The *in vitro* and *in vivo* effects of a fast-dissolving mucoadhesive bi-layered strip as topical anesthetics

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To overcome pain on injection, the dentist can apply a topical anesthetic spray. Despite the convenience, it is not easy to apply it locally. So, we developed an oral mucoadhesive bi-layer film containing an anesthetic. We used polyvinylpyrrolidone (PVP)/hydroxypropyl methylcellulose (HPMC) and HPMC-only layer as the drug-containing layer and ethyl cellulose (EC) as the backing layer. The lidocaine released was tested *in vitro* together with the adhesion time and cytotoxicity of the film. Mucosa permeability was tested *in vivo*. Statistical analysis was performed, with *p* at 0.05 taken to be significant. The lidocaine was released significantly faster in the PVP/HPMC than HPMC-only group and 80% of the drug was released within 1 min (*p*<0.05) and they attached at least 3 h. The test groups showed no toxicity and the drug effectively permeated the mucoa (*p*<0.05). We suggest this new mucoadhesive anesthetic may reduce dental phobia.

**Keywords:** Dental phobia, Mucoadhesive, Bi-layered film, Anesthesia, Lidocaine

**INTRODUCTION**

Dental phobia may cause recurrent and severe problems for both the patient and the dentist, as it often gives rise to many deleterious effects⁴. There are reports that people with high dental phobia refuse to keep their dental appointments²,³. The dental injection is one of the causes of their refusal; therefore, in the clinic, the dentist applies a topical anesthetic to minimize pain and fear. Unlike a local anesthetic, the topical anesthetic is used for reducing the pain by numbing a limited area and reversibly blocking the signal flow of the nerve fibers.

The local anesthetic most often used in dentistry is lidocaine hydrochloride [2-diethylamineoacetate-2', 6'-xylidide]. This anesthetic is commercially available as a 2 or 3% solution with 1:50,000 to 1:100,000 epinephrine⁴⁵. Different forms of lidocaine have been developed for use as topical anesthetics. These forms include ointments, gels and sprays⁶⁻⁹. Despite its convenience, the single application can be washed away quickly by the saliva¹⁰.

Currently, there are many studies concerning buccal films that counter the disadvantages of using lidocaine as an oral tablet. Lidocaine in a buccal film form can be easily applied and adheres to the local mucosa. The buccal film has the advantage of not being as uncomfortable as other foreign substances. In this study, we have selected a specially designed buccal film. Ethyl cellulose (EC) was used as the backing layer and hydroxypropyl methylcellulose (HPMC) and polyvinylpyrrolidone (PVP) were used as the drug-containing layer¹⁰⁻¹⁴. Therefore, the aim of this study was to develop a bi-layered strip consisting of a tooth-adhesive layer and a non-adhesive protective layer to deliver lidocaine. After blending and making the bi-layered film, we examined its sectional image to check its uniformity. Additionally, we evaluated the effect of a fast lidocaine release profile, *in vitro* and *in vivo* adhesion times and *in vivo* drug permeation. The null hypothesis of the study is that there is no difference in the two formulas, PVP/HPMC and HPMC, used for the release of lidocaine.

**MATERIALS AND METHODS**

**Materials**

Polyvinylpyrrolidone (PVP) type Kollidon K30 with a molecular weight of 50,000–55,000 was obtained from BASF (Bask, Ludwigshafen, Germany). Hydroxypropylmethylcellulose (HPMC) type Methocel K4M and ethylcellulose (EC) were obtained from Sigma Chemical (No.E-8003, Sigmaaldrich, MA, USA). Lidocaine was obtained from Supriya Chemicals (Mumbai, India). The bi-layered strip was prepared with a casting-solvent evaporation technique using different concentrations of polymers.

**Preparation of the bi-layered film**

For the bi-layered film, the backing layer was made first, and then the adhesive layer was completed. Ethyl cellulose (EC) was dissolved in 100 g of ethyl alcohol for 5 h, and 1% dibutyl phthalate (DBP) was added as a plasticizer. Ten milliliters of backing solution was poured into a 10 mm petri dish, and the backing solution was evaporated until the blistering effect on dried strips. For the adhesive layer, 5 wt% of PVP and 2 wt% of HPMC were individually
dissolved in distilled water to create two homogeneous solutions. Then, 10 mL of glycerin was added as a plasticizer (60°C, 24 h) to each of the solutions. The two solutions were mixed in differing amounts under stirring for 12 h (HPMC-PVP). Then, the required amount of lidocaine was slowly added into the HPMC-PVP solution. The HPMC-PVP-LIDO solution was poured onto the dried backing layer and allowed to dry in an oven at 80°C until a flexible bi-layered strip was formed. The strip was cut into 6 mm diameter pieces and stored at (23±2)°C and (50±2)% relative humidity until further analysis was performed.

**Bi-layer film analysis**

The Fourier Transform Infrared Spectroscopy (FT-IR) spectra of the HPMC-PVP blended thin strips were obtained using a FT-IR spectrophotometer (Nexus-870, Thermo Nicolet, USA). To avoid any moisture effects, the samples were dried overnight in a desiccator. The IR spectra in absorbance mode were obtained in the spectral regions of 1,000–4,000 cm⁻¹. The spectrum of each sample was acquired by accumulation of 32 scans with a resolution of 4 cm⁻¹. To observe the morphology, cross-sections of the bi-layered strips were observed using an image analyzer (HIROX KH-1000, HIROX, Seoul, Korea).

**In vitro adhesion time of the bi-layered strip**

To measure the in vitro adhesion time and the release of lidocaine, the film was attached to the bottom of a beaker. Under the film, 15 μL of simulated saliva fluid (SBF) was used to hydrate the adhesion surface. After the film attached, the beaker was filled with 800 mL of SBF and kept at 37°C. A 150 rpm stirring rate was applied to simulate the buccal cavity environment, and strip adhesion was monitored for 24 h. The time necessary for complete erosion or detachment of the strip from the beaker surface was recorded.

**In vitro lidocaine release from the bi-layered strip**

The 100 mL of phosphate buffer solution (PBS, pH 7.0) was stirred at 100 rpm and (37±1)°C. At appropriate time intervals, 1 mL samples were withdrawn and then replaced with the same volume of buffer. Lidocaine concentrations were calculated spectrophotometrically at a wavelength of 264 nm.

**Cytotoxicity test (WST-1) of the film**

To evaluate the cytotoxicity of the film, a WST-1 test was conducted with L-929 mouse fibroblasts. The 5x10⁴ well of fibroblasts were seeded in 96-well microplates for 24 h. To make an extraction solution, we immersed the test groups in cell culture media according to ISO 10993-12. The cell culture media (with no test material) was used as control. After 24 h, the cell culture medium was changed to an extraction solution. After another 24 h of incubation, the supernant solution was replaced to 100 μL of premixed WST-1 solution to each well and then incubated for 4 h. The wells were read using an ELISA reader at 540 nm, and then the % viability was calculated. For each WST-1 test, the control was defined as 100%.

**In vivo testing: adhesion time and lidocaine permeability of the bi-layered strip**

To evaluate the mucous adhesion time in vivo, we euthanized two male mongrel dogs, each 18–24 months old and weighing approximately 30 kg. Animal selection, management, preparation, and surgical protocol were followed the routine procedure approved by the Animal Care and Use Committee at the Yonsei Medical Center, Seoul, Korea (No.09-125). The bi-layered strips were attached to the canine oral cavity. The surfaces of the strips were moistened with 15 μL SBF, and then the strips (size 6 mm²) were brought immediately into contact with an initial force for 5 s. The entire experiment was performed at room temperature. The adhesion of the strips was monitored for 30 min, and the number of strips that detached from the oral cavity was recorded.

**Statistical analysis**

The IBM SPSS 20.0 program (SPSS, Chicago, IL, USA) was used for the statistical analysis. All results were analyzed by one-way ANOVA followed by the Tukey test at a level of significance of 0.05.

### RESULTS

The spectrum from FT-IR (A) and the sectioned image of FA (B) are shown in Fig. 1. In Fig. 1A, the FTIR shows homogeneous results for three randomly selected films. The benzene ring and bonded-OH of lidocaine helped produce the signal in the approximately 3,030 cm⁻¹ range. The C=C produced a signal in the 1,450–1,600 cm⁻¹ range. The sectioned surface of the bi-layered film is shown in Fig. 1B. The EC layer was dried in the lower part of the film as the backing layer; the HPMC-PVP layer was dried onto the backing layer as the adhesive layer. Thus, the bi-layered strips had the mechanical and physical combination of the EC layer and the HPMC-PVP layer.

The result of the in vitro test of adhesion time of the bi-layered strip is shown in Fig. 2. The PVP and EC groups, which were made of only one layer, detached immediately from the beaker wall. The FA group showed that 2 out of 3 specimens were detached from the beaker wall after approximately 4 h. In contrast, all FB groups

<table>
<thead>
<tr>
<th>Code</th>
<th>HPMC (g)</th>
<th>PVP (g)</th>
</tr>
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<tbody>
<tr>
<td>FA</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>FB</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1 Test groups used in this study. The composition of the experimental groups FA and FB was listed.
Fig. 1  The spectrum of FTIR (A) and a cross-section image of FA (B).
In Fig. 1A, the randomly selected film showed homogeneous and there were same spectrum with lidocaine, HPMC and PVP spectrum. The sectioned surface of the bi-layered film is shown in Fig. 1B. The EC layer was dried in the lower part of the film as the backing layer; the HPMC-PVP layer was dried onto the backing layer as the adhesive layer. There was tightly attached cross-sectional image.

were only detached after 5 h.

The accumulated release of lidocaine is shown in Fig. 3. The lidocaine release profile of the FA was rapid, and 16 mg of lidocaine had been released within the initial 5 min. As a result, the FA group with HPMC-PVP showed a fast lidocaine-releasing property that was 4 times faster than in the FB group ($p<0.05$).

Fig. 3  In vitro lidocaine-release profiles of bi-layered strips in the FA group and the FB group (There were significant differences in all time points). The lidocaine release profile of the FA was rapid, and 16 mg of lidocaine had been released within the initial 5 min. The FA showed 4 times faster lidocaine-releasing property than in the FB group ($p<0.05$).

The cytotoxicity results for each film are shown in Fig. 4. All experimental groups except the FB group were non-toxic. The highest cell viability was in the EC strip at 94.61%, with a rank order of FA (HPMC-PVP blended strip) and lidocaine. However, there were no significant differences in cytotoxicity ($p>0.05$).

Fig. 4  Cell viability [* means significant difference ($p<0.05$)].

All experimental groups except the FB group were non-toxic. The highest cell viability was in the EC strip at 94.61%, with a rank order of FA (HPMC-PVP blended strip) and lidocaine. However, there were no significant differences in cytotoxicity ($p>0.05$).
Lacks the property of adhesion. HPMC is a water-soluble polymer through the body when taken orally. However, PVP is a water-soluble tablet binder, and it simply passes through the body when taken orally. Methylcellulose (HPMC) as the drug-containing layer. and polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose, which is hydrophobic, as the backing layer corresponded to an injection point and not to affect other unwanted regions. For this design, we used ethyl cellulose, which is hyrophobic, as the backing layer and polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC) as the drug-containing layer. PVP is a water-soluble tablet binder, and it simply passes through the body when taken orally. However, PVP lacks the property of adhesion. HPMC is a water-soluble polymer with good film-forming properties. The degree of substitution, types of functional group substitution, and chain length of this polymer affect its permeability, mechanical properties and water solubility. In a previous study, HPMC showed a slow drug-release behavior. Therefore, we added various concentrations of PVP in the film and received the injection in a clinical situation. The thickness and weight of the polymer samples differed in spite of maintaining a uniform volume. Depending on the volume of the adhesive polymer, the lidocaine content within each specimen also differed. Therefore, we fixed the content in relation to the volume of the adhesive polymer and found the appropriate volume for the proper film lidocaine contents. In terms of uniformity, the formula was confirmed by FTIR. When we randomly made the films, the functional groups of lidocaine and HPMC-PVP were produced at the same peaks, as confirmed by FTIR.

In Fig. 3, it is shown that, in the FA group, the HPMC-PVP-blended strips showed faster drug release than that of the FB group, which had a pure HPMC strip. During the first minute, 16 mg of lidocaine was released, and lidocaine release continued until the film was peeled off. The mucoadhesive film was designed for applying the topical anesthetic before the injection, so we considered 5 min to be sufficient time to allow between applying the film and receiving the injection in a clinical situation. Therefore, the lidocaine-release test was conducted for 5 min. Of course, the two groups had shown clinically acceptable adhesion time in vitro (Fig. 2).

A mucoadhesive film applied to the mucosa tissue might irritate the subject. Therefore, we conducted cytotoxicity tests of each film. There was no toxicity for the backing layer, the FA group and for lidocaine. This lack of toxicity was reported in previous studies. However, the FB group, which had only HPMC, showed a significantly lower cell viability than other groups (p<0.05).

Even though the FA group with low HPMC concentration had the weakest attachment, it adhered for sufficient time to show effectiveness. We chose the blending concentrations of the FA group with the quickest release time, and this combination seemed best for our needs. On the basis of the results, we chose the blending proportion of HPMC:PVP=10:90 when manufacturing mucoadhesive drug layers.

The bioadhesive force of the prepared film specimen was an important property. If film specimens detach from oral mucosa before the clinical application time, they might pose a danger to the patient, who might be anesthetized in unwanted regions. We measured the bioadhesive force indirectly using the in vivo adhesion
time test. The results showed that the film specimens did not detach within 30 min, which was the expected application time for each film specimen. The permeation of lidocaine was evaluated for the labial, buccal, and alveolar mucosa, which are typical local injection points. The alveolar mucosa was more permeated with lidocaine than the buccal mucosa and labial mucosa. In general, the oral mucosa is classified as a somewhat leaky epithelium with a permeability rank order of alveolar mucosa > buccal > labial mucosa, based on the thickness and degree of keratinization of the tissues.

In conclusion, from the above results, the null hypothesis was rejected, and we confirmed that the prepared strip, for the FA group, showed proper oral mucosa adhesion and fast drug release. Therefore, if the prepared strip is clinically applied, it might show positive effect for the patients with dental phobia. Also, to support its effect, clinical study needed to be assessing in the future.

ACKNOWLEDGMENT

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REFERENCES