—Regular Articles—

In Vitro and Clinical Evaluation of an Oral Mucosal Adhesive Film Containing Indomethacin

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To develop a new mucoadhesive film containing an analgesic combining clinical efficacy and patient comfort, we prepared and evaluated a two-layered film consisting of an adhesive layer containing indomethacin (IM) as the active ingredient and carboxyvinyl polymer (CP) as a bonding agent and a nonadhesive layer containing polyethylene glycol (PEG) to improve film texture. In *in vitro* and *in vivo* adhesive tests, the optimal concentration of CP that could be applied to the mucous membrane was 0.2% or 0.3%. Stability testing determined that the optimal storage conditions and expiration period were 4℃ without shade and 4 weeks, respectively. The film was clinically evaluated in patients with oral pain. IM at concentrations of 0.5% and 1% provided optimum analgesic effects, and the effects were the greatest in the 1% IM group. The addition of PEG to the nonadhesive layer reduced the number of patients experiencing discomfort at the site where the film was applied. Therefore this film formulation may be useful for local analgesic application due to its low dose requirement, moderate adhesion, and comfortable texture.

Key words——mucoadhesive film; analgesic; hydroxypropyl cellulose; indomethacin

INTRODUCTION

Oral mucosal pain can affect the activities of daily living, such as eating and sleeping, and result in disorders that may considerably reduce patient quality of life (QOL).[1] Oral adhesive films containing local anesthetics[2] or steroids[3] have been developed to improve QOL in patients with oral pain, although these films have several drawbacks including discomfort due to numbness or hypoesthesia resulting from local anesthetic action, film exfoliation from the affected part, and film hardness. In addition, the current method used for film preparation requires repeated expansion and drying of the base, making it difficult to prepare a film of uniform thickness. We investigated a simple method for preparing a film of uniform thickness and developed a film with moderate adhesion and good texture.

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MATERIALS AND METHODS

Film Preparation Hydroxypropyl cellulose (HPC) 150–400 cP, indomethacin (IM), and polyethylene glycol (PEG) 400 were purchased from Wako Pure Chemical Industries (Osaka, Japan). carboxyvinyl polymer (CP) (Hivis Wako105) was a gift from Wako.

Films used in the adhesion test were composed of a single layer of various concentrations of CP (0%, 0.1%, 0.2%, or 0.3% in the *in vitro* adhesion test and 0%, 0.1%, or 0.2% in the *in vivo* adhesion test) as an adhesive adhering to an HPC base. The film used in the clinical evaluation had two layers (bicast), the first of which was film evaluated in the adhesion test (mucous membrane layer). The first layer adhering to the mucous membrane is composed of HPC, IM (0.5% or 1%) as an analgesic, and CP (0.2%). IM (0.0015 g or 0.003 g) was first completely dissolved in ethanol, HPC was added, and then ethanol was added to make a final volume of 30 ml for 0.9 g of HPC. Ten milliliters of the mixed solution was cast in a flat

Teflon dish 75 mm in diameter using a graduated pipette at a flow rate of 10 ml/4 min, followed by drying overnight on a clean bench. For the second layer, 0.006 g of PEG as a softening agent[4] and HPC dissolved in ethanol were cast in a Teflon dish, as described above. No precipitation of IM was detected.

Each application was assumed to use 1 cm^2 of film with 0.5% or 1.0% IM (IM content: 0.03 mg/cm^2 or 0.06 mg/cm^2, respectively), 0.1%, 0.2%, or 0.3% CP (CP content: 0.012 mg/cm^2, 0.024 mg/cm^2, or 0.036 mg/cm^2, respectively), or 2% PEG (PEG content: 0.24 mg/cm^2). Concentrations of IM or additives were calculated based on the amount of IM solution[5] or local anesthetic artificial dentifrice (AD) film[6] reported previously.

***In Vitro* Adhesion Test** One layer of experimental film consisted of four concentrations (0%, 0.1%, 0.2%, and 0.3%) of CP as an adhesion agent and HPC as a base component and was cut into 2-cm squares. After the experimental film was placed in the center of a nonwoven cloth (4C cloth, FK900–0138 EVA80, Kuraray Kuraflex, Tokyo, Japan) that was cut into 3-cm×10-cm sections, the cloth was wetted with 39.6 μl of phosphate-buffered saline (PBS) and folded in half, and 500 g of flat weight was placed on the cloth for 5 s and then removed. Five minutes after the experimental film was placed in the center of the cloth, one end of half of the cloth was pulled at a speed of 300 mm/min. The maximum force [kilogram-force (kgf)] of peeling was measured with a digital force gauge (ZP-50N, Imada, Aichi, Japan) at an angle of 90° by adjusting the slide system. The volume of PBS applied to the wet nonwoven cloth was calculated from the volume of saliva secreted in a Saxon test in a fixed time so that it would adequately permeate the entire experimental film.[7]

The experimental results were compared using Scheffe's multiple-comparison test.

***In Vivo* Adhesion Test** Thirteen healthy adult individuals gave informed consent to evaluate the adhesion of the film. Before the adhesion test, they gargled with water to cleanse the entire mouth, and the designated site was dried by lightly wiping with gauze. The CP-containing adhesive side of the film was then placed on the buccal mucosa. Three types of film (CP concentration 0%, 0.1%, or 0.2%) cut into 1-cm squares were placed in the center of the buccal mucosa, and the adhesion of the film was checked with a mirror after 5, 10, and 15 min and then every 15 min for a total of 120 min. Eating, drinking, conversing, and exercising strenuously during the adhesion test were prohibited, and the mouth was kept closed as much as possible. Three percent gentian violet (1 μg/cm^2) was added to the film to permit visual assessment.

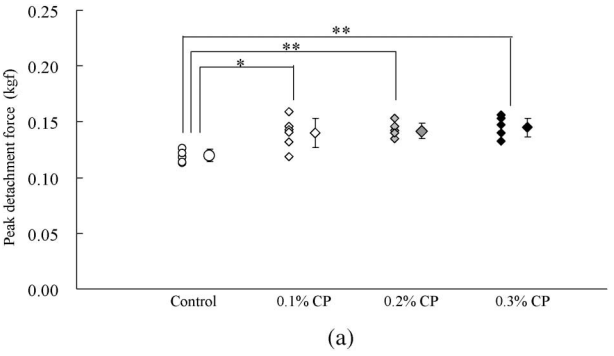
***In Vitro* Release of IM** Four types of film (0.5% IM, 0.5% IM+0.2% CP, 1.0% IM, 1.0% IM+0.2% CP) were prepared and cut into circles with a diameter of 21 mm containing IM 30 μg (0.5%) and IM 60 μg (1.0%). A piece of film was placed in the center of a membrane filter (type HA, pore size 0.45 μm, Millipore, Billerica, MA, USA) in a vertical-diffusion cell system (Hanson Research, Chatsworth, CA, USA), which was filled with 15 ml of PBS 0.1 M and kept at 37°C. The units used in this study had an effective diffusion area of 21 mm in diameter and a receptor compartment volume of 15 ml. The solvent was maintained at 37°C and continuously stirred using a magnetic stir bar. Diffusion sample aliquots were removed through the sampling port using a syringe and replaced with an equivalent volume of fresh solvent. Released sample aliquots of IM were collected at 5, 10, 15, 30, 60, 120, and 360 min. The amount of IM that diffused into the collected sample was measured using an HPLC system. The HPLC system was composed of two LC-10AD_{VP} pumps, an SPD-10A_{VP} ultraviolet detector, and an SIL-10AD_{VP} autosampler from Shimadzu (Kyoto, Japan). The analysis was performed as previously described[8] on an octadecylsilica (ODS) column (150 mm×4.6 mm i.d.) with 5-μm particle size (Wakopak, Wakosil-II 5C18, Wako Pure Chemical Industries). The mobile phase of the assay consisted of sodium monophosphate buffer 0.1 M and sodium acetate buffer 0.2 M (8 : 2 v/v) at a flow rate of 1.0 ml/min. Standard solution was prepared for each assay set at 0.25, 0.5, 2, 20, and 40 mg/ml.

Stability Test Two groups of sample film (0.5% IM+0.2% CP, 1% IM+0.2% CP) selected for clinical evaluation were cut into 1×1-cm^2 sections and preserved under three different conditions, all in the shade: 37°C, room temperature, and 4°C for 0, 7, or 28 days. The amount of IM in the film sections was measured after dissolving in 5 ml of phosphoric acid buffer solution 0.1 M (pH 7.0), using the HPLC system described above.

Clinical Evaluation Sixty-five patients who had

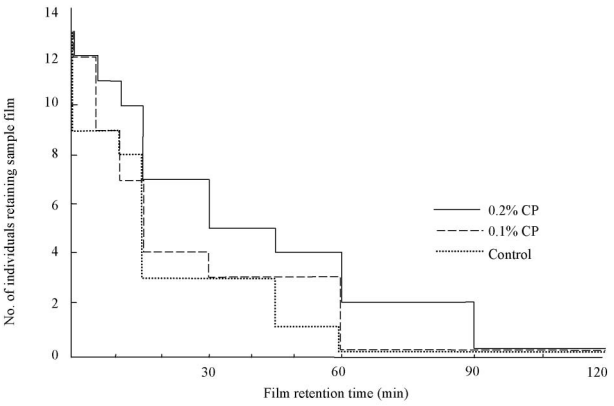
oral pain and visited the Maxillo-facial Surgery Department of Teikyo University Hospital gave written informed consent to participate in this evaluation. They were randomly allocated to four groups (0% IM, 0.5% IM, 0.5% IM with PEG, 1.0% IM). There were 21 cases of oral stomatitis, 14 cases of pain after surgery that included tooth extraction, 12 cases of ulcers (7 decubitus ulcers and 5 ulcers inside and on the edge of the tongue), 12 cases of wide painful areas of mucous membrane (2 lichen planus, 4 glossitis, 6 periodontal disease), and 6 other conditions.

The analgesic efficacy of the film was evaluated 1, 3, and 5 min after application using the Visual Analogue Scale (VAS), with a horizontal line, 100 mm in length. The patients marked the line at the point they felt represented their perception of their current state compared with the pain before applying the film, which was taken as 100% (100 mm). The VAS score is determined by measuring in millimeters (%) from the left end of the line to the point marked by the patient. Exclusion criteria were used when the applied film was removed. Fifty percent or greater pain relief was judged as effective. In addition, patients evaluated whether the texture of the film would induce discomfort at the localized site of application. Any adverse effects of the film were monitored for 1 week, which was the maximum period of clinical evaluation. This evaluation protocol was approved by the Committee for Medicinal Products of Teikyo University Hospital.



(a)

Fig. 1(a). *In Vitro* Adhesive Strength of Samples
Each sample was checked six times. The data shown are mean±S.D. $^{*}p<0.05$, $^{**}p<0.01$, Scheffe's *F*-test.



(b)

Fig. 1(b). *In Vivo* Adhesive Strength of Samples
13 healthy adult volunteers applied each sample film at the center of their buccal mucosa.

RESULTS AND DISCUSSION

Optimization of Mucoadhesive Film Preparation When casting frequency was examined to produce a uniformly thick film, two or three castings were found to provide adequate thickness (data not shown). The best method for producing the film required two castings, which makes two-layered film. Films composed of two layers had a thickness of 79.6±8.9 μm.

Efficacy of CP in the Adhesion Test Based on the results of the *in vitro* adhesion test, the film containing 0.1% CP showed greater adhesion compared with control films (0.1 % CP *vs.* 0% CP, $p<0.05$). The addition of 0.2% or more CP resulted in greater adhesion, but the adhesion was approximately the same between 0.2% CP and 0.3% CP (0.2% CP, 0.3 % CP *vs.* 0% CP, $p<0.01$) (Fig. 1(a)). More individuals with 0.2% CP film maintained the film in place during the entire test period compared with 0.1 % CP film or control film in the *in vivo* adhesion test (Fig. 1(b)).

Based on the results of the *in vitro* and *in vivo* adhesion tests, the addition of 0.2% CP increased the adhesion and the amount of CP for each use was 12 μg, which was well within safety limits compared with the maximum dose of 40 mg for dental or oral use and 150 mg for oral use.[9]

Efficacy of IM Release IM was released within 5 min from the 0.5% or 1.0% IM film (6.7 μg–8.0 μg), and a 2-fold greater amount of IM was released from the 1.0% film compared with the 0.5% film. The addition of CP did not significantly change the amount of IM released (Fig. 2). The amount of IM released from 0.5% and 1.0% IM films was constant for 60 min, and 80% of the IM was released within 120 min from all samples. The addition of CP did not affect IM release.

Drug-release tests showed that IM was released from the film within 5 min of film wetting. If the film swelled or dissolved in saliva in the oral cavity, a rapid-onset analgesic effect would be expected. Eighty percent of IM was released within 120 min, indicating that disintegration of the film was sufficiently slow to allow drug release. Therefore this film formulation is expected to provide an adequate analgesic effect. Moreover, the results suggested that the addition of 0.2% CP delays dissolution of the film, thus providing a protective effect at a localized site.

Optimization of Storage Conditions and Expiration Period Stability tests showed no change in the content of IM in all samples for 4 weeks after preparation (Table 1). However, at 28 days, the 0.5% IM film showed a reduction in the IM content to 98%. The expiration period for clinical use was therefore set at 4 weeks, and storage conditions were set at room temperature or cool conditions in the dark.

Clinical Evaluation All patients evaluated the taste and texture of the film, and 50 patients evaluated pain relief with film use at specified times. Thirty-seven of 50 patients reported an analgesic effect after application of the IM film. Furthermore, in the non-IM control samples, 7 patients still experienced oral pain despite the use of the film. The mean maximum pain relief ratio in effective cases within 5 min was 84.4%±16.9%, 82.2%±10.3%, and 86.1%±16.1%, and the mean time until analgesic relief occurred was 2.0±1.6 min, 1.7±0.9 min, and 1.3±1.0 min in the 0.5% IM, 0.5% IM with PEG, and 1.0% IM groups, respectively (Table 2). An analgesic effect was achieved within 1 min after applying the film in 13 cases (61.9%) with 0.5% IM and in 15 cases (93.7%) with 1.0% IM. Maximum pain relief rates for 0.5% IM and 1.0% IM were significantly greater than the controls ($p<0.01$) (Fig. 3). In addition, when classified by disease, the average maximum pain relief rate was 93% of stomatitis, 88% of ulcer (decubitus ulcer and tongue ulcer), and 80% of pain after surgery and wide mucous membrane lesion patients (data not shown). The number of individuals complaining of discomfort at the localized site where the film was applied was 3 of 17 (17.6%) in the film with

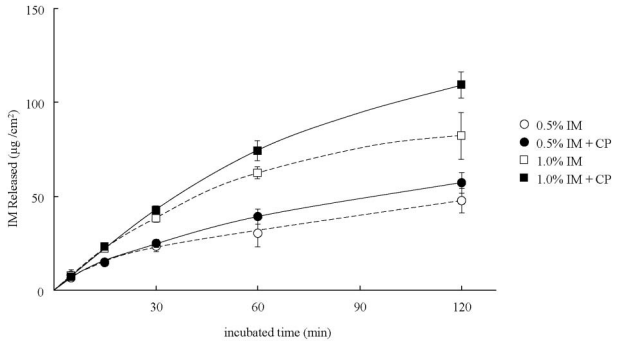


Fig. 2. IM Released from Sample Film

ϕ22 mm diameter film was incubated in phosphate buffer at 37℃, and the amount of IM released was measured using the HPLC system. Each sample was measured four times. The data shown are mean±S.D.

Table 1. IM Stability in Film Containing 0.2% CP

a) 0.5% IM

Storage condition	IM content (%) after preparation (days)		
	0	7	28
4℃	100.0±0.5	100.4±2.2	98.9±2.6
Room temperature	100.0±0.5	102.2±2.1	99.7±0.6
37℃	100.0±0.5	99.7±3.0	98.9±0.5

b) 1% IM

Storage condition	IM content (%) after preparation (days)		
	0	7	28
4℃	100.0±1.3	99.5±2.3	100.1±2.2
Room temperature	100.0±1.3	99.4±1.5	99.4±1.5
37℃	100.0±1.3	98.6±3.0	100.0±0.7

Each sample was checked four times. The data shown are mean±S.D.

Table 2. Clinical Analgesic Effects of IM Film

Sample	Sample contents			No. of effective cases	Effective cases		Pain ratio relief (%)[a] in all cases
	IM (%)	CP (%)	PEG (%)		Time until analgesic effect (min)	Pain ratio relief (%)[a]	
Control	—	0.2	—	0/7	—	—	3.43±4.54
0.5% IM	0.5	0.2	—	12/15	2.00±1.60	84.42±16.91	68.81±31.75**
0.5% IM+PEG	0.5	0.2	2	9/12	1.67±0.87	82.22±10.26	
1% IM	1.0	0.2	—	16/16	1.25±1.00	86.13±16.08	86.13±16.08**

a) Pain ratio relief=100 (pain before film application) − pain after film application. ** $p<0.01$, Kruskal-Wallis H test and Scheffe's F-test.

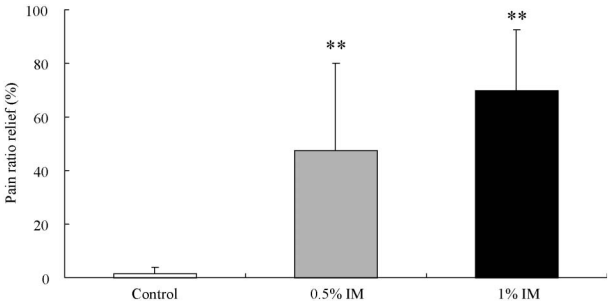


Fig. 3. Pain Relief Ratio[a] in Clinical Use 1 min after Film Application

[a] Pain relief ratio=100 (pain before film application) − pain after film application

**$p<0.01$, Kruskal-Wallis *H*-test and Scheffe's *F*-test *vs.* control

Table 3. Complaints of Discomfort after Film Application

Application site	No. of complaints/total no. of cases	
	Film with PEG	Film without PEG
Lips	0/2	4/6
Gingiva	1/5	10/29
Tongue	2/5	2/6
Mucosa of palate	0/2	0/1
Buccal mucosa	0/3	0/5
Hypoglottis	—	0/1
Total	3/17	16/48

PEG group and 16 of 48 (33.3%) in the non-PEG group (Table 3). The safety of film application was also monitored in this period among the individuals who received either of those film preparations. Two of 65 individuals reported numbness at the film site 1–4 min after application. Exfoliation of the film or gargling was recommended in those cases, the symptoms were relieved, and there was no effect on treatment or patient condition after film application. There were no other complaints regarding film application.

In terms of clinical efficacy, 6 of 16 individuals in the high-dose IM film group reported 100% pain relief within 1 min, and all (100%) showed greater than 50% pain relief using the VAS method (effective). Rapid, effective analgesia was therefore obtained with the film formulation containing 1% IM compared with the lower dose. In individual cases, marked analgesic effects were seen, especially when pain was localized and the trigger point of pain was clear, in cases with spontaneous pain, or in cases with severe, intolerable pain. However, the duration of the analgesic effect with film application could not be definitively determined in this study, although a maximum sustained analgesic effect of 7 h was observed in 6 patients in whom investigation was possible. The persistence of the analgesic action of the film in this study was almost equal to that of dibucaine film prepared using conventional methods and used in other medical fields, which maintains its analgesic effects for less than 5.5 h.[6]

In addition, the IM content of the film for one application was approximately 1/400 or 1/800 that of the standard oral dose. Therefore this formulation is expected to be useful in patients in whom normal doses cannot be used due to adverse side effects.

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