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Chlorhexidine-releasing orthodontic elastomerics

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The aim of this study was to identify the best combination of chlorhexidine (CHX) with orthodontic elastomerics for prevention of oral disease in orthodontic patients. We used ethyl cellulose (EC) as the polymer, and experimental groups were divided into five groups according to differences in solvent (*i.e.*, ethanol; EtOH, dichloromethane; DCM). CHX release from the coated elastomerics was evaluated with a UV spectrophotometer and observed by scanning electron microscope (SEM). The antimicrobial release increased over time for 48 h in Group 3 (CDA+EC+30% EtOH/70% DCM), exhibiting the longest sustained-release characteristics ($p<0.001$). It also showed the highest antimicrobial properties, which was confirmed by inhibition zone testing using *S. mutans* ($p<0.05$). All groups were not affected when tensile force was tested in the coated elastomerics. We conclude that the antibacterial effect of CHX can be adjusted according to combinations of polymers and solvents. Group 3 exhibited the best substantivity and antimicrobial properties.

Keywords: Chlorhexidine, Drug release system, Elastomeric, Polymer

INTRODUCTION

Patients receiving orthodontic treatment tend to exhibit plaque build-up around their appliances and enamel demineralization around their brackets because oral hygiene is more difficult for these patients[1]. For this reason, orthodontic patients can easily develop white spot lesions (WSLs) that might cause aesthetic problems. This finding implies there are aesthetic problems with the enamel following orthodontic treatment that, in severe cases, might require restorative treatments[2,3]. Moreover, gingival and periodontal diseases can develop if the accumulated plaque is not sufficiently removed well, and these diseases can lead to tooth loss in severe cases.

Plaque control methods can largely be divided into physical and chemical methods. Bis-biguanide chlorhexidine (CHX) is widely used in chemical methods and is a representative antimicrobial with broad-spectrum antibacterial activity that is known to act against gram-positive/negative germs, aerobic and anaerobic microorganisms, yeast, and fungi[4,5]. Various products containing CHX (*e.g.*, mouthwashes, gels, and varnishes) can be used to prevent dental disease in orthodontic patients[6]. The largest advantage of CHX is its sustained substantivity over long periods of time. This property is known to result in sustained antimicrobial effects due to the easy combination of CHX with the hydroxyapatite of tooth enamel, oral mucosa, and oral bacteria, which results in slow release over 12–24 h[7,8]. However, because saliva is continuously secreted into the mouth and the oral membranes move, the effects of such products do not last long. If antimicrobial materials could remain inside the oral cavity for longer periods of time, they would help to prevent dental disease over the course of orthodontic treatment.

Here, we examined the use of a drug delivery system (DDS). The DDS is a method for adjusting drug release rates or a method for the efficient delivery of the drug to the target site. This method is an administration technique that increases safeness, efficacy, or reliability of the biological drug and improves the formulation of the drug *in vivo* to control the behavior of the system. DDSs are widely used in the medical field[9-11]. In this study, we used a DDS method to maintain CHX inside the mouths of orthodontic patients for longer periods of time. A polymer is a matrix that is capable of prolonging the duration of antimicrobial substances.

Orthodontic patients regularly visit the dental clinic to change the elastomeric ligatures of fixed orthodontic wires to move the teeth. Therefore, antibacterial effects would be expected if the elastomeric ligatures that are employed also consistently maintain a constant concentration of CHX. Thus, we used CHX-releasing elastomerics in this study. Specifically, we used ethyl cellulose, which has been proven to be safe and efficacious in the oral cavity, as the polymer, we used ethanol and dichloromethane as the polymer solvents due to their safety and solubility.

The first aim of this study was to determine the appropriate chemical combination to achieve the sustained release of CHX that had been coated onto the orthodontic elastomerics that are replaced periodically for the prevention of dental disease in orthodontic patients. The second aim of the study was to assess the

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amounts of antimicrobial agents released by each tested combination.

MATERIALS AND METHODS

The solutions that were to be coated on the orthodontic elastomerics were prepared by combining polymers that are capable of controlling the release of the antimicrobial agents, chlorhexidine using two selected solvents (Table 1). In this research, we used ethyl cellulose (EC; SIGMA ALDRICH, Inc., St. Louis, MO, USA) as the polymer; ethyl cellulose has been proven to be safe and efficacious for use in DDSs in the oral cavity. We used ethanol (EtOH; SIGMA ALDRICH, Inc., St. Louis, Missouri, USA) and dichloromethane (DCM; SIGMA ALDRICH, Inc.) as the polymer solvents due to their safeness and solubility. We dissolved ethyl cellulose completely in the prepared solvent to make a coating solution and added chlorhexidine diacetate (CDA; SIGMA ALDRICH, Inc.) as antimicrobial agents using a stirrer[12].

Preparation of the elastomerics

Four mm diameter and 0.005 g elastomerics (TP Orthodontics, Inc., La Porte, USA) were washed in distilled water and dried in a dry oven at 60°C. The elastomerics were then placed in 1 mL of coating solution for simple packing and dried for 1 h at room temperature. The sixteen coated elastomerics were then fixed in 1-mm intervals onto a 2-mm diameter stainless steel wire and fixed onto a vial lid. Next, 4 mL of distilled water was added to an 8 mL vial so that the elastomerics fixed on the lid were submerged. Additionally, distilled water was replaced at set times (1, 3, 6, 9, 12, 36, and 48 h). All procedures were performed at approximately 20ºC with stirring and were repeated four times[13].

Amounts of chlorhexidine released from the coated elastomerics

To measure the amounts of antimicrobial agent that were released every hour, we collected 1 mL of CDA released distilled water and used an ultraviolet spectrophotometer (Spectrophotometer CM-3500d, KONICA MINOLTA SENSING, INC., Osaka, Japan) in 10 s intervals for light stabilization. In accordance with previous studies, the 254 nm wavelength was converted into the CDA release concentration within the standard curve range of 0.1–40 μg/mL (r=0.98). Moreover, a scanning electron microscope (SEM; FE-SEM S-800, Hitachi, Japan) was used to observe microscopic changes of the surfaces of the coated elastomerics.

Antibacterial effect of elastomerics coated with chlorhexidine

S. mutans (*streptococcus mutans* ATCC 25175, Korea Research Institute of Bioscience and Biotechnology, Dae-jeon, South Korea) of 100 μL was pre-incubated in Brain Heart Infusion (BHI) liquid medium to a level of 10^7 CFU/mL and was then smeared on BHI solid medium. Eight coated elastomerics were fixed on the solid medium smeared with the bacteria and were cultured in conditions of 37°C and 10% CO_2 for 48 h. Next, the width and height dimensions of the inhibition zone centered on the elastomeric that were free of the presence of any bacteria were measured using an image analysis program (Image-Pro® Plus 6.0, Media cybernetics, Inc., GA, USA).

Tensile force of the coated elastomerics

In order to know that the original tensile forces of elastomerics are not disturbed by the antibacterial coating process, we evaluated the tensile forces of the CHX-coated elastomerics using a Universal Testing Machine (Unite-O-Matic FM 20, Instron, United Calibration Corporation, Garden Grove, Calif). Based on the time that orthodontists spend mounting elastomerics on bracket for orthodontic patients, we measured the strengths (N) of the elastomerics as they were stretched at 2 mm/s and 3 mm/s to perform group-to-group tensile force comparisons.

Statistical analyses

The volumes of CHX released over time were compared between the groups using repeated measure ANOVA, and the tensile forces of the coated elastomerics were compared according to the tensile lengths *via* two-way ANOVA. The inhibition zones of the elastomerics were compared between groups using one-way ANOVA by

Table 1 Compositions of the coating solutions

Group	Drug (mg)	Polymer (mg)	Solvent (mL)	
	CDA	EC	EtOH	DCM
1 (Control)	10	–	–	1
2	10	100	–	1
3	10	100	0.3	0.7
4	10	100	0.5	0.5
5	10	100	0.7	0.3

CDA: chlorhexidine diacetate, EC: ethyl cellulose N 100, EtOH: ethanol, DCM: dichloromethane

Scheffe's test at a 5% significance level. The statistical analyses were performed using PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA), and the confidence level was set to 95%.

RESULTS

The amounts of CHX released were measured with a UV spectrophotometer as shown in Fig. 1. While there were significant differences between the groups as time elapsed, the antimicrobial release tended to increase over time (p<0.001). The release stopped within 2–3 h in the Control group (Group 1) that did not involve polymers. The groups with polymers and different combination of the solvents exhibited gradual releases of the antimicrobial substance, and there were some differences in the actual amounts released. Group 2, which involved a CDA+EC+DCM combination, exhibited increasing antimicrobial release that was approximately four-fold greater than that of the control group. And we could observe sustained-release property up to 24 h but there was no significant release after 48 h. Among these differences, Group 3, which involved a CDA+EC+30% EtOH/70% DCM combination, exhibited increasing antimicrobial release over the first 48 h that was approximately six-fold greater than that of the control

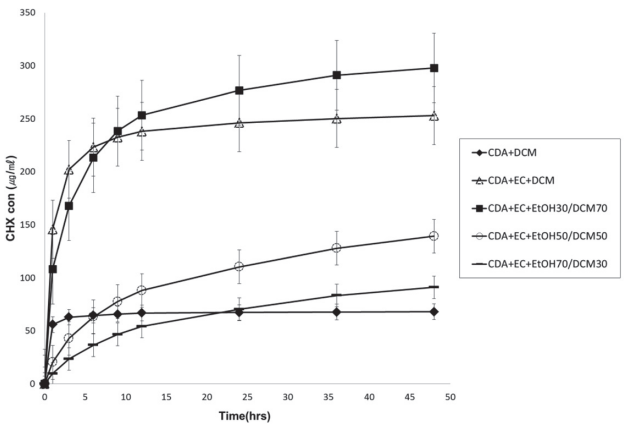
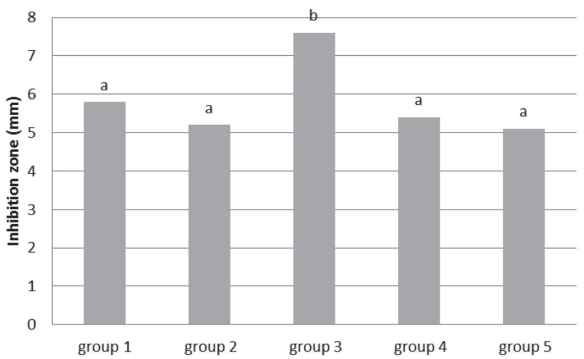


Fig. 1 Cumulative volumes of chlorhexidine released from the polymer film coated elastomerics.
Coating polymers and solvents: Group 1, DCM only; Group 2, EC+DCM; Group 3, EC+30% v/v EtOH:70% v/v DCM; Group 4, EC+50% v/v EtOH:50% v/v DCM; and Group 5, EC+70% v/v EtOH:30% v/v DCM.



Group	1	2	3	4	5
Mean(SD)	5.8(0.18)	5.2(0.15)	7.6(0.42)*	5.4(0.17)	5.1(0.49)

Fig. 2 Inhibition zones of the polymer film-coated elastomerics.
*Significantly different from the experimental group (p<0.05).

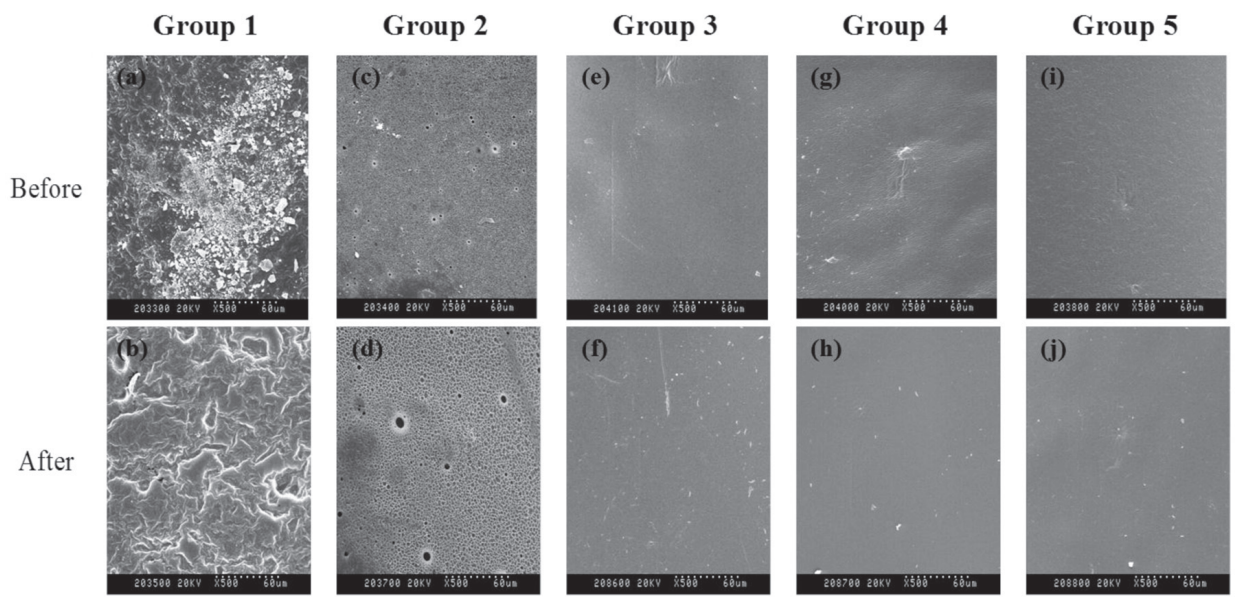


Fig. 3 SEM images (magnification: ×500) of the coated elastomeric surface in the antibacterial substance release test.

Table 2 Tensile test of the coated elastomerics (Unit: N)

Group	2 mm tensile strength	3 mm tensile strength
Group 1 (Control)	5.64±1.23	6.66±1.33
Group 2 (CDA+DCM)	5.64±0.45	6.79±0.37
Group 3 (CDA+EC+30EtOH/70DCM)	5.62±0.30	6.81±0.38
Group 4 (CDA+EC+50EtOH/50DCM)	4.97±0.70	6.19±0.64
Group 5 (CDA+EC+70EtOH/30DCM)	5.59±0.29	6.79±0.28

The data were analyzed with a two-way ANOVA test ($p<0.001$). All values are presented as the mean ±the SD.

group (Fig. 1).

We compared the inhibition zone sizes to evaluate the antibacterial effects of the coated elastomerics using *S.mutans*. All groups were treated with the same amount of CHX, but the size of the inhibition zone of Group 3 was the largest; *i.e.*, we found Group 3 contained a significantly greater amount of antibacterial substance compared to the other groups ($p<0.05$). Also, you can see that there are no significant differences among the other groups through Fig. 2. Group 3 released the antibacterial substance at a concentration that was approximately 23.7% higher than that of the control (Group 1) (Fig. 2).

Before and after the tests of the release of the antibacterial substance, the surfaces of the elastomerics were observed using SEM (Fig. 3).

Group 1, the control group without polymers, exhibited small particles on the elastomerics surface before the release experiment. However, after completing the release experiment, the particles were no longer visible and the original elastomeric surface was exposed. Group 2 involved the use of EC as the polymer and exhibited microscopic holes on the surface before the release experiment, and these holes were enlarged after the experiment was completed. In contrast, Groups 3–5 did not exhibit changes in the elastomeric surfaces following the experiment.

Each coated elastomeric group was compared in terms of tensile force due to stretched lengths that were increased at 2 mm/s and 3 mm/s. The elastomerics in each group were tested 4 times. Although statistically significant differences were observed in terms of stretched lengths, the results revealed no significant differences among the groups according to solvent combinations (Table 2).

DISCUSSION

In the present study, we proposed a method for reducing the risk of oral disease in patients with fixed orthodontic appliances that employed antimicrobial DDSs. DDSs were primarily developed by researchers in the field of pharmacy and have been used in various dental procedures, including implants, root canal treatments, and periodontal treatments, to control infectious diseases[14,15]. However, attempts to apply DDSs with orthodontic elastomerics using antimicrobial substances have been limited.

A polymer is a matrix that is capable of prolonging the duration of antimicrobial substances. There are synthetic polymers, such as polylactide, polyglycolide, and polymethylmetacrylate, and natural polymers, such as gelatin, cellulose, and chitosan[12,16,17]. In this experiment, we used ethyl cellulose[18] as the polymer; EC has been approved by the FDA and has excellent biocompatibility[19,20]. EC is a water insoluble polymer, and its main mechanism for the drug release is the osmotically driven release[21]. The safety of EC in terms of physiochemical safety and low toxicity has been proven in large pharmaceutical studies[22,23]. Thus, EC was deemed appropriate as a polymer capable of producing sustained CHX release from orthodontic elastomerics.

In this study, dichloromethane (DCM) and ethanol were chosen as the solvents because they dissolve polymers well and are highly volatile. Unlike Group 2, in which a single solvent was used, different combinations of solvents were used in Groups 3–5 to identify the best conditions for CHX release. EC was used as the polymer, and CHX release continued for approximately 48 h (Fig. 1). Differences in solvent compositions resulted in different CHX release amounts. This could be due to the fact that different solvent compositions lead to different evaporation rates which in turn, change the physical status of coated surfaces, affecting CHX release amounts.

Our results revealed that the solvent combination producing the largest release rate was 70% DCM and 30% EtOH while a previous research that used CDA in film form found that the combination of 30% DCM and 70% EtOH resulted in sustained antibacterial release over approximately 25 days[12]. This is because when the concentration of DCM is relatively higher, films form dense polymer networks, causing prolonged releases of CHX in distilled water. In comparison, when the EtOH concentration is higher, the resulting polymer network becomes relatively sparse which results in greater CHX release amount[24].

The solubility parameter of DCM is 19.8 $MPa^{0.5}$ and that of EtOH is 26.0 $MPa^{0.5}$ with respect to Ethyl Cellulose[25]. EC with a solubility parameter of 21.18 $MPa^{0.5}$ dissolves in DCM which possesses a similar

solubility parameter as well[26].

Also in Fig. 1, when producing coating solutions using two solvents, Group 3, which had a relatively higher DCM ratio dissolved EC faster than Group 5 with lower DCM concentration. Furthermore, Group 5, which had a higher ratio of EtOH, required a longer agitation time which was approximately 3 times greater than that of Group 3.

Agitation time is one of various factors that determine drug-releasing properties[27]. It seems as agitation time increased, the intermolecular chain structures of the polymer became denser. An increase in EtOH ratio resulted in an increase in agitation time; hence Group 5 which required the longest agitation time probably showed the least amount of CHX release. As coated elastomerics are used in the oral cavity, proper and consistent manufacturing techniques might be needed to achieve optimal CHX release in the oral cavity.

Excessive amounts of drugs in the oral cavity could be toxic, while minute amounts could be ineffective. Therefore, a concentration of ≤1 μg/mL, which is the minimal inhibitory concentration (MIC) of CHX[28], must be sustained in the oral cavity. In our study, Group 3, which exhibited sustained high-volume antimicrobial release for up to 48 h, continued to release CHX at 1 μg/mL across each hour of the experiment (Fig.1). The properties of the elastomerics are influenced by several factors such as moisture, temperature, and pH variations. Even biofilm adsorption and mineral precipitation alter the surface properties and the structural conformations of these accessories[29]. Thus, further experiments are necessary to ascertain whether the same condition can be achieved in the dynamic environment of the oral cavity.

The SEM evaluations of the coated elastomerics revealed that Group 1 (no polymers) exhibited small particles before the release experiment (Fig. 3) and the original elastomeric surface was subsequently exposed by the end of the experiment. This result indicates that it is difficult to achieve sustained CHX release if a polymer is not used in the coating. Group 2 used only DCM as a solvent which probably evaporated very rapidly, forming small pores on the surface. Groups 3–5, which used varying combinations of the DCM and EtOH solvents, exhibited smooth surfaces. Although results from SEM did not allow clear comparisons among surfaces of Group 3 to 5, a smoother surface was observed when EtOH was included, which would have changed the rate of evaporation.

The stretching of the coated elastomerics to 2 mm and 3 mm revealed no significant inter-group differences (Table 2). Thus, the polymers used to coat the elastomerics did not affect the tensile forces of the elastomerics, and coated elastomerics can thus be applied clinically without compromising their originally intended use. However, a previous study used a tensile length of approximately 5 mm/min[30], but our tests were based on the estimated lengths of the elastomerics when ligated to the brackets in clinical application. Additionally, we measured only the tensile force, whereas previous studies have been conducted in the described oral environment. Due to force decay and deformation that can occur with time, ligatures are subjected to environmental factors during their setting into the oral cavity. Additionally, immersion in artificial saliva at 37°C causes tensile force decay rates that vary from 50.89% to 76.34% according to the sample[29]. Therefore, it will be necessary to assess feasibility of the clinical application based on tensile length in future studies. We believe that elastomeric ligatures with sustained-release characteristics can help prevent oral diseases due to plaque accumulation and are an excellent alternative for solving the most common adverse effects suffered by orthodontic patients while maintaining the original aim of the orthodontics. Moreover, we used elastomeric rings in this study, but in the future, the same method can be applied to elastomerics of all types that are used in orthodontics, such as elastomeric chains, to effectively prevent oral diseases.

Ultimately, we found that EC is an appropriate polymer that is capable of producing sustained CHX release and that CHX release periods can be adjusted *via* the use of different proportions of solvents. However, because most orthodontic patients visit the clinic on a monthly basis, methods that further extend the time period of antimicrobial release are needed. Moreover, because polymers tend to add to the thickness of the coating, the coating itself tends to break off at long tensile lengths. Therefore, a new coating method needs to be developed.

In future studies, we will vary the polymer contents to identify conditions for longer CHX release, and the CHX content will be measured in the elastomeric coatings to determine the optimal CHX concentrations for the maintenance of both safeness and antibiotic efficacy in the oral cavity. Moreover, further studies using a microcosm biofilm model for the oral environment are needed.

CONCLUSION

We determined that the time period of the release of the antimicrobial agents, CHX can be adjusted by varying the combinations of polymers and solvents. Group 3 used a CDA+EC+30% EtOH/70% DCM combination, and released CHX for 48 h with the highest level of antimicrobial properties.

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