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Improving the efficacy of chlorhexidine-releasing elastomerics using a layer-by-layer coating technique

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The aims of this study were to identify the optimal concentration of coated orthodontic elastomerics using a layer-by-layer technique that can release chlorhexidine (CHX) as an antimicrobial material, and to measure the physical properties and antimicrobial effects of the coated elastomerics. Ethyl cellulose (EC) was used as the polymer, and five study groups with various combinations of solvents (*i.e.*, ethanol and dichloromethane [DCM]) were included. The coated elastomerics were evaluated with a spectrophotometer to confirm the release of CHX, and their surfaces were observed by SEM. The CHX+EC+DCM group sustained antimicrobial release for the longest period (168 h, $p<0.001$) and exhibited the largest antimicrobial effect in an inhibition zone test using *S. mutans* for 7 days ($p<0.05$). This group had most effective physical properties and antimicrobial effects of coated elastomerics produced using a layer-by-layer technique, and so its composition should be considered for use in clinical applications in orthodontics.

Keywords: Drug delivery system, Elastomeric, Chlorhexidine, Layer-by-layer technique

INTRODUCTION

Orthodontic patients visiting dental clinics tend to exhibit greater numbers of bacterial retentions due to their irregular dentition and orthodontic appliances attached to their teeth[1,2]. Also, maintaining oral hygiene is more difficult for orthodontic patients due to plaque attachment around their orthodontic wires and brackets[3]. For this reason, 84% of orthodontic patients were found to exhibit white spot lesions during visual examination after their orthodontic treatment[4-6], with 97% of patients exhibiting more than one such lesion when examined using quantitative light-induced fluorescence (QLF)[7]. In other words, inappropriate oral care can result in irreversible tooth damage during orthodontic treatment[4,6,7]. Orthodontic patients should generally visit a dental clinic to change orthodontic elastomerics. Thus, if antimicrobial-releasing elastomerics could be developed, they might be useful in preventing oral disease[8,9].

A drug delivery system (DDS) can be used to regulate drug release or absorption rates or to deliver drugs to specific target sites, which ultimately ensures the effective presence of certain drugs over required treatment periods. Since the late 1940s, when the concept of the DDS was first applied within the fields of medicine and pharmacy[10,11], DDSs have been applied in various dental areas such as dental implants, endodontics, and periodontal treatments for preventing infectious diseases[8,12-15]. Bis-biguanide chlorhexidine (CHX) is an antimicrobial agent with broad-spectrum antimicrobial activity that is known to be effective at inhibiting Gram-positive and Gram-negative, aerobic and anaerobic microorganisms, yeast, and fungi[16]. Furthermore, CHX is widely acknowledged as one of the most effective antimicrobial agents due to its superior antiplaque and antigingivitis effects compared with other antimicrobial agents[17,18]. Based on this background, a previous study developed a local DDS for applying CHX to orthodontic patients as an elastomeric[8,19].

A DDS needs to be able to maintain and then release a CHX coating on orthodontic elastomerics. A previous study that used a dipping method to coat elastomerics as a single thick layer found limitations in maintaining the coating layer and sustaining the release of CHX. A layer-by-layer (LBL) technique can be used to build up a thin multilayer film[20,21], and so the present study utilized a LBL technique to build up multilayer films using spray to overcome the limitations associated with a single thick layer.

Applying a DDS to the oral cavity requires determination of the optimal concentration of coating solution for releasing antimicrobial materials with the appropriate antimicrobial effect level while not exerting adverse effects on the oral cavity. Although, previous study evaluated the properties of CHX-releasing elastomerics developed with various coating solution compositions[8] but, the results of our pilot study confirmed that consistency of coating solution is need lower when applying a spray LBL technique than when

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using a dipping method. Thus, the properties when using different concentrations for CHX-releasing orthodontic elastomerics should be evaluated and effects on antimicrobial release also need to be determined in order to confirm the antimicrobial effects on *S. mutans*[22].

The aims of the present study were to determine the optimal concentration of coated orthodontic elastomerics when using the LBL technique for releasing CHX as a typical antimicrobial material, and to identify the physical properties and antimicrobial effects of coated elastomerics on *S. mutans*.

MATERIALS AND METHODS

Preparation of coating solutions

Chlorhexidine diacetate (CDA; SIGMA-ALDRICH, St. Louis, MO, USA) was used as the antimicrobial agent, and ethyl cellulose (EC; N100, SIGMA-ALDRICH) was used as the polymer for preparing the coating solution. The volume of polymers used was 2% of that of the solvents, and five study groups were designated, comprising a group with mixtures without polymers as the control, and other groups with polymers and various solvent combinations assigned as the experiment groups (Table 1). In attempts to ensure chemical stability and solubility, ethanol (EtOH; DUKSAN Pure Chemicals, Ansan, Republic of Korea) and dichloromethane (DCM; DUKSAN Pure Chemicals) were used as solvents when varying the ratios of dissolved powdered CDA and EC. EC was dissolved completely in the prepared solvent using a stirrer, and then the antimicrobial agent (CDA) was added to produce the coating solution.

Preparing the elastomerics

The orthodontic elastomerics (TP Orthodontics, LaPorte, IN, USA) with a diameter of 4 mm and weighing 0.005 g were washed in distilled water and dried in an oven at 60°C. The mixed coating solution (8 mL) was sprayed five times, with both sides sprayed twice each and dried for 5 min. After being sprayed five times, the elastomerics were dried completely for 1 h at room temperature.

Amount of chlorhexidine released from the coated elastomerics

The 16 elastomerics that had been dried for 1 h were then fixed onto a vial lid using orthodontic ligature wire. Distilled water (4 mL) was added in an 8-mL vial so as to submerge the elastomerics fixed on the lid. The distilled water was replaced at set times: once every 3 h for the first 12 h, due to the large amount of antimicrobial released, followed by once every 12 h until at least 168 h from the beginning point. All procedures were performed at room temperature with stirring, and they were repeated four times.

The amounts of CHX released from the coated elastomerics were measured using an ultraviolet spectrophotometer (CM-3500d, KONICA MINOLTA SENSING, Osaka, Japan). In accordance with previous studies, the measurements made at 254 nm were converted into the concentrations of released CHX using a standard curve ranging from 0.1 to 40 μg/mL (r=0.98)[8,23].

The CHX released into 1 mL of distilled water was collected for measurement, and the ultraviolet spectrophotometer was used at 10 s intervals to ensure light stabilization.

Observation of coated elastomeric surfaces

The elastomeric surfaces were observed before and after the release experiment. To facilitate clear viewing, the elastomerics were first coated with gold at a thickness of 150 nm in a vacuum using an ion sputter (Hitachi, Tokyo, Japan). Scanning electron microscopy (SEM; FE-SEM S-800, Hitachi) was then applied at an accelerating voltage of 20 kV so as to observe the surfaces at 500-fold magnification.

Antimicrobial effects of coated elastomerics

To observe the coated elastomerics and whether their antimicrobial effect was sustained over a long period of time, elastomerics coated with 2% EC solution were evaluated as they exhibited prolonged release over 168 h in the release experiment.

S. mutans (*Streptococcus mutans* ATCC 25175, Korea Research Institute of Bioscience and Biotechnology, DaeJeon, Republic of Korea) was incubated in liquid

Table 1 Compositions of the coating solutions for a drug delivery system on orthodontic elastomerics in the five study groups

Group	Drug (mg)	Polymer (mg)	Solvent (mL)	
	CDA	EC	EtOH	DCM
Group 1 (control)	10	—	—	1
Group 2	10	20	—	1
Group 3	10	20	0.3	0.7
Group 4	10	20	0.5	0.5
Group 5	10	20	0.7	0.3

CDA, chlorhexidine diacetate; EC, ethyl cellulose; EtOH, ethanol; DCM, dichloromethane

brain heart infusion (BHI) medium to a concentration of 10^7 CFU/mL, and then 100 mL was plated on solid BHI medium. Eight coated elastomerics were fixed on the solid medium smeared with the bacteria, and then cultured at 37°C under 10% CO_2 for 24 h. The coated elastomerics were then moved onto a new solid BHI medium smeared with the bacteria, and the sustained antimicrobial effects of the elastomerics were observed over the following 7 days. In order to measure the size of inhibition zones centered on the elastomerics, images were acquired using a digital camera (model 450D, Canon, Tokyo, Japan). The horizontal and vertical diameters of the inhibition zones were then measured using an image analysis program (Image-Pro Plus 6.0, Media Cybernetics, Washington, GA, USA).

Statistical analyses

The differences in the amounts of CHX released over time among the groups with different coating methods were examined by repeated-measures ANOVA, which was also used to examine the differences in inhibition zones over time among the different groups. Statistical analyses were performed using SPSS (version 20, SPSS, Chicago, IL, USA), with the confidence level set to 95%.

RESULTS

The release levels of CHX coated using the LBL technique were investigated over 168 h. The amount of antimicrobial materials released increased over time (Fig. 1, $p<0.001$). Although group 3 (CDA+30% EtOH+70% DCM) exhibited the greatest amount of antimicrobial released, the CHX release stopped within 9 h. The amount released in group 2, in which only DCM was used a solvent, was small (73.2%) compared to being more than 90% within the first 1 h in the other groups. However, the release in group 2 had increased by 48 h and was maintained throughout the evaluation period

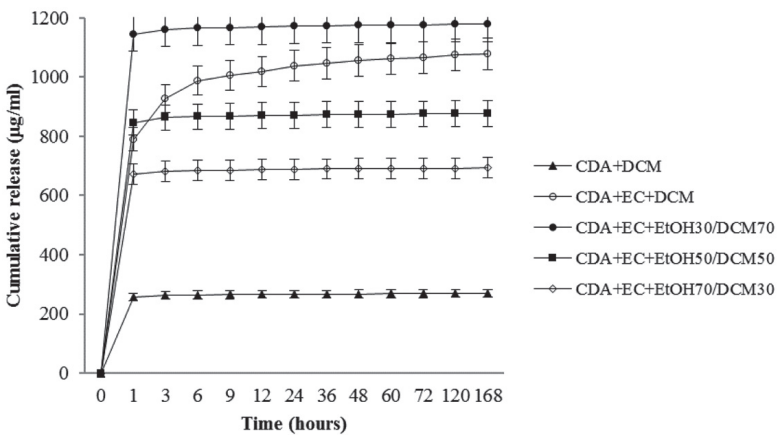

Fig. 1 Cumulative mass of chlorhexidine released from coated elastomerics. Each group has 16 coated elastomerics. Data were analyzed by repeated-measures ANOVA ($p<0.001$), and are mean and SD values.

Table 2 Changes in the size of the inhibition zone when using CHX-releasing elastomerics over time

(Unit: mm)

Time (days)	Group 1	Group 2	Group 3	Group 4	Group 5
1	16.05±0.50 ^{A}	16.25±0.70 ^{A}	16.44±0.83 ^{A}	16.78±1.52 ^{A}	16.93±0.89 ^{A}
2	14.55±1.29 ^{B}	13.41±1.15 ^{B}	15.71±1.25 ^{B}	15.29±0.99 ^{B}	14.83±1.10 ^{B}
3	14.21±2.37 ^{B}	15.44±1.13 ^{C}	14.74±0.79 ^{C}	12.97±0.93 ^{C}	12.18±1.24 ^{C}
4	6.62±1.69 ^{C}	8.29±1.45 ^{D}	7.58±0.90 ^{D}	6.93±0.56 ^{D}	6.96±0.49 ^{D}
5	3.77±0.98 ^{D}	11.25±0.96 ^{E}	8.08±0.51 ^{D}	7.76±0.53 ^{E}	7.67±0.79 ^{E}
6	3.71±3.03 ^{D}	11.28±1.23 ^{E}	8.55±1.86 ^{E}	9.13±0.66 ^{F}	8.34±0.83 ^{E}
7	1.43±2.57 ^{E}	10.86±0.66 ^{E}	9.11±0.58 ^{E}	9.23±1.00 ^{F}	8.30±1.11 ^{E}
p	<0.001	<0.001	<0.001	<0.001	<0.001

Each group has 8 coated elastomerics. p-Values are for repeated-measures ANOVA. Different upper-case letters indicate statistically significant differences as analyzed by the paired t-test.
Data are mean and SD values.

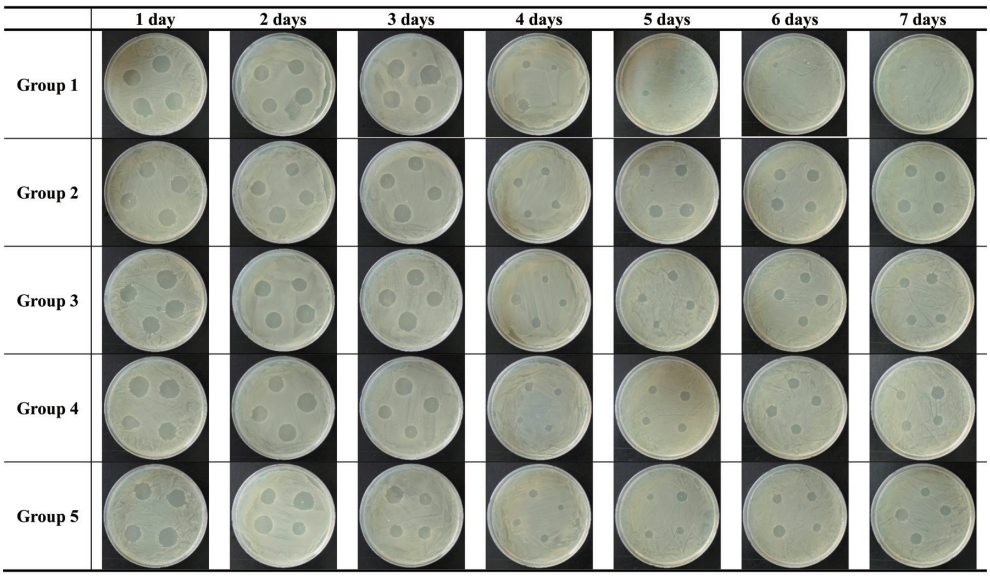

Fig. 2 Antimicrobial effects on *S. mutans* inhibition zone of CHX-releasing elastomerics for 7 days.

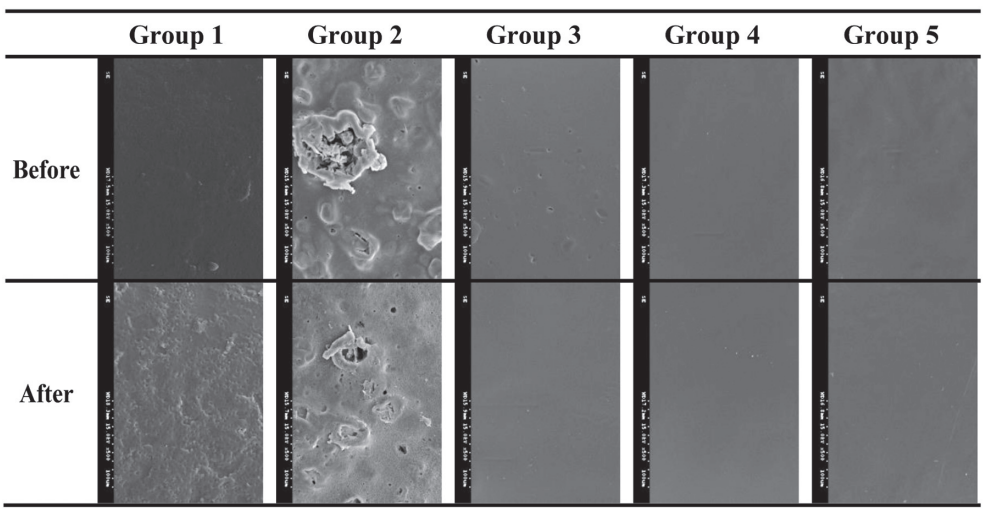

Fig. 3 Scanning electron microscopy images (magnification, ×500) of coated elastomeric surfaces before and after the release experiment.

(to 168 h) (Fig. 1).

The size of the inhibition zone on the medium that was smeared by *S. mutans* for evaluating the durability of antimicrobial effect differed significantly between the groups over time (Table 2, Fig. 2, $p<0.001$). The antimicrobial effect in group 1, in which polymer was not used, decreased rapidly after 4 days, while in groups 3–5, in which a different solvent composition was used, shown an inhibition zone that decreased rapidly after 3 days and was then sustained at a similar level. Group 2 exhibited sustained antimicrobial release and also the largest inhibition zone at 7 days (Table 2, Fig. 2).

After including elastomerics in each coating solution and drying them, the surfaces were observed using SEM after the end of the release experiment (Fig. 3). Group 1 (the control group) exhibited smooth surfaces before the release experiment and smooth elastomeric surfaces after the release experiment. Lumpy surfaces were observed in group 2, which used DCM as the sole solvent, and small holes were observed on rough surfaces after the release experiment. Groups 3–5, with varying solvent combinations, exhibited no significance differences between before and after the release experiment, with their surfaces appearing to be fairly smooth.

DISCUSSION

The area around the brackets in orthodontic patients can exhibit tooth demineralization due to this being a high-risk area for bacterial retention. A method for continually releasing antimicrobial material in order to decrease bacterial viability could be useful in decreasing these adverse effects of orthodontic treatment.

The use of elastomeric rings as ligature wires in orthodontic treatment increases the risk of oral disease since plaque is deposited more easily on these rings, which means that they have to be changed periodically[24]. We expected that a DDS could be useful for preventing oral disease if the elastomerics could be utilized as a medium for providing antimicrobial material. This study aimed to determine the optimal concentration to apply a DDS by estimating the antimicrobial effects and sustainment properties of elastomerics that were coated by a solution with various concentrations.

The elastomerics were coated with solutions of various compositions and were evaluated based on the CHX released. The release of antimicrobial material increased over time, but the amount released differed according to the composition of the coating solution (Fig. 1, Table 2). Group 3 (CDA+30% EtOH+70% DCM) exhibited the greatest CHX release, with the amount of CHX released when using the current LBL technique being approximately four times greater than when using the previously reported dipping method. However, group 3 did not show prolonged release. On the other hand, group 2, which comprised DCM only, exhibited the second largest amount of CHX released as well as prolonged release over 168 h (Fig. 2). These differences in the CHX release rate and sustainment duration are attributed to the drying condition of the coating solution on the elastomeric surface varying with the evaporation rate. That is, when the concentration of DCM is higher, the produced film will form a denser polymer network resulting in the prolonged release of CHX. In contrast, the inclusion of EtOH increases the water penetration and also the rate of CHX release[25]. Therefore, differences in the concentrations of DCM and EtOH may affect the release rate and sustainment duration of antimicrobial materials[8,26].

A previous study found that the releasing effect was longest for a group corresponding to our group 3 (CDA+30% EtOH+70% DCM)[8], which might be due to differences in the concentration of EC as a polymer. That previous study used 10% EC for the elastomeric coating in a dipping method. In contrast, the present study needed a thin coating solution so that the LBL technique could be implemented using spraying, and so a 2% EC coating solution was used.

The water penetration effect by EtOH increases as EC decreases, and this may result in the prolongation property of CHX being lost due to the release being faster than in a previous study in which the CHX was dissolved in a high concentration of EC[27].

The antimicrobial effect of CHX has been known for a long time, but it is important for the appropriate concentration to be maintained when applying a DDS in oral applications to ensure both efficacy and safety. Therefore, it is suggested that the composition used in group 2 —which showed continuous and prolonged antimicrobial material release— is better for preventing oral disease in orthodontic patients than that used in group 3, in which large amounts were released but only over a relatively short period of time.

Group 5 exhibited the second lowest release, while group 1 (only DCM) showed the lowest release, with these results thought to be due to differences in agitation times for mixing the various materials in order to prepare the coating solutions. The agitation time is one of the factors determining drug-releasing properties[28]. The solubility of DCM is similar to that of EC, and so it can dissolve better in EC than in EtOH[29]. In other words, the increased agitation time required to dissolve EC completely in groups 3–5 (containing EtOH) relative to group 2 would have resulted in the intermolecular chain structures of the polymer becoming denser. Group 5, which needed the longest stirring time due to the higher EtOH ratio, probably had a denser polymer network and therefore showed the smallest amount of CHX release.

Group 1, which did not contain polymers, exhibited a sharp decline in the size of the inhibition zone after 3 days, while in group 2, which exhibited prolonged release, the inhibition zone lasted for 7 days. It was estimated that the antimicrobial effect increased significantly compared with the rapid decrease after 48 h found in the previous study that used elastomerics coated by a dipping method[8]. In particular, the inhibition zone was typically two- to threefold bigger than that in the previous study after 48 h, and its size was maintained after 7 days (1.3–2-fold difference). These results confirm that the LBL technique that involves producing a thin multilayer film has a greater antimicrobial effect than the dipping method that produces a single thick layer. However, the present study evaluated only a single strain of bacteria, and so future studies should attempt to estimate biofilm formation inhibition using a microcosm biofilm model in order to reproduce the real oral condition.

Finally, tiny surface changes in the coated elastomerics before and after releasing were observed using SEM. Group 2, which used only DCM as a solvent, exhibited small pores on the elastomeric surface, with the pore size increasing to become large after the releasing experiment. These surfaces would allow diffusion through the aqueous pores, which is the antimicrobial release mechanism of EC[30] and osmotically driven release[31]. The enlarged pores and the increase in their number before and after the release experiment in group 2 may have resulted in the release of a large amount of antimicrobial materials. On the other hand, smooth surfaces were evident in groups 3–5, which had different amounts of DCM and EtOH, and so the difference between group 2 and groups 3–5 were probably due to the differences in solvent compositions[32]. Although the SEM results did not allow clear comparisons among surfaces in group 2 and groups 3–5, the surfaces were

smoother when EtOH was included, and this would have changed the rate of evaporation. The coating method is considered to have only a smaller effect on the elastomeric surfaces because the present results are similar to those found in a previous study using elastomerics coated using a dipping method[8].

This study has confirmed the possibility of using elastomerics in the dental clinic by measuring release rates and sustainment period of coated antimicrobial materials. Although the CHX release was the most stable and long term in group 2, which used only DCM as a solvent, elastomerics that are fixed inside the oral cavity for long periods are often influenced by multiple factors such as moisture, temperature, and pH variations. Therefore, further experiments are required to examine whether similar antimicrobial effects would occur in the dynamic environment of the oral cavity. The possible effect of long-term use of CHX on tooth discoloration also needs to be assessed.

In conclusion, a mixture of CDA with only DCM as a polymer (*i.e.*, group 2) exhibited the most effective physical properties and antimicrobial effects, and thus may be useful in clinical applications in orthodontics.

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