ORIGINAL ARTICLE

The Healing of Post-curettage Chronic Periodontitis on the Implantation of Carbonate Apatite-gelatin Film as a Chlorhexidine Delivery System (A Randomized-Controlled Trial)

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ABSTRACT

Introduction: The use of a delivery system to administer an anti-microbial agent offers advantages in the treatment of periodontal diseases. We compare the periodontal tissue healing of chronic periodontitis patients after administrating a combination of curettage with chlorhexidine digluconate-delivery membrane. The membrane are collagen and gelatin-carbonated apatite based. **Materials and methods:** We assign total of 45 periodontal pocket with 3-5mm depth of 9 patients into 3 groups of 15 to receive curettage and one of membranes: collagen-chlorhexidine digluconate (CCH), gelatin-carbonated hydroxyapatite (GC), or gelatin-carbonated hydroxyapatite-chlorhexidine gluconate (GCCH). Evaluation before treatment as baseline and 7, 21, as well as 28 days after the treatment include parameters Pocket Depth (PD), Relative Attachment Loss (RAL), and Bleeding on Probing (BOP). **Results:** TAll three combinations promote decreasing PD, RAL, and BOP after 7, 21, and 28 days. The differences among groups are insignificant. **Conclusion:** Carbonate apatite gelatin membrane itself decreased PD, RAL, and BOP greater on the 21st day. This work shows treatment options for clinician to manage a chronic periodontitis case.

Keywords: Carbonated hydroxyapatite, Gelatin, Collagen, Chlorhexidine digluconate, Chronic periodontitis, Healing

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INTRODUCTION

Periodontal disease is an inflammation of the tooth supporting tissue which can cause tooth loss and contributes to systemic inflammation (1). The main goal of chronic periodontal treatment is to control periodontal infections and inflammation and stop the development of attachment loss (2). In deep pockets, especially on concave root surfaces or furcation involvement, curettage is less effective, because in long junctional epithelium its presence interferes with the direct apposition of connective tissue and cementum, thus limiting the height to which periodontal fibers can insert to the cementum, so that anti-microbial agent addition is recommended (3).

Chlorhexidine is a broad-spectrum anti-microbial agent, which has been used for more than 30 years. Its benefits as a dental plaque inhibitor and gingivitis have been studied for a period of 2 years and without the development of bacterial resistance (4). The use of chlorhexidine after subgingival instrumentation as an adjunctive antiseptic may be considered (5). The application of chlorhexidine after curettage can increase the effectiveness of periodontal treatment (6). The main problem when applying topical chlorhexidine is the loss of unrestricted chlorhexidine in the first 48 hours by more than 40% (7).

A drug delivery system with controlled release property is needed for maintaining chlorhexidine therapeutic concentration locally. The drug delivery system must be prepared from a chemically stable materials that biocompatible, biodegradable, and adapt well with drugs component. The material able to release and allow drug penetration into tissues (8). Examples of material that have biocompatible, biodegradable properties and can be used as drug and protein carriers is gelatin (9). Gelatin survives in the gingival pocket for 7-10 days and is able to restore attachment loss and reduce pocket depth (10). Evidence showed locally administered chlorhexidine in a sustained-release mode improves clinical outcome of subgingival instrumentation (5).

It is also necessary to treat the loss of alveolar bone in chronic periodontitis. Advances in medical technology has developed rapidly in creating solutions to the

problem of replacing damaged or lost bones (11). One bone graft developed in the medical field is Carbonate Hydroxyapatite (CHA). Many studies have shown that CHA is able to stimulate and induce new bone growth, is biocompatible, and bioresorbable (12). The combination of Gelatin-CHA in membrane preparations as a drug delivery system becomes a useful material to manage periodontitis (13, 14). This study investigates the potential of gelatin-CHA membrane as a delivery system of chlorhexidine in treating chronic periodontitis.

MATERIALS AND METHODS

Patient's selection

Procedures approval from The Ethics Committee of Research Faculty of Dentistry Universitas Gadjah Mada was declared by the ethical clearance document number 0066/KKEP/FKG-UGM/EC/2019. This study was part of thesis project entitled "The effect of gelatin-carbonated apatite membrane as chlorhexidine delivery system as an adjunctive to curettage on the healing of chronic periodontitis" of Periodontology Specialist Program, Faculty of Dentistry, Universitas Gadjah Mada, year 2019 (15).

Patients of the Universitas Gadjah Mada Dental Hospital Prof. Soedomo, Yogyakarta, Indonesia was acceptable to participate if suffering a mild to moderate chronic periodontitis, refusing history of systemic diseases, smoking, and alcohol consumption. A mild chronic periodontitis was patients who suffered periodontal destructions with CAL less than 1-2 mm and moderate periodontitis with CAL about 3-4 mm. Patients with pregnancy or taking a long-term medication (antibiotics, anti-inflammation and medication for systemic disease) were ineligible for participating. We only involved patients who voluntary signed the informed consent form after receiving information about this study. Patients who receive other regenerative therapies, reject the curettage treatment, or fail the evaluation period were excluded.

Sample size was calculated according to Kim and Dailey (31):

$n_1 = n_2 = (2\sigma^2 (z_{(\alpha/2)} + z_\beta)^2)/(\mu_1 - \mu_2)^2$

n_1 = number of samples treatment group
n_2 = number of samples control group
z = value in the standard normal distribution
σ = standard deviation
μ_1 = mean outcome of the treatment group
μ_2 = mean outcome of the control group

We calculated the sample size to be n = 11.18 ≈ 15 individuals. We added more samples to avoid loss follow up, the number of sample size was 15.

Subject selection has a randomized controlled trial design trial design.

Treatment

Previous publications described the preparation of gelatin-CHA (GC) membrane (13, 14, 16). Briefly, carbonated hydroxyapatite is chemically mixed with the type B bovine gelatin in 3:7 w/w composition. The membrane was obtained by casting the solution into a polypropylene dish, then freeze-dried. The membrane was finally crosslinked using dehydrothermal treatment in a vacuum condition and cut to have similar shape and dimension with collagen-chlorhexidine digluconate (CCH) membrane (Periochip, Perio Products Ltd, Jerusalem, Israel). The gelatin-carbonated hydroxyapatite-chlorhexidine gluconate (GCCH) membrane was prepared by dropping 12,5 µl chlorhexidine gluconate 0.2% (Minosep, Minorock Mandiri, Jakarta, Indonesia) onto the GC membrane to be fully absorbed. Both GC and GCCH membranes are sterilized with low temperature oxide ethylene gas in an individual pack. Forty-five periodontal pockets with 3-5 mm depth were randomly assigned to receive GC, CCH, or GCCH membranes after curettage procedure.

All subjects received scaling and root planing (SRP) and oral hygiene instruction at the initial appointment (Figure 1). A week after, they underwent a clinical examination to evaluate the baseline of bleeding on probing (BOP), pocket depth (PD), and relative attachment loss (RAL). Sites with pocket depth 3-5 mm were treated with a curettage procedure by an operator who is blinded to the condition of the site and is not involve in the treatment randomization.

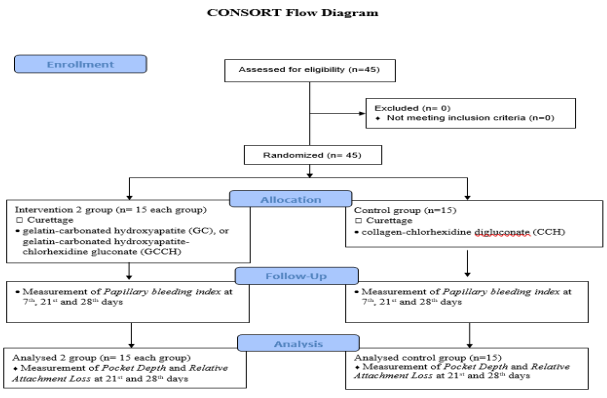

Figure 1: CONSORT Flow Diagram

The BOP score was determined according to papillary bleeding index by Saxer and Muhlemann (17). A periodontal probe (UNC 15, Osung MND Co., Ltd., Seoul, South Korea) was inserted through gingival margin. The bleeding was observed for 30 seconds, and the corresponding score was recorded.

The PD was measured as the distance between the gingival margin and the pocket base at 6 points, that was mesiofacial, midfacial, distofacial, mesiolingual or mesiopalatal, midlingual or midpalatal, and distolingual or distopalatal. The RAL was determined from the

distance between the point on the acrylic stent and the pocket base. Both PD and RAL are measured using the periodontal probe (UNC 15, Osung MND Co., Ltd., Seoul, South Korea). We evaluated BOP after 7, 21, and 28 days while PD and RAL are measured after 21 and 28 days.

RESULTS

A total of 9 patients (7 male and 2 female) aged 21-54 years participated in this study. All subjects were completed the study. Data were statistically analyzed to evaluate the differences between groups with a significance level of 0.05. The BOP results from GC, GCC, and GCCH groups were declining over time as shown in Table I. The BOP data were analyzed using Kruskal-Wallis dan Mann Whitney tests to evaluate the significance of different between groups.

Table I: Median (minimum-maximum) of score of bleeding on probing

Time (day)	CCH Group	GC Group	GCCH Group
Baseline (0)	2 (1-3)	2 (1-3)	2 (1-2)
7	1 (0-1)	0 (0-1)	0 (0-1)
21	0 (0-1)	0 (0-1)	0 (0-1)
28	0 (0-0)	0 (0-0)	0 (0-0)

CCH: collagen-chlorhexidine digluconate; GC: gelatin-carbonated hydroxy-apatite; GCCH: gelatin-carbonated hydroxyapatite-chlorhexidine gluconate. Interpretation:
Score 0 no bleeding.
Score 1 bleeding point in the gingival sulcus 20-30 seconds after probing.
Score 2 a thin line of bleeding or a few of bleeding point is observed in the margin of gingiva.
Score 3 interdental papilla is full of blood after probing.
Score 4 blood drains into interdental area. Bleeding covers tooth and or it's gingival after probing.

The median BOP at baseline had scored of 2 in all groups. After 7 days, the median BOP on GCC group is 1, while GC and GCCH groups have a score of 0. On the 21st day, all of three groups has median BOP score of 0 with a range of 0-1. While on the 28th day the median BOP of all groups was at a score of 0. However, on the 28th day BOP score of CCH group was significantly higher than GC group (p=0.035).

The comparison of clinical measurements of PD and RAL at baseline, day 21st, and 28th after treatment is summarized in Table II. Data of PD and RAL were in ratio scale. We applied for the Saphiro-Wilk test to identify data distribution. The test showed both parameter data were not normally distributed. Kruskal-Wallis and Mann Whitney test were performed to determine significance of difference between group.

Table II: Measurement result of pocket depth and relative attachment level

Variable	CCH Group (n=15)	GC Group (n=15)	GCCH Group (n=15)
Pocket depth (mm, mean ± standard of deviation)			
Baseline	4.03 ± 0.67	4.27 ± 0.46	3.37 ± 0.52
Day 21	2.02 ± 0.77	1.70 ± 0.49	1.73 ± 0.59
Day 28	2.00 ± 1.31	1.63 ± 0.61	1.57 ± 0.68

CONTINUE

Table II: Measurement result of pocket depth and relative attachment level (CONT.)

Variable	CCH Group (n=15)	GC Group (n=15)	GCCH Group (n=15)
Pocket depth differences (mm, mean ± standard of deviation)			
Day 21 – Baseline	1.83 **± 0.75**	2.57 **± 0.53**	1.63 **± 0.58**
Day 28 – Baseline	2.03 **± 1.11**	2.63 **± 0.72**	1.80 **± 0.62**
Day 28 – Day 21	0.20 **± 1.15**	0.07 **± 0.65**	0.17 **± 0.65**
Relative attachment level (mm, mean ± standard of deviation)			
Baseline	12.23 **± 1.88**	12.33 **± 1.50**	11.57 **± 1.94**
Day 21	10.40 **± 1.96**	9,83 **± 1.80**	9.93 **± 2.22**
Day 28	10.20 **± 2.11**	9.57 **± 1.78**	9.83 **±1.96**
Relative attachment level differences (mm, mean ± standard of deviation)			
Day 21 – Baseline	1.83 **± 0.75**	2.50 **± 0.65**	1.63 **± 0.58**
Day 28 – Baseline	2.03 **± 1.11**	2.77 **± 0.56**	1.73 **± 0.65**
Day 28 – Day 21	0.20 **± 1.15**	0.27 **± 0.53**	0.10 **± 0.60**

Compared to baseline data, PD and RAL parameters of all groups decrease over time. In this study, GC and GCCH membrane lower the periodontal pocket depth to a healthy gingival sulcus depth range starting at day 21st. This finding was consistent to day 28th. However, CCH membrane application showed more unpredictable results. At day 21st and 28th, mean of PD on GC group are 2.02 ± 0.77 and 2.00 ± 1.31, respectively. On day 21st, PD reduction of GC group was statistically higher than CCH group (p=0.007) and GCCH group (p=0.000). The difference between GC and GCCH groups was still significant after 28 days (p=0.003).

GC group shows greatest RAL decrease at day 21st and 28th compared to baseline. At day 21st, RAL decrease of GC group was significantly greater than CCH and GCCH groups (p=0.016 and p=0.019, respectively). But the difference between CCH and GCCH groups was not significant (p=0.449). The situation is similar at day 28th. In GC group, RAL decrease was significantly greater than CCH and GCCH groups (p=0.001 and p=0.000, respectively).

DISCUSSION

In this study, curettage was administered with the addition of drug delivery system as an adjunctive to increase the clinical result of curettage. The system is designed to locally introduce antiseptic into periodontal pocket. Operator blinding is partly impossible to implement because of dissimilarity of CHH and GC membrane appearance. However, GC and GCCH membranes are difficult to distinguish (Figure 2).

Figure 2: Periodontal membranes used in this study are (A) CCH: collagen-chlorhexidine digluconate; (B) GC: gelatin-carbonated hydroxyapatite; (C) GCCH: gelatin-carbonated hydroxyapatite-chlorhexidine gluconate.

Chlorhexidine was a chosen agent for periodontal treatment as it is a widely used broad-spectrum antimicrobial to inhibit bacterial growth and, thus, an adjunctive mean to control oral hygiene in patients with periodontal disease (3, 7, 18). A two-phase release profile of chlorhexidine from the chip has been observed. An initial burst of unbound chlorhexidine release was found over the first 24 to 48 hours during which 40% of the chlorhexidine was released into a buffer solution containing collagenase. Thereafter, a slower release of the remaining chlorhexidine, bound to the matrix, occurred until complete biodegradation of the chip was completed (19, 20).

Local administration of chlorhexidine using a delivery system does not increase the adverse effects, but the clinical results are heterogeny. A delivery system of chlorhexidine is available in the market, but it is costly and limited in the availability, for example in European countries (5). In search of the alternative, we incorporate chlorhexidine into gelatin-carbonated hydroxyapatite which was previously prepared as a scaffold for periodontal regeneration and a delivery system (13, 14, 16).

The phenomenon of bleeding upon probing indicates gingival inflammation. Inflammation promotes vascular changes, including capillary vessels dilatation and increasing blood flow (17). Treatment using GC, CCH, or GCCH membrane in this study showed similar pattern of BOP scores, which were declining during the first 7 days. This result supports the study that shows beneficial effect of local administration of chlorhexidine digluconate in improving early wound healing in the first 24 hours after application (21). Interestingly at 28th days after treatment, BOP score of CCH group is statistically higher than that of GC group but not with GCCH group. This indicated that local administration of an antimicrobial agent is not a necessity to reduce gingival inflammation post-curettage of 3-5 mm PD cases. However, a careful consideration needs to be taken in deciding antimicrobial local administration as reduce susceptibility against chlorhexidine digluconate was found on several strain of bacteria, for example *Pseudomonas aeruginosa* and *Staphylococcus aureus* (22).

The parameter of PD is usually, but not always, correlated with attachment level (2). In this study, both PD and RAL were showing the tendency to be decrease over time on all treatment group. However, PD and RAL decrease observed on GC group is greater than CCH and GCCH group. GC membrane can reduce PD and RAL through its CHA role in the healing process of periodontal tissue, which increases fibroblast proliferation at week 2 as collagen connective tissue cells which are the main compounds in the formation of collagen fibers (23, 24). Antimicrobial effects on the CHA were also investigated. An alkaline CHA stable at 168 hours and decreasing thereafter. This alkaline property is associated with antimicrobial effects because the alkaline environment will interfere with bacterial proliferation (23). This study showed no advantage was gained from merging chlorhexidine into the GC membrane which contain CHA. Chlorhexidine forms a physical interaction with CHA, so that no ion exchange was formed and results in a weak bond. This weak bond promotes an initial burst release of chlorhexidine when the GCCH membrane was applied to the pocket (26, 27). The degree of acidity of chlorhexidine also influences incorporation into GC membrane. An acidic environment causes autocatalysis or hydrolysis of membranes and increases their degradation (28).

CHA contains various ions, including Ca^{2+} and OH-, which are also components of CaOH (28). Previous study indicated the combination of CaOH and chlorhexidine causes the deposition of CHX molecules and decreases their effectiveness (29). An in vitro study showed no additional antibacterial effect of chlorhexidine incorporation to CaOH. In the study, CaOH does not lose its antibacterial properties. CaOH creates an alkaline environment. At pH more than 10, chlorhexidine release H^+ ion thus reducing its solubility. Its interaction with the surface of bacteria is also altered due to changes in molecular charge (30). CHA is also alkaline. The incorporation of apatite carbonate and chlorhexidine remains to be investigated.

CONCLUSION

There is no additional advantage of using gelatin membrane carbonate apatite as a delivery system for chlorhexidine after curettage to the healing of chronic periodontitis which is evaluated from parameters pocket depth, relative attachment loss and bleeding on probing. Gelatin-carbonated apatite membrane itself promotes greater improve the clinical parameters starts from day 21st compared to collagen-chlorhexidine digluconate or gelatin-carbonated apatite-chlorhexidine gluconate membranes. This study showed the use of local antimicrobial agent as adjunctive treatment after curettage failed to promote better periodontal regeneration clinically. Further risk-benefit consideration needs to be taken before administering an antimicrobial agent.

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