ORIGINAL ARTICLE

Doxycycline Incorporation Into Gelatin- Carbonate Apatite Membrane as Adjuvant Post Gingival Curettage

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ABSTRACT

Introduction: Tissue destruction in periodontitis caused by chronic inflammation and form of host tissue response. Local drug delivery, such as doxycycline has antibacterial and host modulation properties. Drug vehicle like gelatine and carbonate apatite has good biocompatibility and enhance regeneration process periodontal defect. Objective: This study aimed to examine effect of doxycycline incorporated into gelatine-carbonate apatite membrane as adjuvant for periodontitis after gingival curettages. **Methods:** Gingival curettages were performed to forty-five periodontal pocket points from eight patients, then adjuvant material was applied to each pocket according to the treatment group: gelatine– carbonate apatite membrane, gelatine–carbonate apatite–doxycycline membrane, or doxycycline solution. Treatment was chosen by simple randomization. Periodontal pack was placed for seven days. Clinical evaluation was carried out 7-, 21-, and 28-days post-operative for Papillary Bleeding Index (PBI) and 21-,28-days for Pocket Depth (PD) and Relative Attachment Level (RAL). The data were statistically analysed using the Kruskal Wallis test, followed by the U Mann Whitney test. **Results:** PBI scores were reduced since the 7th day then remained 0 until the last day of examination in all groups. All groups showed reduction of PD and Ral from day 0 to day 28. Greatest reduction of PD and RAL were examined on the gelatine– carbonate apatite group ($p<0.05$). Pocket with gelatine–carbonate apatite–doxycycline membrane and doxycycline solution as adjuvant materials showed no significant difference. **Conclusion:** Doxycycline incorporated into gelatine-carbonate apatite membrane was not as effective as gelatine-carbonate apatite membrane to treat shallow pockets.

Keywords: Doxycycline, Membrane, Gelatin-carbonated apatite, Adjuvant therapy

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INTRODUCTION

Chronic periodontitis is a multifactorial dental and oral disease, not only involving the virulence of periodonto-pathogenic bacteria, but also involves the response of the host body (1). Condition of periodontal tissue with chronic inflammation will worsen the condition of oral bacterial imbalance, or dysbiosis, causing inflammation to continue with the response host resulting in more severe destruction (2). Tissue destruction is facilitated by inflammatory mediators, such as Prostaglandin E-2 (PGE-2), Interleukin-1 (IL-1), and Tumor Necrosis Factor (TNF). Host response in producing collagenase enzymes also influences the severity of periodontal tissue destruction, clinical signs of periodontitis include inflammation of the gingiva, periodontal pocket, attachment loss, to bone damage and tooth mobility (3,4). As an initial phase therapy, scaling and root planing therapy have a role in the removal of dental plaque and calculus, as the main etiology of chronic periodontal inflammation. Gingival curettage is the debridement of granulation tissue from the gingival wall needed to treat the shallow to moderate periodontal pocket (4). Bacterial elimination is an important part of the success of periodontal treatment (5,6). Mechanical debridement or mechanical cleansing, such as gingival curettage, root planing, and subgingival scaling, followed by therapy using additional therapeutic agents or adjuvant proved to be better in clinical and biochemical parameters in gingival crevicular fluid, both in the periodontal tissue and in the peri-implant area, healthy individuals and with diabetes mellitus (7-10).

Doxycycline is a type of tetracycline-derived antibiotic that has a long half- life (18-24 hours) if administered orally or intravenously. Doxycycline has lower adverse effects compared to other tetracycline derivatives.

Some of the characteristics of doxycycline make doxycycline more preferred than tetracycline (11). Doxycycline also has good affinity to the network so that it can already be applied locally. Doxycycline resistance in most bacteria has been shown to be low (12). In its application, doxycycline is also combined with polymers and growth factors in trans-genetic therapy locally (13). The local application of antibiotics directly in periodontal pockets has long been used as a local therapy option. Drug delivery technology, or Drug Delivery System (DDS), allows the drug to last longer and release slowly or in a controlled release. DDS technology has been developed using several types of materials as drug delivery or other active agents (14).

DDS application technology locally can affect the length of time an active ingredient is in the pocket. This will increase the efficacy of local drug treatment (15). DDS technology utilizes several types of polymers. One example of the use of polymer carriers for local therapy of periodontitis that has been marketed is Periochip ™. The polymer used in this product is gelatin hydrolysis which is carried out cross-linking using glutaraldehyde (16). Previous studies carried out research into the incorporation of gelatin-apatite carbonate membrane with metronidazole (17). Other polymers, such as ε-caprolactone copolymer, are also used as a carrier matrix in DDS membrane technology - apatite carbonate with metronidazole. with doxycycline drugs.

Gelatin is a polymer that can be formed in a thin membrane and is suitable as an intra-pocket carrier in periodontal treatment (1,18). The combination of gelatin with apatite carbonate material has been widely applied to bone graft treatments (19). Apatite carbonate also functions as a carrier for DDS, both in drugs and protein carriers (17,20). Membranes consisting of gelatin and apatite carbonate have been used as additional therapeutic agents after scaling and root planing therapy. The therapeutic results showed improvement in the condition of the periodontal tissue after therapy, judging by the depth of the pocket and attachment loss (21). Local doxycycline has also been shown to be an additional therapeutic ingredient after scaling and root planning (22). This study aimed to examine the effect of doxycycline incorporation in the gelatin-apatite membrane on the healing periodontitis after gingival curettage treatment with clinical parameters.

MATERIALS AND METHODS

1. Subjects

Subjects were chronic periodontitis patients at the Periodontal Clinic of the Dental and Oral Hospital of Gadjah Mada University (RSGM UGM) Prof. Soedomo, Yogyakarta; with inclusion criteria: both female and male patients with chronic periodontitis, age ranging from 17 - 45 years, have minimum one periodontal pocket with of 3-5 mm depth, do not have systemic disease, approve and sign informed consent; exclusion criteria: taking antibiotics in the last 6 months, pregnant or breastfeeding women, smokers, history of doxycycline allergy or other tetracycline derivatives, history of surgery on periodontal tissue in the last 6 months

2. Materials

Gracey curettes (CRGR 3-4; CRGR 7-8; CRGR 11-12; CRGR 13-14, Osung, Korea), probe UNC-15 (BPUNC-15, Osung, Korea), ultra-sonic scaler (Bonart- ART-P1, ART), diagnostic set, analytical balances (Mettler Toledo, Swiss), gelatin carbonate apatite membrane (Dept. of Dental and Biomedical Science, Faculty of Dentistry, UGM), gelatin – carbonate apatite – doxycycline (contain 6µL 1% doxycycline hyclate solution), 6µL 1% doxycycline hyclate solution, periodontal dressing (Coe- pack, GC, Japan)

3. Methods

This research method has been ethically approved by the Research Unit of Research Faculty of UGM FKG (00156/KKEP/FKG-UGM/EC/2019).

a. Sample recruitment

Sample were recruited based on the inclusion and exclusion criteria of the subjects.

b. Initial phase therapy

At the initial meeting, a clinical examination is performed and scaling and root planing, polishing, and other etiological removal treatments, such as occlusal adjustments.

c. First evaluation and baseline data record

One week after scaling and root planing, baseline data were measured: Papillary Bleeding Index (PBI), pocket depth (PD), and Relative Attachment Level (RAL) measurements. Visual examination and periodontal probe (UNC-15) used to examine the inflammation of the gingiva, using PBI four-grade scores by Saxer and Mьhlemann in 1975 [23]. Pocket depth was measured from the base of the pocket to the gingival margins. Acrylic stent was placed to measure the RAL.

d. Gingival curettages and adjuvant applications

After recording the baseline data, gingival curettage is carried out on pockets that have a depth of 3-5 mm. Under aseptic procedure, 2% lidocaine local anesthesia was injected using infiltration technique. Gingival curettage was performed as seen in Figure 2. Gracey's curettes was used to scrape the gingival wall of the pocket up until there was no more granulation tissue, then saline irrigation and gauge pressure was applied gently. Adjuvant materials, doxycycline solution, apatite gelatin-carbonate membrane, or apatite- doxycycline-gelatin-carbonate membrane, was applied according to the treatment groups. Periodontal pack was placed, and patients were prescribed with mefenamic acid 500 mg two to three times a day for three days.

e. Reevaluation and data recording

Reevaluation was carried out on the day 7, 21, and 28 after surgery. On day 7, periodontal dressing was only taken and PBI measurement was taken. All measurement was performed on day 21 and 28.

RESULTS

This research showed that all inflammation in gingival and periodontal area healed in all groups. The use of local adjuvant therapy using the local delivery system membrane gelatin – carbonate hydroxyapatite showed better result than the doxycycline gelatin – carbonate hydroxyapatite and doxycycline solution.

1. Papillary Bleeding Index (PBI)

The Papillary Bleeding Index is measured quantitatively using the Papillary Bleeding Index (PBI). Measurements were performed on day 0 (baseline), 7, 21, and 28 after curettage. All PBI data from all groups were score 2 at day 0, then it was all decreased to score 0 started at the day 7. The score was all remained 0 until day 28 for all groups, as shown in Table I.

2. Pocket Depth (PD)

Pocket depth (PD) was measured on baseline, day 21 and 28 post curettage. Mean and standard deviations of PD in all treatment groups and times are presented in Figure 1. Reduction of PD was calculated from baseline to day 21 and baseline to day 21, presented in Table II. Biggest reduction was baseline to day 21 from CHA group. However, additional PD was shown. The normality and homogeneity of the data was tested through Shapiro-Wilk and Levene's statistical tests, respectively. The data obtained are homogeneous ($p > 0.05$) but not normal ($p < 0.05$). The statistical test used is the Kruskal- Wallis test and is presented in Table III.

Probing Depth of day 0 to 21 between groups were statistically different ($p<0.05$). Table IV shows that there was significance between CHA groups with CHA-DOX and DOX ($p < 0.05$). PD reduction between the CHA-DOX and DOX groups did not differ statistically significant.

3. Relative Attachment Level (RAL)

The RAL measurements were carried out on baseline, 21 and 28 post curettage. The mean and standard deviation of RAL in all treatment groups and times are presented in Figure 1. Reduction of RAL showed in all groups, it was calculated between day 0 to 21, day 0 to 28, and day 21 to 28, then presented in Table II. The biggest reduction was day 0 to day 28 in the CHA group. The RAL reduction data was not normally distributed ($p<0.05$) but homogenous ($p>0.05$). Kruskal-Wallis test was performed and presented in Table III.

Table I. Papillary Bleeding Index (PBI) scores on day 0 (baseline), 7, 21, and 28 after curettage

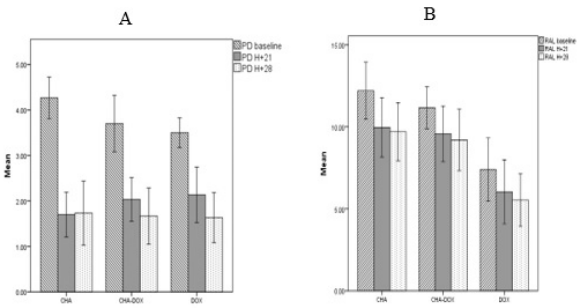
Group	n	PBI scores			
		Day 0	Day 7	Day 21	Day 28
DOX	15	2	0	0	0
CHA	15	2	0	0	0
CHA-DOX	15	2	0	0	0

CHA : curettage + gelatin carbonate apatite membrane
CHA-DOX : curettage + gelatin carbonate apatite membrane – doxycycline
DOX : curettage + doxycycline

Table II. Averages and standard deviation of Pocket Depth (PD) reduction and Relative Attachment Level (RAL) (mm)

Group	n	PD reduction (mm)		RAL reduction (mm)			
		Day 0 to day 21	Day 0 to day 28	Day 0 to day 21	Day 0 to day 28	Day 21 to day 28	Day 21 to day 28
DOX	15	1.37 ± 0.14	1.87 ± 0.17	1.37 ± 0.14	1.87 ± 0.17	0.50 ± 0.14	0.50 ± 0.14
CHA	15	2.57 ± 0.14	2.53 ± 0.22	2.33 ± 0.23	2.50 ± 0.23	0.27 ± 0.17	-0.03 ± 0.18
CHA-DOX	15	1.67 ± 0.19	2.03 ± 0.24	1.60 ± 0.17	1.97 ± 0.25	0.37 ± 0.10	0.37 ± 0.10

CHA : curettage + gelatin carbonate apatite membrane
CHA-DOX : curettage + gelatin carbonate apatite membrane – doxycycline
DOX : curettage + doxycycline



CHA : curettage + gelatin carbonate apatite membrane
CHA-DOX : curettage + gelatin carbonate apatite membrane – doxycycline DOX
DOX : curettage + doxycycline

Figure 1 : Mean and standard deviations of pocket depth (A) and relative attachment level (B) in all treatment groups and days.

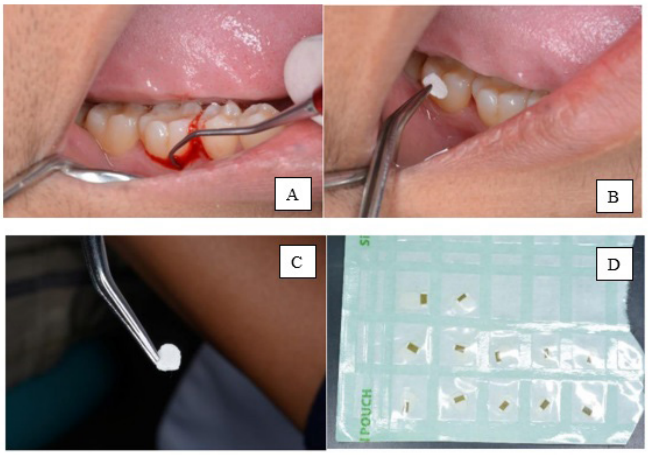


Figure 2 : Gingival curettage treatment using adjuvant (A) Debridement using Gracey's Curette, (B) Adjuvant placement, (C) and (D) The adjuvant membrane.

Table III : Kruskal Wallis test result of PD and RAL reduction

	Groups	N	Mean Rank	Sign.
PD day 0 to day 21	DOX	15	14.90	0.000*
	CHA	15	34.40	
	CHA-DOX	15	19.70	
	Total	45		
PD day 0 to day 28	DOX	15	19.27	0.055
	CHA	15	29.47	
	CHA-DOX	15	20.27	
	Total	45		
PD day 21 to day28	DOX	15	26.87	0.160
	CHA	15	17.63	
	CHA-DOX	15	24.50	
	Total	45		
RAL day 0 to day21	DOX	15	17.23	0.008*
	CHA	15	31.23	
	CHA-DOX	15	20.53	
	Total	45		
RAL day 0 to day28	DOX	15	19.57	0.032*
	CHA	15	30.07	
	CHA-DOX	15	19.37	
	Total	45		
RAL day 21 to 28	DOX	15	25.03	0.732
	CHA	15	21.67	
	CHA-DOX	15	22.30	
	Total	45		

*) : p<0,0 05
CHA : curettage + gelatin carbonate apatite membrane
CHA-DOX : curettage + gelatin carbonate apatite membrane –doxycycline
DOX : curettage + doxycycline

Table IV : U Mann Whitney statistical test result for day 0 to day 21 PD reduction

	DOX	CHA	CHA-DOX
DOX	-	0.000*	0.304
CHA		-	0.002*
CHA-DOX			-

*) : p<0,005
CHA : curretage + gelatin carbonate apatite membrane
CHA-DOX : curretage + gelatin carbonate apatite membrane – doxycycline
DOX : curretage + doxycycline

Table V : U Mann Whitney statistical test result for day 0 to day 21 and day 0 to day 28 RAL reduction

	Day 0 to 21			Day 0 to 28		
	DOX	CHA	CHA-DOX	DOX	CHA	CHA-DOX
DOX	-	0,003*	0,539	-	0,019*	0,847
CHA		-	0,028*		-	0,037*
CHA-DOX			-			-

*) : p<0,005
CHA : curretage + gelatin carbonate apatite membrane
CHA-DOX : curretage + gelatin carbonate apatite membrane – doxycycline
DOX : curretage + doxycycline

It shows that there were statistically significant differences between treatment groups in the reduction of RAL day 0 to 21 and day 0 to 28 ($p < 0.05$). Mann Whitney U test was performed to find out the differences between treatment groups on the reduction of RAL day 0 to 21 and day 0 to 28. Table V shows that there was significance difference between CHA groups with CHA-DOX and DOX groups on the reduction of days 0 to 21 and day 0 to 28 ($p < 0.05$). The reduction of RAL between the CHA-DOX and DOX groups on RAL day 0 to 21 and day 0 to day 28 were not statistically different.

DISCUSSION

1. Papillary Bleeding Index (PBI)

Papillary Bleeding Index is a sign of gingival inflammation. Linear PBI scores with pocket depth and biofilm index on the gingiva (24). Gingival bleeding can be used as a predictor of gingival conditions (25). All treatment groups experienced a decrease in PBI since day 7. Subgingival curettage treatment along with adjuvant in all treatment groups has been shown to improve gingival conditions and reduce gingival inflammation. Subgingival curettage therapy is performed with the aim of reducing the accumulation of microbiota in the subgingival as the main cause of inflammation. The addition of adjuvant can be used to increase the effectiveness of the treatment (26).

Gelatin is a polymer that is widely used in tissue engineering technology because it has regenerative properties and accelerates healing of tissue affected by lesions. Other properties of gelatin that are easily combined with other materials are also advantages of gelatin (27). The addition of gelatin in periodontal therapy has been applied and combined with several additional active agents, both antibiotics, antiseptics, and growth factors (26, 28). Apatite carbonate in wet environmental conditions can release calcium ions locally. This results in a higher concentration of calcium ions in the area around the apatite carbonate (29). During the healing process of tissues, calcium ions are useful for accelerating wound healing (30).

Controlled growth of microbes that cause periodontal disease also plays a role in the healing of gingival bleeding (24). Application of antibacterial properties is useful for such control, in this research, we applied doxycycline as an antibiotic. There are three theories about the antibacterial mechanism of apatite due to ionic activity. The first mechanism is the ion penetrates the bacterial cell and interferes with the process of DNA replication by affecting the intracellular ATP production process. The second mechanism is related to the accumulation of bacterial cell ions in the bacterial cellular membrane so that the permeability to change is characterized by the gradual release of proteins and lipopolysaccharides. This causes proton

transport through the cell membrane to be inhibited then the cell will die. The third mechanism is the induction of Reactive Oxygen Species (ROSs) that can react with bacterial cell membrane components and other cell components, such as mitochondria, causing irreversible changes in cell structure that will continue to cell death (31).

2. Pocket Depth (PD)

Reduction in pocket depth is one of the evaluations in periodontal tissue healing (32). Pocket depth (PD) decreases occurred in all groups in the reduction of PD on day 0 to 21. Decrease in pocket depth is a sign of linear periodontal tissue healing with other healing variables (24). All treatment groups experienced clinically significant PD decrease in all treatment groups to under 3 mm.4 This indicates that all treatment groups have reached the normal value of gingival sulcus depth on day 21. Gingival condition after curettage healed well on day 21, marked by the re-attachment of the gingiva and its normal color and shape, then maturation will occur from day 28 (33).

Doxycycline, gelatin, and apatite carbonate are materials that can support the healing process after curettage. Gelatin as a carrier material while at the same time has regenerative properties and accelerates the healing of tissues affected by lesions (34). The ability of gelatin as a drug delivery system allows the active ingredients to be released in a controlled and long-term manner (35). Doxycycline has a role in inhibiting tissue damage by inhibits MMP (36). The difference in PD reduction values between treatment groups was statistically significant in the reduction of PD in days 0 to day 21. Statistically, the CHA treatment group had the highest reduction on the baseline and 21st days. The CHA-DOX and DOX treatment groups did not differ significantly.

Carbonate apatite in soft tissue healing can add calcium ions so that it helps in wound healing (30). In addition, calcium plays a role in cell signaling for proliferation and differentiation during healing. Doxycycline which is incorporated with CHA has no significant difference with doxycycline solution. Therefore, it is estimated that doxycycline can inhibit the signaling of calcium for tissue healing so that the healing process is not optimal (37). The incorporation of doxycycline into the apatite gelatin-carbonate membrane did not affect the decrease in pocket depth. Several material and biological aspects in the administration of doxycycline to the apatite gelatin-carbonate membrane may influence the results of the pocket reduction. The pH of doxycycline, embedding process, the amount of cross-linking affects the degradation of gelatin in apatite-doxycycline-gelatin-carbonate membrane. Faster degradability will affect the length of material to contact locally in the pocket (38).

3. Relative Attachment Level (RAL)

Reduced RAL is a good form of evaluation after periodontal therapy. RAL evaluation is accurate in describing periodontal tissue healing (39). The difference in RAL reduction was statistically significant at the reduction of days 0 to 21 and days 0 to 28. All groups showed improvement in the attachment of periodontal tissue in all groups on the day 21 and 28. The evaluation is in accordance with the healing process and re-attachment after periodontal pocket treatment. The complete maturation process takes place after 4 weeks or 28 days (33). The tissue healing mechanism is maximized with the CHA membrane because it consists of gelatin and apatite carbonate. Both compound function in improving tissue healing (30,35). RAL reduction is more accurate in describing re-attachment after periodontal treatment (39). The results of RAL reduction at day 0 to 21 and day 0 to 28 were highest in the CHA group. This shows that there is a re-attachment of periodontal tissue after curettage treatment (4). The treatment group with CHA-DOX membrane did not differ significantly from DOX solution. This can be because doxycycline inhibits the release of calcium which plays an important role for wound healing (37).

The application of doxycycline in the treatment of chronic periodontitis is expected to increase the effectiveness of therapy. Provision of doxycycline with materials, for example apatite gelatin-carbonate is expected to improve treatment results. The incorporation of doxycycline in the apatite gelatin-carbonate membrane in this study did not increase the effectiveness of doxycycline. In vitro studies have shown that doxycycline release can occur slowly, but this does not occur in clinical studies (34). Some possibilities occur, for example, a condition that affects gelatin degradation in apatite gelatin-carbonate membranes embedding with doxycycline (40).

All treatment groups in this study experienced improvement in post curettage periodontal tissue. Curettage therapy is a standard therapy in mild periodontal pockets by removing the granulation tissue in the pocket wall, although its effectiveness needs to be increased (41). Doxycycline adjuvant has been shown to improve clinical signs in periodontal tissue (1). Doxycycline incorporation in apatite gelatin carbonate membrane is expected to increase the effectiveness of doxycycline in periodontal pockets (42). In this study, differences in treatment results between the doxycycline (DOX) group and apatite gelatin-carbonate membrane (CHA- DOX) had no difference, both statistically and clinically.

The doxycycline incorporation in the apatite gelatin-carbonate membrane can have several possible bonds. There are two interactions that can occur in the process of loading drugs inside hydrogels, namely physical

interactions, and chemical bonds (43). The process of incorporation of doxycycline into the apatite gelatin-carbonate membrane is carried out by an embedding process so that the possibility of the bonding is physical. The process of incorporation by embedding is an easy process and the process of releasing antibiotics occurs with an initial burst release (44). The release of an initial burst release drug allows a drug to achieve a high concentration of therapeutic effects at the start (44).

The level of acidity (pH) of doxycycline is in acidic condition (11). During the embedding process, there is a possibility of reaction of gelatin in the membrane with doxycycline. Acidic pH conditions will affect the decrease in the number of cross-linking gelatin bonds. Fewer cross-linking bonds will affect the degradability of apatite gelatin-carbonate membranes. Faster degradation will affect the therapeutic ability of the membrane (38). This event is likely an explanation that the incorporation of doxycycline to apatite gelatin-carbonate has no significant difference from doxycycline alone.

Epithelial cells and fibroblasts require a more acidic pH in tissue proliferation. These conditions also play an important role in preventing bacterial accumulation (46). The difference between the healing conditions of in vivo tissue is likely to lead to differences in results with previous in vitro studies (41).

CONCLUSION

This study concludes that gelatin– carbonate apatite membrane has better effect on pocket reduction in shallow pockets. There was no effect of incorporation of doxycycline into gelatin-carbonate apatite membrane, measured by clinical parameters Papillary Bleeding Index (PBI), Pocket Depth (PD) and Relative Attachment Level (RAL).

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REFERENCES

1. Garg S. 2015. Local drug delivery systems as an adjunct to cure periodontitis- the novel dental applicant. Pharm Methods 2015;6(1): 1–8. doi: 10.5530/phm.2015.6.1
2. Meyle J, Chaple I. Molecular aspects of the pathogenesis of periodontitis. Periodontology 2000 2015;(69): 7 – 17. doi: 10.1111/prd.12104
3. Kinane DF, Stathoupoulou PG, Papapanou PN, 2017. Periodontal diseases. Nat Rev Dis Primers. 2017;(3):1078. doi: 10.1038/nrdp.2017.38
4. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Clinical Periodontology. 12th ed. Philadelphia: WB Saunders Co; 2015.
5. Prakasam A, Elavarasu SS, Natarajan RK. Antibiotics in the management of aggressive periodontitis, J Pharm Bioallied Sci. 4 2012;(Suppl 2): S252-S255. doi: 10.4103/0975-7406.100226
6. Kopytynska-Kaspercyk A, Dobrzynski P, Pastusiak M, Jarzabek B, Prochwicz W. Local delivery system of doxycycline hyclate based on ε- caprolactonecopolymers for periodontitis treatment. I J Pharm 2015;(6):34. doi: 10.1016/j.ijpharm.2015.06.034
7. Al Mubarak S, Abou Rass M, Alsuwyed A, Al Zoman K, Al Sohail A, Sobki S, et al. A new paradigm between mechanical scaling and root planing combined with adjunctive chemotherapy for glycated hemoglobin improvement in diabetics. Int J Diabetes Dev Ctries 2010;(2): 158 – 164. doi: 10.1016/j.ijdm.2010.08.006
8. Eickholz P, Kim TS, Schacher B. Subgingival topical doxycycline versus mechanical debridement for supportive periodontal therapy: A single blind randomized controlled two-center study. Am J Dent 2005;18: 341 – 346. Available from: https://pubmed.ncbi.nlm.nih.gov/16433404/
9. Singh N, Chandel S, Singh H, Agarwal A, Savitha AN. 2017. Effect of scaling & root planing on the activity of ALP in GCF & serum of patients with gingivitis, chronic and aggressive periodontitis: A comparative study. J Oral Biol Craniofac Res. 2017;7(2): 123 – 126. doi: 10.1016/j.jobcr.2017.03.006
10. Javed F, BinShabaib MH, Alharthi SS, Qadri T. Role of mechanical curettage with and without adjunct antimicrobial photodynamic therapy in the treatment of peri- implant mucositis in cigarette smokers: A randomized controlled clinical trial. Photodiagnosis Photodyn Ther. 2017;18: 331–334. doi: 10.1016/j.pdpdt.2017.04.015
11. Kogawa AC, Salgado HRN. Doxycycline hyclate: the review of properties, applications, and analytical methods. Int J Life Sci Biotechnol Pharma Res 2012; 2(4): 11 – 25. Available from: https://www.ijlpr.com/admin/php/uploads/152_pdf.pdf
12. Cunha BA, Baron J, Cunha CB. Similarities and differences between doxycycline and minocycline: clinical and antimicrobial stewardship considerations. Eur J Clin Microbiol Infect Dis 2017;2017: 1 – 6. doi: 10.1007/s10096-017-3081-x
13. Bara JJ, Dresing I, Zeite S, Anton M, Daculsi G, Eglin D, et al. A doxycycline inducible, adenoviral BMP-2 gene delivery system to bone. J Tissue Eng Regen Med 2018 Jan;12(1): e106-e118. doi: 10.1002/term.2393
14. Moura LA, Ribeiro FV, Aiello TB, De Rezende Due EA, Sallum EA, Nociti Jr FH, et al. Characterization of the release profile of doxycycline by PLGA microspheres adjunct to nonsurgical periodontal

therapy. J Biomater Sci Polym Ed 2015; 26(10):573-84. doi: 10.1080/09205063.2015.1045249
15. Flemming TF. Locally delivered antimicrobials adjunctive to scaling and root planing provide additional PD reduction and CAL gain in the treatment of chronic periodontitis. J Evid Base Dent Pract 2006; 6:220-1. doi: 10.1016/j.jebdp.2006.06.006
16. Puri K, Dodward V, Bhat K, Puri N. Effect of controlled-release Periochip™ on clinical and microbiological parameters in patients of chronic periodontitis. J Indian Soc Periodontol. 2013; 17(5): 605–611. doi: 10.4103/0972-124X.119299
17. Ardhani R, Setyaningsih S, Hafiyyah OA, Ana ID. Preparation of Carbonated Apatote Membran as Metronidazole Delivery System for Periodontal Application. KEM 2016; 696: 250 – 258. doi: 10.4028/www.scientific.net/KEM.696.250
18. Ardhani R, Susilowati R, Ana ID. Functional recovery of axonal injury induced by gelatin hydrogel film and PRP: an initial study in rats, JBiSE 2015; 8(3): 160 – 169. doi: 10.4236/jbise.2015.83016
19. Salim S, Ariani MD. In vitro and in vivo evaluation of carbonate apatite-collagen scaffolds with some cytokines for bone tissue engineering, J Indian Prosthodont Soc 2015;15: 349-55. doi: 10.4103/0972-4052.171821
20. Tada S, Chowdhury EH, Cho CS, Akaike T. pH-sensitive carbonate apatite as an intracellular protein transporter. Biomaterials 2010; 31(6):1453-9. doi: 10.1016/j.biomaterials.2009.10.016
21. Komara I, Rusminah N, Hendiani I, Sopiatin S, Utami ND. The effect of Apatite Carbonate Membrane Application on Periodontal Tissue after Scaling and Root Planing Treatment. IJCRGG 2018;11(09): 162-169. doi: 10.20902/IJCTR.2018.110921
22. Lyons LC, Weltman RL, Moretti AJ, Trejo PM. Regeneration of Degree II Furcation Defects With a 4% Doxycycline Hyclate Bioabsorbable Barrier, J Periodontol 2008; 79(1):72-9. doi: 10.1902/jop.2008.070161
23. Checchi L, Montevecchi M, Marucci G, Checchi V. A proposed new index for clinical evaluation of interproximal soft tissues: the interdental pressure index [published correction appears in Int J Dent. 2014;2014. doi:10.1155/2014/586537
24. Checchi L, Montevecchi M, Checchi V, Zapulla F. The relationship between bleeding on probing and subgingival deposits. An endoscopical evaluation. Open Dent J. 2009; 3: 154 – 160. doi: 10.2174/1874210600903010154
25. Gonzales S, Cohen CL, Galvan M, Alonaizan FA, Rich SK, Slots J. Gingival bleeding on probing: relationship to change in periodontal pocket depth and effect of sodium hypochlorite oral rinse. J Periodontal Res 2015;50(3): 397 – 402. doi: 10.1111/jre.12219
26. Matezan- Рйrez P, Garcна-Gargall M, Figuero BA, Sanz M, Herrera D. A systematic review on the effects of local antimicrobials as adjuncts to subgingival debridement, compared with subgingival debridement alone, in the treatment of chronic periodontitis. J Clin Periodontol 2013; 40(3): 227 – 41. doi: 10.1111/jcpe.12026
27. Echave MC, Saenz del Burgo L, Pedraz JL, Orive G. Gelatin as biomaterial for tissue engineering. Curr Pharm Des. 2017;23(24):3567-3584. doi: 10.2174/092986732466617051112310l
28. Tamura T, Yokoya S, Kamata Y, Kinoshita Y, Tabata Y, Matsumoto G. Periodontal regeneration using gelatin hydrogels incorporating basic fibroblast growth factor. Biomed J Sci & Tech Res 2018; 4(2): 3820 – 4. doi: 10.26717/BJSTR.2018.04.001025
29. Ana ID, Matsuya S, Ishikawa K. Engineering of Carbonate Apatite Bone Substitute on Composition –Transformation of Gypsum and Calcium Hydroxide. Engineering2010;2: 344 – 352. doi: 10.4236/eng.2010.25045
30. Moe A, Golding AE, Bement WM. Cell healing: calcium, repair, and regeneration. Semin Cell Dev Biol 2015;45: 18-23. doi: 10.1016/j.semcdb.2015.09.026
31. Kolmas J, Groszyk E, Kwiatkowska-Ryhycka D. Substituted Hydroxyapatites with Antibacterial Properties. BioMed Research International 2014: 1 – 14. doi: 10.1155/2014/178123
32. Kour A, Kumar A, Puri K, Khatri M, Bansal M, Gupta G. Comparative evaluation of probing depth and clinical attachment level using a manual probe and Florida probe. J Indian Soc Periodontol 2016;20(3): 299–306. doi: 10.4103/0972-124X.181241.
33. Pippi R. Post-Surgical Clinical Monitoring of Soft Tissue Wound Healing in Periodontal and Implant Surgery. Int J Med Sci. 2017;14(8): 721–728. doi: 10.7150/ijms.19727
34. Echave MC, Saenz del Burgo L, Pedraz JL, Orive G. Gelatin as biomaterial for tissue engineering. Curr Pharm Des. 2017;23(24):3567-3584. doi: 10.2174/092986732466617051112310l
35. Santoro M, Tatarab AM, Mikos AG. Gelatin carriers for drug and cell delivery in tissue engineering. J Control Release. 2014;28: 210–218. doi: 10.1016/j.jconrel.2014.04.014
36. Salim S, Ariani MD. In vitro and in vivo evaluation of carbonate apatite-collagen scaffolds with some cytokines for bone tissue engineering, J Indian Prosthodont Soc 2015;15: 349-55. doi: 10.4103/0972-4052.171821
37. Landson AB. Calcium: a potential central regulator in wound healing in the skin. Wound Repair Regen. 2002;10(5ding):271-85. doi: 10.1046/j.1524-475x.2002.10502.x.
38. Yun Y H, Lee BK, Park K. Controlled Drug Delivery: Historical perspective for the next generation. Journal of Controlled Release 2015;219: 2–7. doi: 10.1016/j.jconrel.2015.10.005
39. Kaner D, Soudan M, Zhao H, Gassmann G,

Shoenhausser A, Friedmann A. Early Healing Events after Periodontal Surgery: Observations on Soft Tissue Healing, Microcirculation, and Wound Fluid Cytokine Level. Int. J. Mol. Sci. 2017;18(283):1-14. doi: 10.3390/ijms18020283

40. Scholz OA, Wolff A, Schumacher A, Giannola LI, Campisi G, Ciach T, Velten T. Drug delivery from the oral cavity: focus on a novel mechatronic delivery device. Drug Discov. Today. 2008;13:247-253. doi: 10.1016/j.drudis.2007.10.018
41. Deas DE, Moritz AJ, Sagun RS, Gruwell SF, Powell CA. Scaling and root planing vs. conservative surgery in the treatment of chronic periodontitis. Periodontol 2000 2016;71: 128-139. doi:10.1111/prd.12114
42. Hafiyyah OA, Lastianny SP, Suryono S. Doxycycline Incorporated in Gelatin - Carbonate Apatite Bone Graft Material: In Vitro Evaluation of Osteoblastic Alkaline Phosphatase and Porphyromonas gingivalis Colonies. Mal J Med Health Sci 2020;16(SUPP15): 57-6. Available from:https://medic.upm.edu.my/upload/dokumen/2020123012052210_MJMHS_0319.pdf
43. Herasaki S, Moztarzadeh F, Nemati R, Nezafati N. Preparation and Characterization of Calcium Sulfate–Biomimetic Apatite Nanocomposites for Controlled Release of Antibiotics. J. Biomed. Mater. Res. 2009;B 91(2): 651 – 661. doi: 10.1002/jbm.b.31441
44. Landi E, Cellotia G, Logroscinob G, Tampieria A. Carbonated hydroxyapatite as bone substitute. J Eur Ceram Soc 2003; 23: 2931–2937. doi: 10.1016/S0955-2219(03)00304-2
45. Yildirimer L, Seifalian AM. Three-dimensional biomaterial degradation - Material choice, design, and extrinsic factor considerations. Biotechnol Adv. 2014;32(5):984-99. doi: 10.1016/j.biotechadv.2014.04.014
46. Kruse CR, Nuutila K, Lee CC, Kiwanuka E, Singh M, Caterson EJ, et al. The external microenvironment of healing skin wounds. Wound Rep and Reg 2015; 23: 456-464. doi:10.1111/wrr.1. doi: 10.1111/wrr.12303