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Smart liposomal Chitosan - based Auto gel with Ofloxacin; a new Controlled Release Device used for Treatment of Chronic Periodontitis. A Randomized, Double-blind Controlled Clinical Trials

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Abstract

Background: *Chronic periodontitis is an inflammatory disease caused by groups of specific microorganisms. Localized problem sites raise the concept of use of local drug delivery as effective therapeutic modalities for the treatment of periodontal diseases. The aim of the present study was to evaluate the clinical and microbiological effect of a single administration of a smart controlled-release liposomal autogel system of ofloxacin in adjunct to non- surgical therapy in the management of chronic periodontitis patients.*

Materials and Methods: *In a split mouth design, twenty patients suffering from chronic periodontitis and displaying at least two contra-lateral intrabony defects were randomly selected. Non-surgical treatment (root planing and subgingival scaling) was performed in all sites. One of the two forms of ofloxacin, liposomal autogel, was applied in twenty of the pockets. The other twenty pockets were received non surgical periodontal therapy with ofloxacin solution and act as the control sites. The autogel based on chitosan neutralized by β- glycerophosphate was characterized for mucoadhesion, syringibility and gelation onset. The gel, liposomes afforded 80% of drug release in 7 days.*

Clinical parameters; including plaque index, gingival index, bleeding on probing, probing depth, clinical attachment level (PI, GI, BOP, PD and CAL); were recorded at the base line and 3 months following the non- surgical periodontal therapy. In addition, microbiological examination at the baseline 1, 3 and 7 days after were done to assess the sustained release effect.

Results: *The microbiological assessment revealed that ofloxacin liposomal autogel demonstrated markedly lower anaerobes bioburden in subgingival samples than Ofloxacin solution after 7 days. Moreover, the liposomal autogel formula showed significant improvement in the different clinical parameters evaluated after three months.*

Conclusion: *Based on the microbiological and clinical results of the present investigation, the developed ofloxacin liposomal autogel is thought to be promising in the management of chronic periodontitis.*

Keywords: Ofloxacin, Smart Autogel, Chitosan/B-Glycerophosphate, Liposomes, Controlled Release, Chronic Periodontitis.

Introduction

Chronic periodontitis is a multifactorial infectious disease of the teeth supporting structures, characterized by destruction of the bone and connective tissue. The main etiological factors of periodontal disease are the presence of virulent periodontopathic bacteria that are complex and composed mainly of Gram negative anaerobic bacteria [1]. Moreover, considerable research into the identification of organisms responsible for periodontitis has *implicated Porphyromonas gingivalis, Prevotella intermedia, Eikenella corrodens, Fusobacrerium nucleatum, and Tanerella forsythus* for chronic periodontitis, however, the exact mechanisms of tissue destruction are not completely elucidated [2,3]. These suspected periodontal pathogens have been shown to produce a large number of biological molecules that may act directly on host tissue and destroy its integrity. On the other hand, there is anevidence suggesting that the multitude of inflammatory and immune mediators produced by the host may cause tissue injury [4]. Therefore, periodontal therapy is aimed to remove the bacterial deposits from the tooth surface and to shift the pathogenic microbiota to one compatible with periodontal health [5].

Antimicrobial agents, used as adjunctive to different therapeutic approaches which include mechanical or surgical methods, are one

of most appropriate ways. The main goal of antibiotic therapy is to establish a concentration of drug that inhibits these pathogenic bacteria [6]. With advances in understanding of the etiology and pathogenesis of periodontal disease, attention has been focused on local drug delivery systems. These include both sustained and controlled release polymeric systems which when inserted into periodontal pocket, release antimicrobial agents above the minimum inhibitory concentration (MIC) for a sustained period of time. Thus, intra-pocket devices have high benefit to low risk ratio [7]. Moreover, non- surgical approach (which include mechanical scaling and root planing) with adjunct local antimicrobial therapy proved useful treatment of periodontitis [8].

Ofloxacin is a fluroquinolone antibiotic that has shown marked antibacterial activity against periodontopathic bacteria [9]. Nevertheless, its use in treatment of periodontitis is not adequately reported in literature. The only available forms of ofloxacin reported for treatment of periodontitis was a controlled release strip coded PT-01 and made of poly (methacrylic acid) and hydroxypropyl-cellulose containing 10% ofloxacin that afforded 40% ofloxacin release in 1h and 70% release in 8h [10]. Additionally, Yamagami and co-workers developed a newly water soluble controlled release insert containing ofloxacin as antibacterial agent Pt-01 that resulted in resolution of the periodontal inflammation [11].

More recently, investigated the effectiveness of a single subgingival administration of bio-absorbable controlled release 0.1% ofloxacin gel as an adjunct to scaling and root planing in the treatment of chronic periodontitis [12].

Other studies suggested that this type of delivery system could significantly influence the outcome of therapy [13]. Ofloxacin in carrier polymer ethyl cellulose showed extended spectrum of antimicrobial activity and sustained *in vitro* release for a period of 11 days and could be maintained above MIC for the entire period of release [6]. With this perspective, controlled release delivery systems that can elute the antimicrobial agent at therapeutic level within the periodontal pocket over extended periods are valued by most clinicians. Additionally, systems in the form of injectable gels are more privileged than solid devices in being biocompatible, biodegradable, mucoadhesive and hence no need for surgical removal after complete drug elution [4]. Moreover, thermoresponsive gelling systems are highly appreciated due to their ease of syringeability and high ability to fill the pocket [14].

Over the past few decades, novel approaches for drug delivery have been developed to alter the pharmacokinetic and pharmacodynamics properties of active pharmaceutical ingredient and to fabricate new polymeric materials that respond to external stimuli, such as temperature, light and pH [15]. This environmental responsiveness can be used to favor the encapsulation/release of active molecules [16]. One of the most used nanosystems to encapsulate drug molecules are liposomes, which are spherical bilayer vesicles formed by dispersion of certain polar lipids in aqueous solvents [17]. They have the ability to act as targeted release-on-demand carrier systems for both water- and oil-soluble functional compounds such as antimicrobials, antioxidants, and bioactive ingredients [18]. Encapsulation of functional components in liposomes has been shown to increase their stability and maintain their activity in environments that typically lead to rapid degradation [15]. Furthering, they have high loading capacities for water-soluble components and are biocompatible, biodegradable, and nontoxic [19]. Liposomes generally carry a negative surface charge due to the prevalence of phosphatidylcholine (PC) as a raw material, hence, manufacturing of positively or neutrally charged liposomes requires use of positively charged polar lipids such as phosphatidylethanolamine (PE) [20,21].

Chitosan, that exhibits high antimicrobial activity, is an indigestible polysaccharide and considered as polycationic due to the presence of amino groups. Because of its positive charge, it has been used previously to build secondary layers around dispersed particles such as emulsion droplets and biopolymeric particles [20-23]. Chitosan is obtained by alkaline deacetylation from chitin (poly-N-acetyl-D-glucosamine), a major structural polysaccharide in the exoskeleton of arthropods and the cell wall of fungi. Onsoyen and then Chen reported formation of a variety of structures upon addition of chitosan to liposomes [22,24]. They speculated on the formation of so-called chitosomes, that is, chitosan-coated liposomes.

Chitosan/β-glycerophosphate (C/β-GP) system was recently explored as thermo-responsive gelling systems in many applications with benefits in being biocompatible, biodegradable, mucoadhesive, formulated at low polymer concentration, having reasonable gelation temperature and can sustain drug for a longer period of time [25-29]. Chitosan is an excellent candidate vehicle in periodontics for its reported safety as a promising scaffold for tissue engineering and its antimicrobial activity against periodontopathic bacteria [26, 30-32]. The polyol base, β-GP, serves dual function: first, increase the pH of chitosan to the physiological range (6.2-7.3). Second, prevent immediate precipitation or gelation, but instead, allow for controlled hydrogel formation when an increase in temperature is imposed i.e. confer thermoresponsive character to chitosan [33, 34]. It was also reported that periodontal films of ofloxacin containing chitosan showed an initial burst release of the drug by more than 40%, whereas, the maximum drug release (96.38) over a period of ten days [35].

Therefore, autogels with reasonable pH and temperature of gelation could be prepared after setting a high degree of deacetylation, i.e. >90% for chitosan. Prior encapsulation of the antibiotic in vesicular or particulate systems should afford more controlled ofloxacin release whereby the concentration of the antibiotic could be maintained for a sufficient time (at least a week) above the MIC. The prolonged residence time of ofloxacin in the periodontal pocket, would be beneficial in decreasing the systemic side effects and the frequency of administration. Thus, the main objective of the present study was to evaluate the clinical and microbiological effect of the use of new designed smart intrapocket delivery system for ofloxacin with site-specific gelation and controlled release in patients with chronic periodontitis.

Materials and methods

Study population

This parallel-design-double-blind study was conducted on twenty patients having moderate to severe chronic periodontitis. The participants were selected from the Outpatient clinic, referred to Oral Medicine and Periodontology Department, Faculty of Dentistry, Sinai University during January 2015-June 2016.

Each patient was informed of the objectives and nature of the study, including benefits and risks, and was required to sign an informed consents prior participation in the study. This study complied with the Helsinki Declaration of 1994, as revised in 2004 and approved by the Committee on Ethics Involving Human Subjects.

Patient population consisted of twenty patients (12 female and 8 males) with an age ranged from 39-55 suffering from chronic periodontitis and exhibiting forty intrabony defects. The selected patients were systemically healthy, no history of periodontal therapy in the past 6 months, not receiving any medications or antibiotics known to affect the periodontal status during the past 6 months. Each patient had chronic periodontitis with at least two contralateral periodontal defects with clinical attachment loss ≥ 3 mm.

Exclusion criteria included history of hypersensitivity or allergy to quinolones group; pregnancy or lactation; smokers; a history of alcohol abuse; and participation in other clinical trials.

Study design

Initial visit:

Subjects who met the inclusion/exclusion criteria were assigned numbers in ascending order by the study coordinator and completed a written medical history, which was verbally. Vital signs were taken, together with comprehensive intraoral examination, by a single investigator.

Clinical parameters: The following clinical parameters which include; Plaque index (PI) - ***Silness & LÖe***, Gingival index - ***LÖe & Silness***, bleeding on probing (BOP) - ***Newbrun***, probing depth (PD), and clinical attachment level (CAL) - ***Carranza & Taki***; were recorded using graduated William's probe at base line and three months following the non- surgical periodontal therapy [36-39].

Randomization procedure and treatment protocol

All sites (forty intrabony pockets) were randomly assigned by the study coordinator, using a coin toss, to receive one of the two treatments. They were treated with single course of non-surgical instrumentation followed by administration of either the test formula i.e. ofloxacin liposomal autogel (20 pockets) or ofloxacin (0.1%) solution in phosphate buffer pH 6.8 to serve as positive controls. Both medications were coded by the pharmacist and given to the study coordinator who has the only person who had access to them. The randomization process led to comparable mean values of all investigated clinical parameters at base line in both groups.

All participants were given detailed instructions in self performed plaque control measures but instructed not to use any type of chemical plaque control.

Microbiological evaluation

Baseline sampling (subgingival plaque collection)

After complete isolation and full mouth scaling (removing the supragingival plaque) and root planing, subgingival plaque samples were collected by inserting a sterile paper point into a periodontal pocket and kept for 30s. Each paper point was transferred within 1 or 2s to 5ml thioglycolate transport medium in well closed screw capped bottles. The bottles were collected in anaerobic jar [9,40-42].

At the same session, experimental sites received a single subgingival application of ofloxacin liposomal autogel or ofloxacin solutions. The preparations were slowly delivered to the bottom of the periodontal pocket by using a disposable syringe equipped with a blunt needle until overflowed from the gingival margin. All food and drink were prohibited for 2h after injection. Also, patients were instructed not to use Mouth washes, antibiotics or any anti-plaque agents during the observation interval. All pockets were sampled again at days 1, 3 and 7 for culture studies to measure the bactericidal efficacy of ofloxacin liposomal autogels in comparison to ofloxacin solutions.

Determination of bacterial count

The bacterial deposits were displaced from each paper point and dispersed by vortex mixing for 30s. The bacterial viable count was done using the agar diffusion technique employing the spread plate method [43]. One ml of the homogenized bacterial suspension was placed on sterile Colombia agar plates. The plates were incubated at 37°C for 48h in an anaerobic jar where full anarobiasis was achieved by using gas pack for the generation of hydrogen and carbon dioxide in the jar. The total colony forming units per ml (CFU/ml) were counted on the Colombia agar plates [9,40-42]. Percentage reduction of bacterial count was calculated according to the following equation:

$$\textbf{\% reduction in bacterial count from baseline} = \frac{\textbf{C0 - Ct}}{\textbf{C0}} \textbf{X100}$$

C0 = Bacterial count at day 0 (baseline line)

Ct = Bacterial count at day t (1, 3 and 7)

Statistical Analysis

All tests were conducted in triplicates and the results were expressed as the mean ± standard deviation (SD). Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) followed by the Bonferroni test for multiple comparison at $P<0.05$ using Instat-ANOVA software.

Results

Table of the clinical parameters for control and test pockets receiving ofloxacin solution and liposomal autogel respectively at day 0 and after 3 months.

Control Group			Test Group		
	Day 0	3 months after		Day 0	3 months after
Mean PI[1]	2.25±0.44	0*	**Mean PI**[1]	2.25±0.36	0*
Mean PI reduction[2]					
2.25±0.44[a]			2.25±0.36[b]		
Mean GI[1]	2.42±0.66	1.37±0.42*	**Mean GI**[1]	2.5±0.52	0*
Mean GI reduction[2]					
1.05±0.24[c]			2.5±0.52[d]		
(BOP) frequency	83.33%	50.00%	**(BOP) frequency**	100%	0%
(BOP) frequency reduction[2]					
33.33%[c]			100%[d]		
Mean PD	4.41±1.48[a]	3.54±1.37[b]	**Mean PD**	4.92±1.09	2.54±1.01*
Mean (PD) reduction[2]					
0.87±0.11[C]			2.38±0.08[d]		
Mean CAL	3.41±1.16[a]	3.13±1.17[b]	**Mean CAL**	5.38±1.53	3.33±0.83*
Mean (CAL) gain[2]					
0.28±0.01[c]			2.05±0.70[d]		

[1] PI (Plaque Index) and GI (Gingival Index) = total score/number of surfaces examined.

[2] Mean reduction or gain = difference between the mean at baseline and after 3 months of treatment. [a, b] not significantly different (unpaired 1 test, p = 1.0000). [c, d] significantly different (unpaired t test, P<0.05). *significantly different from the corresponding base line (unpaired t test, P<0.05).

This table showed the mean values and standard deviation of the clinical parameters at the baseline and three months after treatment in

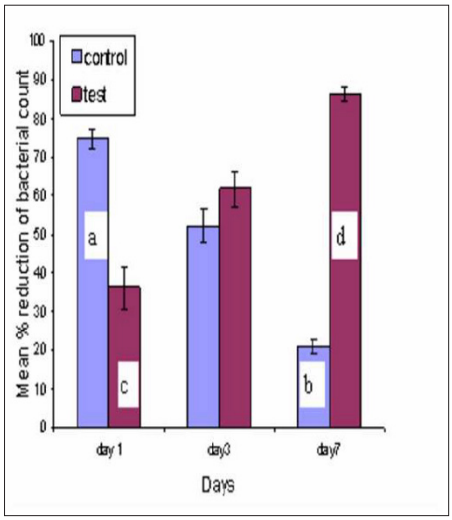
the control and test groups. The mean values of the plaque index in the control and test groups were 2.25 + 0.44 and 2.25 + 0.36, respectively at the baseline. On the other hand, there was no statistical significant difference in this index between both groups at the end of the treatment as all pockets showed no plaque (score 0) two weeks after injection.

Furthering, the mean values of the gingival index in the control group was 2.42 + 0.66 at the baseline and 1.37 + 0.42 after treatment. Regarding the test group, the mean values of this index at before and after treatment were 2.5 + 0.52 and 0, respectively. The mean GI reduction was 1.05 + 0.24 in the control group and 2.5 + 0.52 in the test group. However, the improvement of the gingival condition in the test group was significantly different from the control group (unpaired t test, $p<0.05$).

BOP frequencies before treatment were 88.33% and 100% in the control and test groups, respectively, and were significantly reduced to 50% and 0% at the end of the second week (unpaired t test, $p<0.05$). However, the reduction in BOP frequency in the test group was significantly different from the reduction in BOP frequency in the control group (unpaired t test, $p<0.05$).

In addition, the PD in the test group showed a mean value of 4.41 + 1.48 at the baseline, and 3.54 + 1.37 after treatment. In the control group, the mean values before and after treatment were 4.92 + 1.09 and 2.54 + 1.01, respectively. Considering the CAL, the mean values in the test group at the baseline and after treatment were 3.41 + 1.16 and 3.13 + 1.17, respectively. On the other hand, the mean values in the control group before and after treatment were 5.38 + 1.53 and 3.33 + 0.83, respectively. Moreover, mean PD reduction was 0.87 + 0.11 and 2.38 + 0.08 mm in the control and test groups, respectively. This reduction was in parallel with the mean CAL gain which was about 0.28 + 0.01 and 2.05 + 0.70 mm in the control and test groups, respectively.

Figure of the mean % reduction in bacterial count in both groups. a and b show significant difference (unpaired t test, $p<0.05$). c and d show significant difference (unpaired t test, $p<0.05$). a and d show significant difference (unpaired t test, $p<0.05$).



Regarding the microbiological results, the bacterial count of subgingival plaque at each periodontal site was performed at day 0, 1, 3 and 7 as shown in the figure. Periodontal pockets in the test group (receiving ofloxacin liposomal autogel) had mean bacterial percentage reduction of 36.13%, 61.78% and 86.20 %, while for those in the control group (receiving ofloxacin solution), the mean bacterial percentage reduction values were 74.74%, 52.09% and 20.75% at day 1, 3 and 7 respectively, confirming a long lasting antibacterial action in case of liposomal autogel. The antibacterial effect of ofloxacin liposomal autogel (86.20% bacterial reduction; day 7) was significantly higher than ($p<0.05$) the antibacterial effect of ofloxacin solution (maximum bacterial reduction 74.2%; day 1).

Discussion

Scaling and root planing in conjunction with proper plaque control results in alteration of the subgingival environment that is sufficient, in most instances to improve periodontal health and arrest further loss of attachment. Nevertheless, scaling and root planing alone may not predictably lead to complete elimination of the disease [44]. Poor access to the bottom of deep pockets and anatomical complexities may occasionally limit the efficacy of root planning. Moreover, some bacteria have been shown to invade deep periodontal tissues, making mechanical therapy alone sometimes ineffective and repopulation of scaled teeth from bacterial reservoirs in dentinal tubules may also be responsible for recurrence of the disease [45,46].

Various antimicrobial agents have been administered systemically as well as locally/topically by means of mouth rinses or irrigation solutions as an adjunct to scaling and root planing [47]. However, systemic administration of antibiotics have been associated with side effects, while effectiveness of local delivery of antimicrobial agents in form of mouth rinses and subgingival irrigation has been limited due to inability of the drug to reach the site of action in adequate concentrations and the inability to localize and sustain at disease active sites [45]. Recently, advances in local delivery technology have resulted in control release of drugs that are successful in maintaining effective drug concentration at a lower dosage in the periodontal pocket.

In conjunction with mechanical removal of bacteria and bacterial toxins located on the roots, it appears that the use of smart controlled-release liposomal autogel system of ofloxacin led to reduction of clinical parameters and the bacterial count of subgingival plaque in chronic periodontitis. Ofloxacin is considered as one of the synthetic pyridine carboxylic acid (PCA) derivatives. Although the earlier PCA derivatives were not active against Gram positive bacteria and anaerobes, ofloxacin can kill Gram positive bacteria and anaerobic bacteria and showed marked antibacterial activity against periodontopathic bacteria including Bacteroides species, Fusobacterium species and *Actinobacillus actinomycetemcomitans* [9]. Furthermore, the uptake of ofloxacin by resting polymorphonuclear leukocytes (PMN) appears to be much higher than the uptake of other quinolones. PMN may serve as vehicles for transport and delivery of fluoroquinolones as they migrate from the blood stream to infection sites. By this mechanism, PMN have the potential to enhance resolution of an infection by increasing the local quinolone concentration at sites most beneficial to the host [48]. It was also reported that ofloxacin had high chemical stability and rare adverse effects [9].

Regarding the clinical results, it is worth to report that all the patients receiving ofloxacin liposomal autogel reported no complaints or signs of allergy, inflammation, irritation, pus formation or any other complications suggesting that this formulation as well as ofloxacin solution were well tolerated. Similar results were reported by the study of who evaluated the effectiveness of controlled-release insert

PT-01 and its release profile. The autogel preparation was easy in clinical handling, manipulation and easily injectable inside the pockets [10]. Furthering, it was fluid enough to allow subgingival placement using a simple syringe, requiring only a few seconds to completely fill the periodontal pocket. All pockets (test and control) showed no plaque three months after injection. This might be attributed to plaque removal which was done at the baseline and was followed by constant reinforcement of oral hygiene instruction at each visit. So, there was none significant difference (unpaired t test, p=1.0000) in the effect of both ofloxacin liposomal autogel and ofloxacin solution on the plaque index. However, the mean GI reduction and reduction in BOP frequency, for pockets in the test group were significantly higher (unpaired t test, $P<0.05$) than those in the control group. Thus, the sustainment of ofloxacin in the pocket for a week was more advantageous in reducing gingival inflammation than ofloxacin solution. Additionally, statistical analysis showed that treatment with ofloxacin liposomal autogel achieved extremely significant PD reduction and CAL gain in contrast to the treatment with ofloxacin solution (unpaired t test, $p<0.05$). This could be simply explained by the fact that gel formulation can be easily administered and have relatively faster drug release at the site of application and was also bioadhesive and biocompatible with the oral mucosa [5]. These results were consistent with the results of who reported that weekly application of a water soluble controlled release insert containing ofloxacin PT-01 had a significant effect on the reduction of inflammation in patients with chronic periodontitis [11]. Additionally, similar results were reported after single subgingival administration of bio-absorbable controlled release 0.1% of ofloxacin gel as an adjunct to scaling and root planing resulting in additional PD reduction compared to scaling and root planing alone [12].

Hence, based on the fact that our formula has achieved mean PD reduction of 2.38 mm and mean CAL gain of 2.05 mm, it is clear that this formula achieved clinical improvements greater than those achieved by the marketed products [49].

Regarding the microbiological results, the use of ofloxacin liposomal autogel resulted in 86.20% bacterial reduction at day 7, whereas, the maximum effect of ofloxacin solution on bacterial reduction was 74.2% at day 1. Therefore, the antibacterial effect of gel formulation was significantly higher (unpaired t test, $P<0.05$) than that of the solution. These results were consistent with those of **Kimura et al**. who reported a significant reduction of the total viable counts of bacteria, black-pigmented Bacteroides and Fusobacterium species with the controlled-release insert containing ofloxacin PT-01 [9]. In fact the mean percentage reduction of 86.20% observed after one week treatment with ofloxacin liposomal autogel was considered significantly higher than the reported reduction in bacterial count in the previous studies [9,42]. Hence, it can be concluded that the sustained release achieved by liposomal C/β-GP was important for the bactericidal efficacy of ofloxacin. In addition, the antibacterial effect of chitosan itself might have shared in this high bacterial reduction also proved the bactericidal effect of chitosan based thermosensitive hydrogel against the periodontopathic bacteria [32,50-53]. Hence, three factors might have shared in this extremely significant bacterial count reduction viz, the use of the powerful ofloxacin antibiotic, the use of the bactericidal, slowly eroding, C/β-GP as a vehicle and the sustained release obtained by the liposomal inclusion into the thermoresponsive autogel.

In the present study, floxacin liposomal autogel had proved a greater efficacy in treatment of periodontitis. Two factors might have shared in this significant clinical improvement viz, the significant reduction in bacterial count shown before and the cell proliferation and tissue regeneration-promoting ability of chitosan per se. This proves that C/β-GP is an excellent candidate vehicle in treatment of periodontitis.

Conclusion

Ofloxacin liposomes-containing autogel system released rapidly, therapeutic levels of ofloxacin in periodontal pockets (above the MIC), maintained the antimicrobial levels for at least a week. After mechanical debridement, this smart system markedly suppressed the anaerobes bioburden in the subgingival milieu for at least a week and improved all clinical parameters three months post-treatment proving its superiority over ofloxacin solution. Therefore, this new treatment modality enhances the treatment outcomes for chronic periodontitis patients.

Recommendation

More investigation and long-term studies using this smart system with different concentrations for treatment of other forms of periodontal diseases are suggested to be carried out to affirm the results of our study.

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