A Novel Approach Using Natural 1% (W/W) Chitosan as a Local Drug Delivery System in the Management of Non-Surgical Periodontal Treatment: A Pilot Study

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Abstract

Introduction: Chitosan is a natural polymer found in abundance in nature. It has antimicrobial and anti-inflammatory properties and is biodegradable and biocompatible in nature. It has been used in various forms as an anti-microbial agent in oral care. However, to date, chitosan has not been investigated as a form of local drug delivery (LDD) in the management of non-surgical periodontal treatment (NSPT). Therefore, we aim to investigate the efficacy of natural 1% (w/w) resorbable chitosan membrane as an adjunct to scaling and root planing (SRP) in non-surgical management of chronic periodontitis.

Materials and methods: Ten patients with pocket probing depth (PPD) of ≥ 5 mm were categorized randomly into two treatment groups: test group (SRP plus 1% chitosan membrane) and control group (SRP), in a split-mouth study. The clinical parameters recorded were pocket probing depth (PPD), gingival index (GI) and bleeding on probing (BOP) at baseline before SRP and after 4 weeks. Statistical analysis was performed using Student's *t*-test, Pearson's chi-squared test, the Wilcoxon signed rank test and the Mann-Whitney U test; statistical significance was set at a p value ≤ 0.05.

Results: The test group showed significant improvement in all clinical parameters as compared to the control group. The mean difference between the outcomes of the test and the control groups for PPD was 1.4 mm. Mean ± standard deviation (SD) for GI was 0.20 ± 0.42 at 4 weeks in the test group. All sites (100%) showed a score of '0' for BOP in the test group at 4 weeks. The results were statistically significant.

Conclusion: Within the limitations of this study, it can be concluded that chitosan membranes as a form of LDD could be used as an adjunct to NSPT.

Key words: Chronic periodontitis, local drug delivery, chitosan, membrane

Introduction

Recent developments in the field of dentistry have revolutionized the approach towards the management of periodontal disease (Gupta, 2010). A strong association exists between periodontitis and bacterial plaque (Greenstein and Polson, 1998). The pivotal goal of periodontal therapy is to alter the environment in the vicinity of the gingival tissue, such that it is less conducive for bacterial plaque retention, thus enabling the restoration of function and form of the dentition. Scaling, root planing and soft tissue curettage sometimes demonstrates limited success in treating periodontal disease (Gupta, 2010). This has led to the pursuit for development of drugs to be administered systemically for bacterial elimination. However, the bioavailability of systemically delivered drugs is typically low, thereby requiring re-administration. This expedites decreased patient compliance and increased probability of an overdose (Bhattarai *et al.*, 2010). Hence, an alternate approach of local drug delivery (LDD) was developed to regulate the bioavailability of therapeutic agents by stationing the drug delivery device into a periodontal pocket where gingival crevicular fluid provides a natural reservoir for its insertion with ease (Soskolne, 1997).

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This LDD approach has received a lot of attention in the field of non-surgical periodontal therapy. Arrays of synthetic drugs have been used for LDD such as tetracyclines, including doxycycline and minocycline, metronidazole and chlorhexidine (Greenstein and Polson, 1998). However, the disadvantages of using synthetic drugs are that they induce bacterial-resistant strains (Goodson and Tanner, 1992), degrade inappropriately (Goodson *et al.*, 1991) and are expensive. This shortcoming has been overcome by the inception of natural polymers. Amidst the available natural polymers, the discovery of a novel natural polymer, chitosan, has shown promising potential in various fields.

Chitosan has shown potent antimicrobial action and inhibits periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* (Pichayakorn and Boonme, 2013). Studies have proposed that the positively charged chitosan molecules interact with the negatively charged cell membranes of the microbes. Electrostatic forces mediate this interaction, thereby promoting changes in the permeability of cell membranes, thus provoking an internal osmotic imbalance and eventually inhibiting the growth of micro-organisms. The peptidoglycans in the cell membrane may undergo hydrolysis, causing leakage of intracellular electrolytes such as potassium ions, proteins, glucose, nucleic acids and lactate dehydrogenase (Goy *et al.*, 2009; Koyano *et al.*, 1998).

Chitosan also demonstrates anti-inflammatory activity by modulating prostaglandin E_2 levels (Pichayakorn and Boonme, 2013). The literature suggests that chitosan inhibits inflammatory cytokine IL-6 production in human keratinocytes and IL-12 production in human monocytes. It also downregulates expression of TNF-alpha and IL-6 at the mRNA level. Furthermore, data reveal that signal pathways activated by lipopolysaccharide (LPS), such as c-Jun NH(2)-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), were also found to have been attenuated by chitosan (Azuma *et al.,*2015).

In the field of dentistry, chitosan has been used as an antiseptic in various forms such as gels (Bhattarai *et al.*, 2010) and mouthwashes (Vilasan *et al.*, 2013). Further, chitosan in the past has been used as a carrier system for the local delivery of various drugs (Bhattarai *et al.*, 2010; Pichayakorn and Boonme, 2013) due to its excellent properties such as absorbability, malleability, and cohesive threshold concentration to hold and gradually release drugs with optimal resorption (Benjamin *et al.*, 2009). Because chitosan has limited cytotoxicity, while possessing anti-inflammatory and antimicrobial properties, it may be a good therapeutic option when used alone for local drug therapy in non-surgical periodontal therapy.

Therefore, considering the above-mentioned facts, we aimed to evaluate the efficacy of the adjunctive use of natural polysaccharide, chitosan (1% w/w) as a local drug delivery system with scaling and root planing in the non-surgical management of chronic periodontitis.

Materials and methods

Source of data

The present pilot study consisted of a total of 10 patients, including 6 males and 4 females, attending the outpatient section of the Department of Periodontology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore, from January 2015 to March 2015. The study protocol was reviewed and approved by the institutional ethical committee and review board. The design and nature of the clinical trial and the potential risks, if any, were explained to the patients. A signed written informed consent for their participation was procured from them.

Selection criteria

Systemically healthy patients between the ages of 30 - 55 years who were diagnosed with chronic periodontitis with a single probing depth and clinical attachment loss (CAL) of $\geq$ 5 mm on the distal aspect of posterior teeth without furcation involvement and gingival recession, and who were neither on antibiotic therapy nor had undergone any periodontal therapy in the past 6 months were included in the study. Fifty percent of the selected sites were in the anterior region, whereas the remaining 50% were in the posterior region. Pregnant and lactating women, smokers, patients with suspected or known allergy to chitosan and immunocompromised patients were excluded (Pradeep and Thorat, 2010; Pradeep *et al.*, 2015).

Local drug delivery

A randomized controlled split-mouth study was designed. By means of an envelope technique, 10 sites were randomly allocated as test and 10 sites as control. A well calibrated examiner (MLVP) recorded all clinical parameters to ensure an unbiased evaluation measurement with a UNC 15 (Hu Friedy, Illinois, Chicago) manual probe. Calibration of the examiner was performed by the evaluation of all study parameters on two separate occasions on 10 patients who were not enrolled in the study. Calibration was accepted if the measurements were similar at the 90% level between the two examinations to avoid intra-examiner variability. The other examiner (IB) performed randomization and both the procedures (at test and control sites) during a single session. Thus, the examiner (MLVP) was masked and was unaware of the assignment of sites as test or control during the outcome evaluation.

A resorbable chitosan membrane of 1% (w/w) (Essence Biotech Research Laboratory, Kochi, Kerela, India; Akncbay *et al.*, 2007) was placed in the selected test sites two days after scaling and root planing (SRP) by the examiner (IB). To allow the inflammation to subside, a two-day waiting period was observed prior to membrane placement in the test group in comparison to SRP alone for control group.

The membrane, of pre-determined dimensions 4 mm x 5 mm x 0.1 mm, containing 2 mg of chitosan was inserted into the periodontal pocket using a DeBakey's tissue holding forceps until maximum resistance was felt. Patients were advised to refrain from using antibiotics, mouthwashes and interdental aids following treatment for 7 days to avoid bias (Pradeep *et al.*, 2015).

The clinical parameters assessed were pocket probing depth (PPD; Silness and Löe, 1964), gingival index (GI; Löe and Silness, 1963) and bleeding on probing index (BOP; Ainamo and Bay, 1975) at baseline and 4 weeks post-operatively. A customized pre-grooved acrylic stent with three reference markings, mesio-buccal, mid-buccal and disto-buccal, was used along with a UNC-15 periodontal probe for recording all clinical measurements to ensure reproducibility at subsequent intervals.

Statistical analysis

An SPSS version 21 software program was used to analyze all the data. A Shapiro-Wilks test was done to analyze if the results were parametric or non-parametric. Because the results were parametric, the 4-week post-operative evaluation of PPD was compared to baseline using a paired Student's *t*-test. Unpaired Student's *t*-test was used to compare the results between the test and control groups 4 weeks post-operatively. Pearson's chi-squared test was done to assess the BOP index and compare the 4-week post-operative results to baseline for the test and control groups. Pearson's chi-squared test was also done to evaluate and compare the 4-week post-operative results between the test and control groups. Intra-group analysis for gingival index (GI) was done using the Wilcoxon signed rank test, and inter-group analysis was done using the Mann-Whitney U test. A p value ≤ 0.05 was considered statistically significant, as the sample size was limited.

Results

All 10 patients completed the study. The treated sites were evaluated for clinical parameters at baseline and 4 weeks post-operatively. Uneventful healing was observed. The drug was well tolerated by the subjects and there were no reports of any adverse outcomes or discomfort from chitosan membrane upon post-operative evaluation.

Significant reduction in the PPD and GI was observed between control and test sites at 4 weeks post-operatively compared to baseline. The mean value of PPD at baseline and 4 weeks post-operatively for the test group was 5.90 ± 0.99 mm and 2.70 ± 0.67 mm, respectively ($p = 0.000$), and for the control group it was 5.40 ± 0.51 mm and 3.60 ± 0.51 mm, respectively ($p = 0.000$). The mean score for GI at baseline and 4 weeks post-operatively for the test group was 1.70 ± 0.48 and 0.20 ± 0.42, respectively ($p = 0.004$), and for the control group it was 1.80 ± 0.42 and 0.90 ± 0.31, respectively ($p = 0.003$). According to the BOP index, the control group at baseline showed 100% of sites with a score of 1, which was reduced to 80% at 4 weeks post-operatively (not statistically significant; $p = 0.237$). In the test group, 100% of sites showed a score of 1 at baseline, which was reduced to a score of 0 for all sites at 4 weeks post-operatively ($p = 0.003$). The test group demonstrated a decrease in PPD, GI and BOP index when compared to the control group, and the differences were statistically significant (*Tables 1, 2* and *3*). The mean decrease in PPD in the test group and control group was 3.20 ± 1.03 mm and 1.80 ± 1.03 mm, respectively ($p = 0.007$).

Table 1. Inter-group comparison of pocket probing depth (PPD) between control and test groups (mean ± SD) at baseline and 4 weeks follow-up.

Parameter	Visit	Groups	Mean ± SD	*p* value
PPD (mm)	Baseline	Test	5.90 ± 0.99	0.175
		Control	5.40 ± 0.51	
	4 weeks	Test	2.70 ± 0.67	0.004*
		Control	3.60 ± 0.51	

*$p < 0.05$; unpaired Student's *t*-test

Table 2. Inter-group comparison of gingival index (GI) between control and test groups (mean ± SD) at baseline and 4 weeks follow-up.

Parameter	Visit	Groups	Mean ± SD	*p* value
GI	Baseline	Test	1.70 ± 0.48	0.739
		Control	1.80 ± 0.42	
	4 weeks	Test	0.20 ± 0.42	0.007*
		Control	0.90 ± 0.31	

*$p < 0.05$; Mann-Whitney U test

Table 3. Intergroup comparison of bleeding on probing index (BOP) between test and control group at 4 weeks.

Parameter	Visit	Groups	Score of 1	*p* value
BOP (%)	Baseline	Test	100%	0.175
		Control	100%	
	4 weeks	Test	80%	0.004*
		Control	0%	

*$p < 0.05$; Pearson's chi-squared test

Discussion

After cellulose, chitin is the second most abundant polysaccharide found in nature (Swatantra *et al.*, 2010). Chitin is procured from the exoskeleton of crustaceans (shrimps, crabs, etc). Deacetylation of chitin leads to the commercial production of chitosan (Bansal *et al.*, 2011). It is composed of a linear polysaccharide that is β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units. Amidst the innumerable polymers, the alluring properties of this polysaccharide in comparison to other materials of natural origin are that it fails to transmit the potential risk of pathogens of animal origin and does not induce an immune response (Bhattarai *et al.*, 2010). The commendatory properties of chitosan, such as biodegradability, biocompatibility, non-toxicity, anti-inflammatory (Pichayakorn and Boonme, 2013), anti-bacterial action (Swatantra *et al.*, 2010) and its ability to be sterilized (Bhattarai *et al.*, 2010), make it an ideal material in the management of non-surgical periodontal treatment. The resorbable membrane also demonstrates good retention and is inserted into the periodontal pocket with ease. The resorption time of the membrane is 3 - 5 days.

In the current study, the clinical efficacy of 1% (w/w) chitosan per se was used in the form of a resorbable membrane as a local drug delivery system as an adjunct to SRP for the management of non-surgical periodontal therapy. The results unveiled an improvement in test and control groups that was statistically significant in clinical parameters from baseline to 4 weeks.

Similar studies in the literature using natural polymers have revealed results in accordance with our present study. In a study conducted by Pradeep and Thorat (2010), simvastatin was used in LDD, and the decrease in PPD at the end of 1 month was 3.03 ± 0.99 mm and the GI was 1.95 ± 0.85. In another study, 1.2% rosuvastatin gel was used in periodontal pockets, and the mean decrease in PPD and GI at 4 weeks was 3.14 ± 0.28 mm and 2.21 ± 0.16, respectively (Pradeep *et al.*, 2015).

Reduction in PPD is a beneficial clinical outcome used to assess the success of periodontal therapy. It can be conjectured that the anti-bacterial properties of chitosan aided in decreasing the PPD (Goy *et al.*, 2009). Studies state that positively charged chitosan molecules interact with the negatively charged cell membranes of the microbes, leading to changes in the permeability of cell membranes and eventually inhibiting the growth of micro-organisms (Goy *et al.*, 2009; Koyano *et al.*, 1998).

It can also be surmised that the anti-inflammatory properties of chitosan membrane placed in the test group played a prime role in reducing BOP and GI, as the results were significant at 4 weeks in comparison with baseline. Hence, it could be speculated that the novel properties of chitosan, such as its anti-inflammatory action, mucoadhesion (Swatantra *et al.*, 2010) and promotion of rapid wound healing (Azad *et al.,* 2004) led to overall favorable results.

Even though the current study connotes that chitosan membrane is promising as a suitable adjunct to non-surgical periodontal management, the results of this study should be interpreted with caution, as there are certain inherent limitations. These include limited sample size, the short-term nature of the study and the long-term pharmacological action of chitosan, which is still unknown in the periodontal environment. In the future, there is a need for long-term randomized clinical trials in order to ratify the outcome of this study.

Conclusion

Within the limitations of this study, it can be concluded that chitosan may be a promising adjunct when used in the management of non-surgical periodontal therapy.

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