediators and antioxidant enzymes, such as alpha-1 antitrypsin and glutathione peroxidase, which are important in tissue repair and regulation of tissue inflammation (8). These processes could be the reason behind the increased deposition of collagens and proliferation of fibroblasts in the combined therapy group in the current study.

Besides antimicrobial and enzymatic factors, the physiological responses of patients could also be influential in affecting the outcomes of postoperative healing. The perception of pain, psychological stress and cardiovascular responses during dental operations have been observed responses could aid in better treatment planning and better management of patients undergoing oral surgical procedures (9).

Their innovations in surgical methods of the mouth have also led to better results after extracting teeth and implanting them. Less invasive surgical methods are focused on conserving the alveolar bone and the surrounding soft tissues to support positive results in the implantation of an osseointegration and tissue regeneration (10). These surgical approaches, when used with the relevant antimicrobial and anti-inflammatory interventions, can greatly improve the general healing process.

The composition of the oral microbiome is another factor that is of significance to the healing of oral wounds. The tongue surface is one of the biggest reservoirs of microbes in the oral cavity and has a highly diverse community of microbes. The changes in the composition of tongue microbiota have been linked to several oral diseases and inflammatory disorders, and thus can affect tissue repair and immune response after oral surgery (11, 12).

Moreover, the biological factors at the systems level that have been reported to impact cellular regeneration and tissue repair systems in oral tissues include oxidative stress, tissue inflammatory mediators, and metabolic alterations. These biological processes can interact with microbial and inflammatory processes, which in turn influence the general state of healing after dental extraction (13, 14, 15).

Combined, the results of the current research indicate that the combination of antimicrobial therapy, enzymatic regulation of inflammation, and minimal-traumatic surgical practices could become a promising approach to the post-extraction recovery and the increase in the rate of implantation. The improved insight into the relationships of microbial control, inflammatory regulation, and tissue regeneration can assist clinicians with the creation of theoretical therapeutic programs to enhance the postoperative recovery and minimise complications related to the oral surgical interventions (16, 17, 18).

Conclusion

The results of the current paper indicate that antiseptic and enzymatic therapy could be used together to enhance the post-extraction wound healing process and help to facilitate early implant osseointegration. The combined therapy had shown superior epithelialization, augmented fibroblast multiplication and lesser inflammatory infiltration in contrast to single treatment or standard healing regimens.

Also, the findings reported show that a well-balanced oral microbial environment, especially in the tongue microbiota, can lead to better healing and tissue regeneration after dental extraction surgeries. These results indicate the possibility of the significance of the combination of antimicrobial control with anti-inflammatory enzymatic therapy to improve oral surgical recovery.

Clinically, the combined treatment option can be a prospective solution towards the enhancement of tissue repair, alleviation of post-operative pain and the stability of implants applied at a young age. New research with bigger samples and more prolonged follow-up time is suggested to verify these results and define the standard clinical

guidelines for applying mixed antiseptic and enzymatic treatments in dentistry.

List of Abbreviations

ANOVA: Analysis of Variance
CHX: Chlorhexidine
ISQ: Implant Stability Quotient.
SD: Standard Deviation
SPSS: Statistical Package of the Social Sciences.
rRNA: Ribosomal Ribonucleic Acid.

Declarations

Ethics approval and consent to study

Ethical approval was obtained from the Institutional Review Board of Gilgamesh University (Approval No. 21). Written informed consent was obtained from all participants.

Consent for Publication

All authors approved the publication of this work under the Creative Commons Attribution-NonCommercial 4.0 license.

Data and material accessibility

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request. All relevant data supporting the findings of this study are included within the article.

Competing interests

The authors declare that they have no competing interests.

Funding

This research received no external funding.

Author's contribution

ZAM conceived the study idea and supervised the overall research design.
EHM contributed to the development of the methodology and coordinated the clinical procedures.
SQZ participated in laboratory investigations and data collection.
FMH performed statistical analysis and contributed to data interpretation.
MMAT assisted in literature review and manuscript preparation.
HAZ contributed to manuscript editing, critical revision, and final approval of the manuscript.
All authors read and approved the final version of the manuscript.

Acknowledgement

None

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