Effect of Chitosan on sustained release of chlorhexidine –an in vitro study

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ABSTRACT
Introduction: The aim of the study was to evaluate the sustained release effect of 2% chlorhexidine and its combination with 2% chitosan, in vitro. Method: The sustained release effect of chlorhexidine and its combination with chitosan was determined using a UV spectrophotometer. Results: UV spectrophotometer analysis for the sustained release of chlorhexidine showed that almost 100% of chlorhexidine was released within 4 hours. However when chlorhexidine was combined with chitosan, only 59% of chlorhexidine release was observed even at 24 hours. Conclusion: The percentage drug release of chlorhexidine was almost half when used in combination with chitosan, thus indicating the sustained release property of chitosan.

Key words: Chitosan, Chlorhexidine, Sustained Release Effect

INTRODUCTION
The role of the endodontic microflora in pulpal disease and in endodontic treatment failures is well established. Thus the requirement of the effective microbial control is very important justification for biomechanical procedures. This can be established using mechanical instrumentation and chemical irrigation, in conjunction with root canal medicaments in between each treatment sessions. To reduce or eliminate bacteria, various irrigants have been advocated as an adjunct. Chlorhexidine (CHX) has the ability to adsorb onto dentin as it is a cationic bisguanide. It is widely used as a root canal irrigant and as an intracanal medicament. At higher concentrations (e.g. 2%), CHX is bactericidal and causes cell death because of precipitation of cytoplasmic contents. It has a wide antimicrobial spectrum and is effective against bacteria, as well as yeasts; but mycobacteria, bacterial spores and most viruses are resistant to CHX. Its efficacy is primarily due to interaction between the positive charge of the molecule and phosphate groups on the bacterial cell wall which are negatively charged, which allows the chlorhexidine molecule to penetrate the bacteria with intracellular toxic effects. The beneficial effect of CHX is a result of its antibacterial & substantive properties, its ability to inhibit adherence of certain bacteria, and low grade of toxicity. To achieve long-term substantive antimicrobial effects, chlorhexidine gluconate must be exposed onto the infected root dentin for a longer time than that afforded by irrigation. Numerous studies have shown that controlled release drug devices could effectively sustain the release of chlorhexidine from the device. Chitosan comprised of copolymers of glucosamine and N-acetyl glucosamine and is a natural polysaccharide. Chitosan is produced by the partial deacetylation of chitin which is the second most abundant natural polysaccharide composed of B (1,4)-linked GlcNAc (N-acetyl glucosamine) units. Chitosan is a versatile material with proved antimicrobial activity. Recent data in the literature has the tendency to characterize chitosan as bacteriostatic rather than bactericidal, although several other factors may contribute to the antibacterial action, the exact mechanism is not known. It has been used in drug delivery, peptide delivery, gene delivery, as an absorption enhancer and for colon targeting. Chitosan is also being widely used as a pharmaceutical excipient, which has the significant quality of extending the release of drugs. It has been used in combination with collagen sponge as a barrier membrane in periodontal therapy, and also as an delivery agent for chlorhexidine against C. albicans. Ballal et al. (2009) in an in vitro study, tested the sustained release of chlorhexidine using chitosan and found that release of CHX with chitosan was better than...
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the plain chlorhexidine release. They also reported a significant antimicrobial efficacy of chitosan. Hence, this in vitro study was conducted to evaluate and compare the sustained release effect of 2% chlorhexidine and its combination with 2% chitosan.

MATERIALS AND METHOD

A semi-permeable membrane was formed by immersing an egg shell in 1% hydrochloric acid overnight. A proper ratio of sodium chloride, potassium di-hydrogen orthophosphate & di-sodium hydrogen orthophosphate was used to prepare phosphate buffer saline (PBS) solution. A pH of 7.4 was maintained which was confirmed with the help of a pH meter. The semi-permeable membrane was soaked in phosphate buffer saline. The membrane was clamped carefully to one end of the hollow glass tube having a diameter of 2.3 cm (surface area - 4.16 cm²). One gram of the test solution (equivalent to 10 mg of the drug) was spread uniformly over the membrane. The glass tube was submerged in a glass beaker containing 50 mL of phosphate buffer saline maintained at 37±0.5°C. The PBS solution was continuously stirred by an externally driven Teflon-coated magnetic bar and at predetermined time intervals, 5 mL samples of the solutions were withdrawn and replaced with an equal quantity of PBS. The concentration of drug in the “eluates” using a UV spectrophotometer was determined at 254 nm against an appropriate blank. The experiment was done thrice and average values were recorded.

Fig. 1: Semipermeable membrane (Egg)

Fig. 2: Semipermeable membrane clamped to one end of glass tube
RESULTS

Table 1: Percentage drug release of chlorhexidine and chlorhexidine+chitosan

<table>
<thead>
<tr>
<th>Time</th>
<th>Chlorhexidine</th>
<th>Chlorhexidine + Chitosan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absorbance</td>
<td>Drug release (%)</td>
</tr>
<tr>
<td>0.5</td>
<td>0.099</td>
<td>9.9</td>
</tr>
<tr>
<td>1</td>
<td>0.304</td>
<td>30.8</td>
</tr>
<tr>
<td>2</td>
<td>0.341</td>
<td>35.7</td>
</tr>
<tr>
<td>3</td>
<td>0.587</td>
<td>61.7</td>
</tr>
<tr>
<td>4</td>
<td>0.954</td>
<td>100.7</td>
</tr>
<tr>
<td>24</td>
<td>0.966</td>
<td>105.7</td>
</tr>
</tbody>
</table>

Results of the UV spectrophotometer analysis for the sustained release of chlorhexidine showed that almost 100% of chlorhexidine was released within 4 hours. However when chlorhexidine was combined with chitosan, only 59% of chlorhexidine release was observed even at 24 hours. These results indicate the sustained release property of chitosan.
Graph 1: Percentage drug release of chlorhexidine and chlorhexidine+chitosan

DISCUSSION
Chitosan is a natural polysaccharide comprising of copolymers of glucosamine and N-acetyl glucosamine. It has the significant quality of extending the release of drugs, and also their antimicrobial efficacy, as reported by studies. Recent data in literature has reported chitosan to be bacteriostatic rather than bactericidal in nature (Coma et al. 2002). Chlorhexidine is a cationic molecule, which can be used during treatment. It has a wide range of antimicrobial activity. Few studies have shown that controlled release drug devices using chitosan could effectively sustain the release of chlorhexidine from the device. Unlike the other polymers used to sustain the release of drugs, chitosan possesses the additional quality of inherent antibacterial as well as antifungal action. Ballal et al. (2009) in an in vitro study, tested the sustained release of chlorhexidine using chitosan, and found that release of CHX with chitosan was better than the plain chlorhexidine release. They also reported a significant antimicrobial efficacy of chitosan. Percentage drug release of chlorhexidine and chlorhexidine+chitosan was calculated with the absorbance value obtained with UV spectrophotometer. The results of the UV spectrophotometer analysis for the sustained release of chlorhexidine showed that almost 100% of chlorhexidine was released within 4 hours. (Table 1) However, when chlorhexidine was combined with chitosan, only 59% of chlorhexidine release was observed even at 24 hours. Contrary to this finding, Ballal et al. in their study found 100% release of chlorhexidine within 1 hour and on combination with chitosan, 85% of chlorhexidine was released within 3 hours. In another study done by Lee et al. (2005), 100% of chlorhexidine was released within 2 hours and on combination with chitosan, 80% of chlorhexidine was released within 2 hours. The results of our study and the above-mentioned studies indicate the sustained release property of chitosan.

CONCLUSION
The percentage drug release of chlorhexidine was almost half when used in combination with chitosan, thus indicating the sustained release property of chitosan. Further investigation of the different properties of chitosan is required for a better understanding of the possible role of chitosan in sustained release of chlorhexidine.

REFERENCES
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Conflict of Interest: All authors report no conflict of interest related to this study.