Comparison of Curcumin and Amoxicillin trihydrate Incorporated onto Guided Tissue Regeneration Membrane against Porphyromonasgingivalis: An In vitro Study

P.L.Ravishankar[1], Gayathri.K[2], Kalaivani.V[3], Viola Esther P.S [4], Prem Blaisie Rajula M[5] , Aadhithiyan.S[6]

[1]Head of the department,
Department of Periodontology,
SRM Kattankulathur Dental College and hospital
SRM Institute of science and technology
SRM Nagar, Kattankulathur 603203, Chengalpattu,Tamil Nadu, India

[2]Assistant professor,Department of Periodontology,
SRM Kattankulathur Dental College and hospital
SRM Institute of science and technology
SRM Nagar, Kattankulathur 603203, Chengalpattu,Tamil Nadu, India.

[3]Associate Professor,Department of Periodontology,
SRM Kattankulathur Dental College and hospital
SRM Institute of science and technology
SRM Nagar, Kattankulathur 603203, Chengalpattu,Tamil Nadu, India

[4]Assistant professor,Department of Periodontology,
SRM Kattankulathur Dental College and hospital
SRM Institute of science and technology
SRM Nagar, Kattankulathur 603203, Chengalpattu,Tamil Nadu, India.

[5]Assistant professor, Department of Periodontology,
SRM Kattankulathur Dental College and hospital
SRM Institute of science and technology
SRM Nagar, Kattankulathur 603203, Chengalpattu,Tamil Nadu, India.

[6]Postgraduate student, Department of Periodontology,
SRM Kattankulathur Dental College and hospital
SRM Institute of science and technology
SRM Nagar, Kattankulathur 603203, Chengalpattu,Tamil Nadu, India

ABSTRACT:

Background:
Guided Tissue Regeneration (GTR) is a effective periodontal treatment modality for regeneration, but it is able to be colonised by bacteria. Recently, attention has been drawn to the conflict between using conventional antibiotics and antimicrobial compounds generated from plants.
Aim:

ISSN: 2173-0695

The current study aimed to evaluate the antimicrobial activity of 1% Amoxicillin and 1% Curcumin loaded onto a GTR membrane against Porphyromonas gingivalis in vitro.
Methodology:
To dilute the concentrations to 1% Amoxicillin and 1% Curcumin, an appropriate amount of distilled water was used. Normal saline used as a negative control, and the GTR membrane was a sterile reconstituted Type-I collagen membrane. Membrane was soaked in the diluted solution. The antibacterial efficacy of GTR membrane coated with 1% Amoxicillin solution and 1% Curcumin solution was evaluated in vitro using the agar disc diffusion method.
Results:
The zone of inhibiton for GTR membranes treated with 1% Amoxicillin solution showed antibacterial activity against Porphyromonasgingivalis.whereas 1% Curcumin solution and saline showed no zone of inhibition.Hence amoxicillin exhibited effective antimicrobial action.
Conclusion:
Loading Amoxicillin onto the GTR membrane can help to prevent bacterial colonisation and improve treatment outcomes.

KEYWORDS: Amoxicillin, Curcumin, Disc diffusion, Guided Tissue Regeneration, Normal saline, Porphyromonas gingivalis.

Introduction

Periodontitis, a chronic inflammatory condition impacting the supporting structures of teeth, is closely associated with various bacterial species, and one of the key culprits is Porphyromonasgingivalis. It is a gram-negative anaerobic bacterium, often identified in periodontal pockets contributes to the dysbiosis of the oral microbiota[1] and plays a significant role in the progression and severity of periodontal diseases.

Virulence factors like gingipains, lipopolysaccharides, and fimbriae contribute to the ability of P. gingivalis to evade host immune responses and manipulate the local environment to promote disease progression[2] In the context of therapeutic interventions, targeting P. gingivalis has become a focal point in the management of periodontitis[3].

Guided Tissue Regeneration (GTR) is a pivotal therapeutic technique in periodontics, designed to facilitate the regeneration of periodontal tissues which is the primary goal of periodontal treatment. The efficacy of GTR in promoting new attachment level, bone fill, and overall improvement in clinical parameters is well known[4]. However, a significant challenge in GTR procedures is the potential contamination of the membrane by periodontal pathogens during or shortly after the intervention [5].

Porphyromonasgingivalis and Aggregatibacteractinomycetemcomitans are the most common pathogens, identified on GTR membranes shortly after placement. These bacteria are implicated in periodontal diseases and can compromise the regenerative potential of GTR by triggering an inflammatory response and interfering with tissue healing. Moreover, a study by Lee et al. (2012) examined bacterial contamination of GTR membranes in guided bone regeneration procedures. The results indicated that bacterial contamination occurred within minutes of exposure to the oral environment, raising concerns about the immediate susceptibility of GTR membranes to microbial colonization [6].

To enhance the success of GTR and address concerns related to postoperative infections, researchers have explored the incorporation of antimicrobial agents into GTR membranes. This approach aims to prevent bacterial colonization on the membrane surface, thereby reducing the risk of infections and optimizing the regenerative potential of the treatment[7]. Chen et al[8] conducted a study revealing that in vivo, biodegradable nanofibrous collagen membranes containing amoxicillin exhibited a sustained

release of the antibiotic over 28 days. The released amoxicillin demonstrated significant bioactivity against Staphylococcus aureus.

A research by Siamak Yaghobee et al[9] indicated that incorporating amoxicillin into guided tissue regeneration (GTR) membranes effectively minimizes bacterial penetration and colonization of Streptococcus mutans and Aggregatibacter actinomycetemcomitans. This is crucial in mitigating the risk of membrane-related infections during regenerative intervention. The importance of incorporating antimicrobial agents in GTR extends beyond infection prevention. The effectiveness of conventional antibiotics and plant based antimicrobial agents has recently come under scrutiny [10]. Over centuries, plants with medicinal properties have been utilized for treating various human diseases worldwide.

Curcumin found in Curcuma longa exhibits antimicrobial, antioxidant, immunostimulant, antiseptic and healing properties. Jain et al[11] explored the application of nanofibers loaded with curcumin for bone tissue regeneration and found that these nanofibers exhibited a positive impact on cell proliferation and osteogenesis which was evident from the heightened expression of osteogenic markers and increased mineral deposition observed in the study.Incorporation of curcumin into GTR address challenges associated with traditional antimicrobial treatments, emphasizing the importance of sustainable and effective alternatives in contemporary periodontal care. This approach aligns with the global concern of emerging drug resistance in pathogenic bacteria and the undesirable side effects associated with synthetic antimicrobial agents. This study aimed to evaluate the antibacterial properties of amoxicillin and curcumin loaded onto GTR membranes against P gingivalis in vitro.

Materials and methods:

This study was conducted at SRM in the Department of Periodontology at SRM Kattankulathur Dental College and Hospital in Potheri, Tamil Nadu, and the microbial assay was performed at Nathajirao G Halgekar Institute of Dental Sciences and Research Centre in Belgaum, Karnataka. This study employed an in vitro experimental design. The study received ethical approval from the Institutional Ethics Committee of SRM Kattankulathur Dental College and Hospital in Potheri.

The investigation necessitated the utilization of pure forms of Amoxicillin (Hindustan Pharmaceuticals LTD) and Curcumin (Grenera) at a quantity of 1.0 g each (Figure No.-1). These antimicrobials were dissolved in distilled water to achieve a concentration of 1% Amoxicillin and 1% Curcumin. For the negative control, normal saline was employed. The GTR (Guided Tissue Regeneration) membrane used in this study was a sterile reconstituted Type-I collagen membrane from Healiguide - Advanced Biotech Products (P) LTD (Figure No.-1). Aseptically, the membrane was cut into 1x0.5 cm squares.

In sterile petri dishes containing the GTR membrane, a solution of 10 ml of 1% Amoxicillin and 1% curcumin was poured. The GTR membrane was soaked in the concentrated solution for 15 minutes before being air-dried for five minutes (Figure No.-2).

Microbiological Assay

1% curcumin solution was evaluated using the Agar disc diffusion method. P. gingivalis was cultured and examined on blood agar. Microorganism colonies were transferred to the agar plate using an inoculation loop, ensuring even distribution by rotating the plates at approximately 600 between streaks. Three plates were inoculated in total. The plates were allowed to stand for 3 minutes but not more than15 minutes before introducing the GTR membrane containing different antimicrobial substances for testing. Subsequently, the plates were incubated within 15 minutes of placing the GTR membrane, and P. gingivalis bacteria were cultured for 48 hours (Figure No. -3). The McIntosh and Filde's anaerobic jar were utilized for cultivating anaerobic microorganisms. After the incubation period, the plates were examined for the presence of a confluent growth lawn. The diameter of the zone of inhibition was subsequently assessed with precision, measuring to the nearest millimeter. using Vernier's caliper, and this process was repeated three times.

ISSN: 2173-0695

Results and discussion:

The zones of inhibition against P. gingivalis for 1% amoxicillin, 1% c, 1% curcumin and normal saline are represented in the images, Fig-1,2 and 3.Table 1 showed the zone of inhibition for 1% Amoxicillin ,1% curcumin and normal saline. The mean zone of inhibition measured for 1% amoxicillin was 35.6 mm.Whereas 1% curcumin and normal saline did not exhibit a zone of inhibition against P.gingivalis.Thus , 1% amoxicillin showed the broadest zone of inhibition against P. gingivalis .

The colonization of the GTR membrane by microorganisms poses a threat to periodontal regeneration and may lead to additional bone loss and the recurrence of periodontitis. In a study by Hessam Nowzari et al[12] .the microbiota of polytetrafluoroethylene membranes was investigated, along with the impact of periodontopathogens on healing through GBR procedures. The research found that individuals with exposed membranes exhibited deep pockets, and from three patients with osseous gain <1, Porphyromonas gingivalis or Actinobacillus actinomycetemcomitans were isolated. Peptostreptococcus micros were identified in high concentrations in seven out of eight patients who experienced premature membrane exposure and poor osseous healing. Another study by Horn-Lay Wang et al[13]. compared bacterial accumulation on three different membranes—expanded PTFE, polyglactin 910, and collagen. Using 15 oral microbes for comparison, the study revealed that S. mutans and P. gingivalis adhered the most in all three membranes. The risk of developing periodontitis can accelerate as a result of periodontal pathogen adherence to the GTR membrane[14]. Systemic antimicrobial administration is less effective as the antimicrobial agent may diffuse throughout the body and only a small portion of the entire dose really reaches the periodontal bacteria. Additionally, it increases the organisms' resistance against the antibacterial agent. However, when the same medication is delivered locally it just affects the intended location and becomes more effective even at low dosages [15]. The direct incorporation of antimicrobials onto the GTR membrane might help in overcoming this issue

Research has already been conducted on GTR membranes coated with antibiotics for investigating the potential release of antibiotics. The current study tested the antimicrobial properties of GTR membrane integrated with 1% amoxicillin and 1% curcumin against *P.gingivalis,* and the results showed that *P.gingivalis* was sensitive to 1% amoxicillin but resistant to 1% curcumin.This is similar to the findings of Chi-Fang Cheng et al [16], who discovered that incorporating tetracycline or amoxicillin reduced *S. mutans* or *A. actinomycetemcomitans* adhesion on ePTFE, glycolide fibre, or collagen membranes using SEM. In their study, Neha Mehrotra et al[14] tested amoxicillin and green coffee extract incorporated GTR membranes against two periodontal pathogens, *A. actinomycetemcomitans* and P. gingivalis, and found that amoxicillin had a significant effect on both species while green coffee extract had no effect on either, similar to the results of current study with regard to effect of amoxicillin against P.gingivalis.

A study by Vijay M. Kumbar et al[17] revealed that curcumin prevented bacterial adhesion in a dose-dependent manner with a minimal inhibitory concentration of 125 μg ml^{-1} against clinical stains of P. gingivalis. Previous study by Livada et al [18] has also proved that curcumin inhibits P.gingivalis in a dose dependent manner, However, contrary to expectations, the findings of the present study indicate that a 1% curcumin concentration in the natural plant-based product showed no impact on P. gingivalis, This discrepancy may be attributed to the concentration of curcumin utilized in the study, potentially explaining the deviation from existing literature evidence.Thus elevating the curcumin concentration according to the Minimum Inhibitory Concentration (MIC) is a crucial element in determining antibacterial activity.However, more studies with higher concentrations of curcumin should be tested against *P.* gingivalis and other periodontal pathogens that show greater adherence to the GTR membrane to establish the antimicrobial efficacy of curcumin coated GTR membrane.

References

1. Hajishengallis, G., Darveau, R. P., & Curtis, M. A. (2012). The keystone-pathogen hypothesis. Nature Reviews Microbiology, 10(10), 717-725.

ISSN: 2173-0695

2. Lamont, R. J., &Hajishengallis, G. (2015). Polymicrobial synergy and dysbiosis in inflammatory disease. Trends in Molecular Medicine, 21(3), 172-183.
3. [Herrera, D., & Sanz, M. (2016). The effect of antiseptic mouthrinses on oral bacterial counts. Journal of Clinical Periodontology, 43(5), 468-484.
4. Melcher, A. H. (2000). On the repair potential of periodontal tissues. Journal of Periodontology, 71(1), 8-19.
5. Cortellini, P., & Tonetti, M. S. (2015). Focus on intrabony defects: guided tissue regeneration. Periodontology 2000, 68(1), 218-236.
6. Lee, S. W., Hämmerle, C. H., & Cochran, D. L. (2012). Bacterial colonization of bioabsorbable membranes in GBR. Clinical Oral Implants Research, 23(2), 147-154.
7. Reynolds, M. A., Kao, R. T., Camargo, P. M., Caton, J. G., Clem, D. S., Fiorellini, J. P., ... & Wang, H. L. (2015). Periodontal regeneration—intrabony defects: practical applications from the AAP Regeneration Workshop. Clinical Advances in Periodontics, 5(1), 21-29.
8. Chen DW, Lee FY, Liao JY, Liu SJ, Hsiao CY, Chen JK. Preclinical experiments on the release behavior of biodegradable nanofibrous multipharmaceutical membranes in a model of four-wall intrabony defect. Antimicrob Agents Chemother. 2013 Jan;57(1):9-14.
9. Yaghobee S, Samadi N, Khorsand A, Ghahroudi AA, Kadkhodazadeh M. Comparison of the penetration and passage of Streptococcus mutans and Aggregatibacteractinomycetemcomitans through membranes loaded with tetracycline, amoxicillin, and chlorhexidine: an in vitro study. J Basic Clin PhysiolPharmacol. 2014 Feb;25(1):87-97.
10. Savoia, D. (2012). Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiology, 7(8), 979-990.
11. Jain S, Krishna Meka SR, Chatterjee K. Curcumin eluting nanofibers augment osteogenesis toward phytochemical based bone tissue engineering. Biomed Mater. 2016 Oct 6;11(5):055007.
12. Nowzari H, London R, Slots J. The importance of periodontal pathogens in guided periodontal tissue regeneration and guided bone regeneration. Compendium of Continuing Education in Dentistry (Jamesburg, NJ: 1995). 1995 Oct 1;16(10):1042-4.
13. Wang HL, Yuan K, Burgett F, Shyr Y, Syed S. Adherence of oral microorganisms to guided tissue membranes: an in vitro study. Journal of periodontology. 1994 Mar;65(3):211-8.
14. Mehrotra N, Palle AR, Gedela RK, Vasudevan S. Efficacy of Natural and Allopathic Antimicrobial Agents Incorporated onto Guided Tissue Regeneration Membrane against Periodontal Pathogens: An In vitro Study. Journal of Clinical and Diagnostic Research: JCDR. 2017 Jan;11(1):ZC84
15. Handsfield, H.H., Clark, H., Wallace, J.F., Holmes, K.K., Turck, M. Amoxicillin, a new penicillin antibiotic. Antimicrob Agents Chemother. 1973;3(2): 262–65.
16. Cheng, CF, Lee YY, Chi, LY, Chen, YT, Hung, SL., Ling, LJ. Bacterial penetration through the antibiotics-loaded guided tissue regeneration membranes. J Periodontol. 2009; 80(9): 1471-8.
17. Kumbar VM, Peram MR, Kugaji MS, Shah T, Patil SP, Muddapur UM, Bhat KG. Effect of curcumin on growth, biofilm formation and virulence factor gene expression of Porphyromonas gingivalis. Odontology. 2021 Jan;109(1):18-28.
18. Livada R, Shiloah J, Tipton DA, Dabbous MK. The potential role of curcumin in periodontal therapy: A review of the literature. J Int Acad Periodontol. 2017; 19(3):70-9.

Table1:Disc diffusion results

P.g	Amoxicillin(mm)	Curcumin(mm)	Normal saline(mm)
1	38	0	0
2	35	0	0

ISSN: 2173-0695

3	34	0	0

Figures:

Figure 1: Materials used – GTR membrane, amoxicillin powder,normal saline, curcumin powder

Figure 2: GTR membrane soaked 1% curcumin and amoxicillin solution

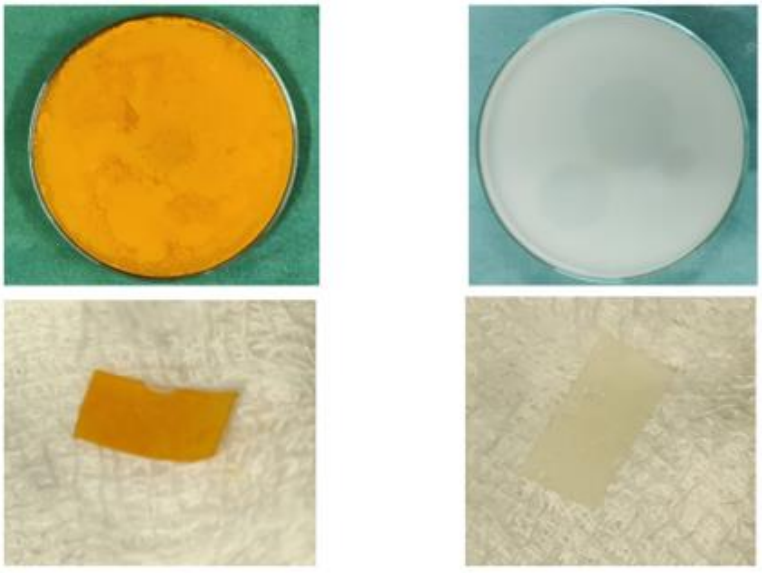
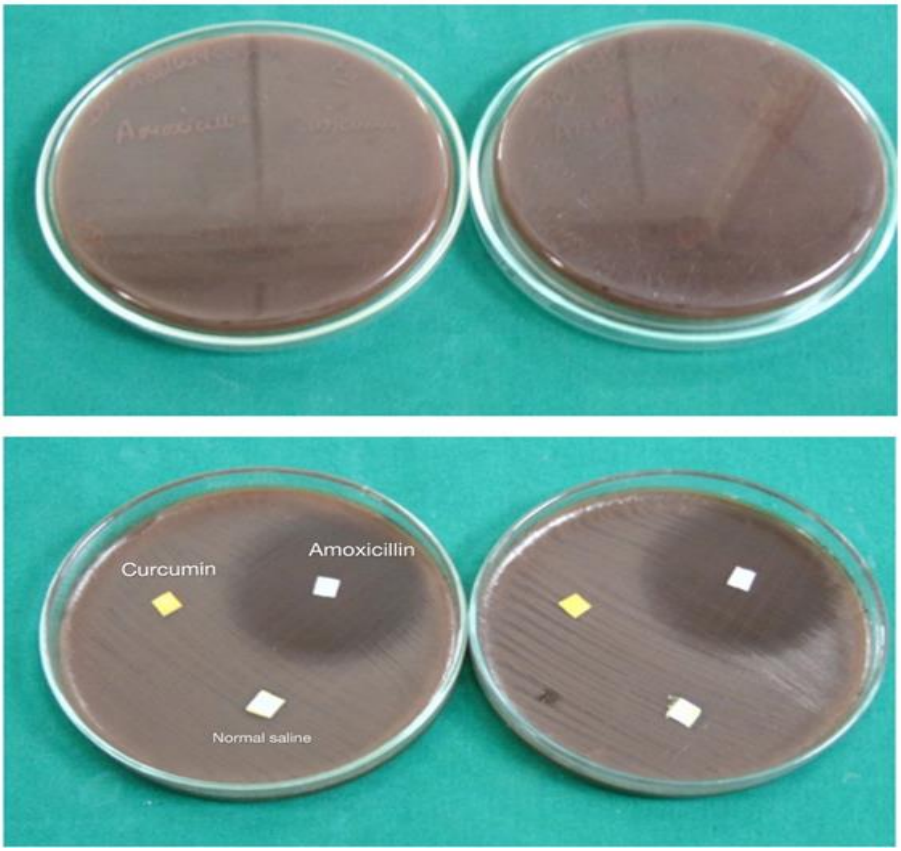


Figure 3: GTR membrane incorporated with antimicrobial agents placed on lawn of P. gingivalis showing zone of inhibition.

ISSN: 2173-0695



\

ISSN: 2173-0695