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Supporting Information

A double-layer thin oral film for wet oral mucosa adhesion and efficient treatment of oral ulcers

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Materials and methods

Materials

All chemical reagents were available purchased from commercial sources and of pure analytical grade and used without further purification. Sodium carboxymethylcellulose (chemically pure CP300-800 mPa.s), polyvinylpyrrolidone K30 and methylene blue were bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glycerol and 2-mercaptobenzothiazole were purchased from Sigma-Aldrich (USA). Zein (from corn) was purchased from Tokyo Chemical Industry Co., Ltd. (TCI, Japan). Artificial saliva and Masson trichrome staining kit were obtained from Solarbio (Beijing, China). Bovine submaxillary mucin was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (China). The control commercially available oral films/patches (propolis oral film, compound chlorhexidine and dexamethasone Pellicles, dexannethasone acetate adhesive tablets) were purchased from Zizhu Pharmaceutical Co., Ltd. (Beijing, China), Xian Kanghua Pharmacy Co., Ltd. (Xian, China) and Joincare Pharmaceutical Group Industry Co., Ltd. (Guangdong, Chnia). Minimum Essential Medium (MEM) and fetal bovine serum (FBS) were bought from Gibco (USA) and PAN (USA). Penicillin–streptomycin liquid and phosphate buffer saline (PBS) were from HyClone (USA). 5% phenol solution was bought from Aladdin (Shanghai, China). Cottonseed oil was purchased from J&K Scientific (Hebei, Chnia). Fluorescein isothiocyanate was from Adamas (Shanghai, China). VECTASHIELD® MOUNTING MEDIUM with DAPI was bought from Vector (USA). Agarose was obtained from Biosharp (Guangzhou, China). Rat IL-6 ELISA kit was bought from Neobioscience (Guangzhou, China). Sirius Scarlet Staining Solution was bought from Huayueyang (Beijing, China). CD68 antibody and CD31 antibody were purchased from Abcam (USA). CD206 antibody was from CST (USA).

Animal experiments

Male Sprague-Dawley rats (SD rats, 280 ± 10 g) were obtained from ENSIWEIER (Chengdu, China). Dunkin-Hartely (Outbred, 307.3-499.5 g) was from CHENGDUSHENGWU (Chengdu, China). Golden Gopher was purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All the animal experiments were conducted following the guidelines outlined in the "Principles of Laboratory Animal Care" (NIH). All the experiments were approved by the Animal Care and Use Committee of West China Hospital of Stomatology (WCHSIRB-D-2022-639). The animals had free access to sterilized water and food in a temperature-controlled room (22 ± 1 °C) with a 12 h light/dark cycle. They were fed adaptively for one week in this circumstance before the experiments.

Preparation of the C-P-G film

The preparation of C-P-G membranes consists of three main steps. Firstly, solution preparation: 4 g of zein was dissolved in 40 mL of 75% ethanol solution to obtain the main components of the hydrophobic layer, and the adhesion layer solution (C-P-G solution) was obtained from sodium carboxymethylcellulose (1 g), polyvinylpyrrolidone (1 g), and glycerol (1 g) together with complete dissolution in 50 mL of ultrapure water. Secondly, film coating: the hydrophobic layer solution was first coated on the platform of the laboratory film coating machine with a thickness of 0.7 mm, and heated at 50°C for about 5 minutes until it was formed; then the adhesion layer solution was applied on the hydrophobic layer with a thickness of 1.5 mm, and heated at 50°C for about 30 min until it was fully shaped. Thirdly, cutting: according to the need to cut the double-layer film into the required shape and size to obtain C-P-G film.

Physicochemical properties of C-P-G film

The thickness of C-P-G film is measured by vernier calipers (AIRAJ, Germany) (n=3). The mass

of a 1-cm diameter round C-P-G film was measured by an accurate balance (METTLER TOLEDO, Switzerland) (n=5).

The surface pH of the C-P-G film was obtained by immersing a 1-cm diameter circular C-P-G film in 2 mL of artificial saliva, and the pH of the artificial saliva was measured at 0 h, 0.5 h, 1 h, 2 h, and 3 h of immersion, respectively using a pH meter (Thermo Fisher Scientific, USA) (n=5).

The contact angle of the C-P-G film was tested by optical contact angle meter (SINDIN, China) (n=5).

Adhesion performance tests

To assess the adhesion performance of the C-P-G film, firstly we used the tack mode of the rheometer (Anton Paar Physica MCR 302 Rheometer, Austria) and fixed the C-P-G film on the sample stage, and then applied a force of 0. 5N for 60 s to make the adhesive layer of the C-P-G film close contact with the PP25 rotor, then unloading the normal force to 0 N for 30 s, waiting for the system to stabilize, and finally, the rotor was raised at a speed of 10 mm/s and the displacement versus force curves of the separation were recorded. The adhesion force of C-P-G film on porcine buccal mucosa was determined by fixing the porcine buccal mucosa on the sample stage, fixing the C-P-G film on the PP25 rotor, and then recording the maximum separation force value (Fn_{max}) after making the adhesive layer of the C-P-G film close contact with the porcine buccal mucosa. The adhesion force was calculated using the following Eq 1:

$$\text{Adhesion force } = \frac{Fn_{max}}{S} \quad (1)$$

where Fn_{max} was the maximum separation force value and S is the contact area (the area of the C-P-G film).

The *in vitro* adhesion time of C-P-G film was determined by the rotary artificial saliva method (Figure S1). After the 1-cm diameter circular C-P-G film was adhered to the isolated porcine oral mucosa, it was placed as a whole in a beaker containing 50 mL of artificial saliva to simulate the wet environment of the oral cavity. Then, a magnetic stirrer was used to rotate the artificial saliva in the beaker at a speed of 1,000 rpm at 35°C to simulate the highly dynamic environment of the oral cavity. Finally, the detachment time of the C-P-G film from the porcine oral mucosa was recorded. The *in vitro* adhesion times of other commercially available drugs were also recorded in this way.

The *in vivo* adhesion time was obtained by adhering the oral films/patches to the oral ulcer lesion in SD rats and then recording their separation time from the ulceration.

Adhesion mechanism

Bovine submaxillary mucin was dissolved in PBS (1 mg/mL) and sonicated for 10 min. The mucin suspension was then reacted with C-P-G solution in different volume ratios for 24 h at 37 °C in a shaker (150 rpm). The atomic force microscope (AMF) image was obtained by evenly dropping the mucin suspension and co-mixture (mucin: C-P-G=7:1) on the mica sheet, and observing their morphology under the atomic force microscope (Shimadzu SPM-9700, Japan) after the sample are dried. The size and zeta potential of the mixture were measured using a Zetasizer Nano ZS90 (Malvern Instruments, UK) (n = 3). The UV-vis spectra were tested by Cary Series UV-Vis Spectrophotometer (Agilent, USA). DSC experiments were conducted on a Differential Calorimeter (TA, Discovery 250) at a programmed ramp of 10 °C/min under nitrogen atmosphere. FTIR spectroscopies were acquired using a Nicolet iS 50 spectrometer in the region of 400-4000 cm^{-1} at room temperature. The hydrated samples were freeze-dried before testing DSC and FTIR.

Cytotoxicity assessment

The cytotoxicity of C-P-G film was evaluated by filter membrane diffusion test. Mouse fibroblasts (NCTC Clone 929, L cell, L-929, derivative of Strain L) were cultured using MEM medium containing two antibiotics (including 100 μg/mL penicillin and 100 μg/mL streptomycin) and 10% serum at 37°C with 5% CO_2 saturated humidity. Cell manipulation was carried out in an ultra-clean bench to be as aseptic as possible. After the cell concentration was adjusted to 2.5×10^5 cells/mL using trypsin digestion, the experiment was performed as described below.

In the 6 cm Petri dish, the 0.45 μm microporous filter membrane was placed, which was fully hydrated before placement. Sterilisation was carried out, and 6 mL of the above cell suspension was added to the sterilised filter membrane (3 dishes) respectively, and in addition, 6 mL of culture medium without cells was added to the control dish. The cells were then incubated for 24 h in a 37 °C incubator.

Remove the above filter membranes, cell side down, and put them firmly on the agar medium. Negative control (filter paper soaked with PBS solution), test samples (n=3) and positive control (filter paper soaked with 5% phenol solution) were placed on the surface of the filter membrane containing cells (2 dishes, Figure 3a 1-iii and 1-iv); in addition, one dish containing cells was placed without samples on the top of the filter membrane (Figure 3a 1-i), and one dish without cells was placed with negative control samples, test samples (n=3) and positive control samples on the top of the membrane (Figure 3a 1-ii). Then, they were incubated in incubator for 2 h.

After 2 h, the test and control samples were removed, and after removing the filter membrane, the membrane was drenched with PBS. Then, the staining was observed after soaking the filter membrane with succinate dehydrogenase staining solution at 37°C for 3 h away from light.

The results of cytotoxic reactions were evaluated according to the scoring system in Table S7. Cytotoxicity was usually considered to be present when the grading was greater than 2.

Allergenic reaction assessment

In this test, saline was used as the polar extraction medium and cottonseed oil was used as the non-polar extraction medium. The C-P-G films were extracted using these two extracting media, and then the allergenic reaction test was performed by the guinea pig maximization test (GPMT)(*1, 2*). Tables S8-S10 show the leaching conditions as well as the state of the leachate. The tests were carried out in four main stages.

The first stage: intradermal induction. Prior to the start of the test, the animals were shaved at the scapulae with a size of approximately 3 cm*4 cm. After the shaved areas were routinely disinfected with iodophor, intradermal injections (0.1 mL per spot) were performed (3 pairs of 6 points) according to the injection sites shown in Figure S5. The groupings were as follows: Test group: 1st pair of injections: emulsifier mixed with Fuchs' complete adjuvant and solvent in a 50:50 volume ratio; 2nd pair of injections: leachate of C-P-G film; 3rd pair of injections: emulsifier mixed with leachate and Fuchs' complete adjuvant and solvent (50%) in a 50:50 (v/v) ratio. Solvent control group: 1st pair of injections: emulsifier mixed with Fuchs' complete adjuvant and solvent at a volume ratio of 50:50; 2nd pair of injections: saline/cottonseed oil; 3rd pair of injections: Fuchs' complete adjuvant and saline/cottonseed oil. Positive control group: 1st pair of injections: emulsifier mixed with Fuchs' complete adjuvant and solvent in a 50:50 (v/v) ratio; 2nd pair of injections: 0.2 g/mL of 2-chromobenzothiazole sodium carbonate solution; 3rd pair of injections: Fuchs' complete adjuvant and 0.2 g/mL of 2-chromobenzothiazole sodium carbonate solution.

The second stage: local induction. Seven days after the intradermal injection, the local induction was started. Firstly, the test area needed to be pre-treated by massaging 10% sodium dodecyl sulphate

into the skin. 24 h later, topical dressing was started, and about 8 cm^2 absorbent gauze dressings soaked with the sample extraction solution was placed to cover the injection sites as shown in Figure S8. Then, the gauze was fixed with a closed bandage, and the gauze and bandage were removed after 48 h. The solvent control and positive control animals were treated using the corresponding liquids in the same way.

The third stage: excitation. After 14 days of local induction, the excitation process was started. The day before the excitation was carried out, the depilatory agent was applied to the white hairs on the flanks of the animal for depilation, then the residual depilatory agent was washed off with warm water and the animal was gently dried. After 24 h of depilation, the test was carried out at the skin of the depilated area according to the method of local induction, and the excitation was carried out with the corresponding specimen, which was removed after 24 h of application.

The fourth stage: animal observation. At 24 h and 48 h after the dressing was removed, the skin condition at the excitation site of the test and control group animals was observed and evaluated, and the skin reactions at different excitation sites and at different observation times were graded in accordance with the scoring rules according to Table S2.

Evaluation of oral mucosal irritation response

After checking that the buccal sacs were free of abnormalities, the buccal sacs of three golden gophers were rinsed with saline. The samples were placed in acute contact with the cheek sacs of one side of the golden gopher, and the other cheek sac was contacted using a 0.9% NaCl-soaked cotton ball. The contact was made for 10 min per hour for a total of 4 h.

Animals were painlessly executed 24 h after the last contact. The buccal mucosa at the contact site of the test samples was observed visually for congestion, oedema, erosion and ulcerative reaction. The degree of tissue reaction was scored according to Table S4. Then the tissues at the contact site were cut and placed in 10% formalin solution for fixation, paraffin embedding, sectioning, H&E staining and microscopic examination. Tissue response scores were first calculated for each animal according to the tissue response scoring system in Table S11; after that, the stimulus index grading was derived from Table S12. The average oral mucosal irritation score (M_1) was calculated according to Eq 2:

$$M_1 = \frac{\text{erythema total scores} + \text{edema total scores}}{\text{number of animals}} \quad (2)$$

The average oral mucosal irritation microscopic score (M_2) was calculated according to Eq 3:

$$M_2 = \frac{\text{epithelium total scores} + \text{leukocyte infiltration total scores} + \text{vascular total scores} + \text{oedema total scores}}{\text{number of observation sites}} \quad (3).$$

Release studies of FITC-loaded C-P-G film

The FITC-loaded C-P-G films were placed on the isolated porcine oral mucosa for 10 min, 20 min, 30 min, 1 h, 1.5 h, 2 h and 3 h. The isolated porcine oral mucosa was frozen at -20°C for 1 h, and then frozen sections were made, sealed with DAPI and observed under fluorescence microscope.

Release profile of Dex from C-P-G film

The Dex-C-P-G film (one piece of 1 cm diameter C-P-G film containing 0.39 mg of dexamethasone sodium phosphate) was placed in a vial containing 2 mL of PBS, which was placed in a constant temperature water bath at 37 °C. 1 mL of aliquots from the upper solution were taken and collected for further analysis at particular time intervals. Subsequently, the same amount (1 mL) of respective fresh PBS was added after every collection of aliquots to maintain a constant volume. The amount of Dex release was analyzed by Ultra-fast liquid chromatography (UFLC)-MS/MS (ThermoFisherScience TSQ

Quantum Ultra, USA)(*3*). The cumulative release mass (M_t) of Dex released from C-P-G film at different time points was measured by Eq 4:

$$M_t = C_t \times V_a + \sum(C_{t-1} \times V_b) \quad (4)$$

where C_t is the concentration of Dex recorded at time t, V_a is the total volume of the PBS in the vials (V_a = 2 mL), and V_b is the volume removed from the vials every time (V_b = 1 mL). The cumulative release percentage of Dex was calculated according to the following Eq 5:

$$Dex\ cumulative\ amount\ released\ (\%) = \frac{M_t}{M_0} \times 100\% \quad (5)$$

where M_0 is the initial quantity of Dex encapsulated inside the C-P-G film (M_0 = 0.39 mg).

***In vivo* therapeutic efficiency**

The C-P-G film, Dex-C-P-G film, and other commercially available oral patches/films (Oral film 1, Oral film 2, and Oral patch 3) were first cut into 4 mm diameter circles for animal testing. SD rats were anesthetized with 1% pentobarbital sodium (40 mg/kg), and oral ulcers were induced by placing a round cotton balls (3 × 3 mm) soaked with 50% acetic acid on the oral mucosa for 50 s. Two days after inducing the oral ulcer (Day 1), C-P-G film, Dex-C-P-G film, and other commercially available oral patches/films (Oral film 1, Oral film 2, and Oral patch 3) were applied onto the oral mucosa ulcer, and the rats with no treatment were set as the blank control group. The animals were treated with the same procedure for seven consecutive days, with one administration per day. During this period, administration was stopped if the ulcers had healed. And gross observation was observed before each film application. The animals were sacrificed on Day 8, and the oral mucosa around the ulcer, major organs (heart, liver, spleen, lungs and kidneys) and blood were collected, and the samples were harvested for histological and immunohistochemical analysis, and enzyme linked immunosorbent assay (ELISA). Immunohistochemistry (CD31, CD68, CD206), Masson staining, Picrosirius Red staining, and ELISA tests were performed according to the manufacturer's instructions. Quantification of CD31-positive blood vessels, CD68-positive and CD206-positive relative area were conducted manually in a blinded manner using Image Pro Plus.

Statistical analysis

All the quantitative experiments in this study were performed at least in triplicate. The obtained data were represented as mean value ± SD (standard deviation). Statistical differences among different groups were determined through descriptive statistics, t-test, one-way ANOVA test and two-way ANOVA with Tukey's multiple comparisons test using the GraphPad Prism 6.0 software, which were considered statistically significant when $P < 0.05$ (* for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$), while $P >$ 0.05 was represented no significance.

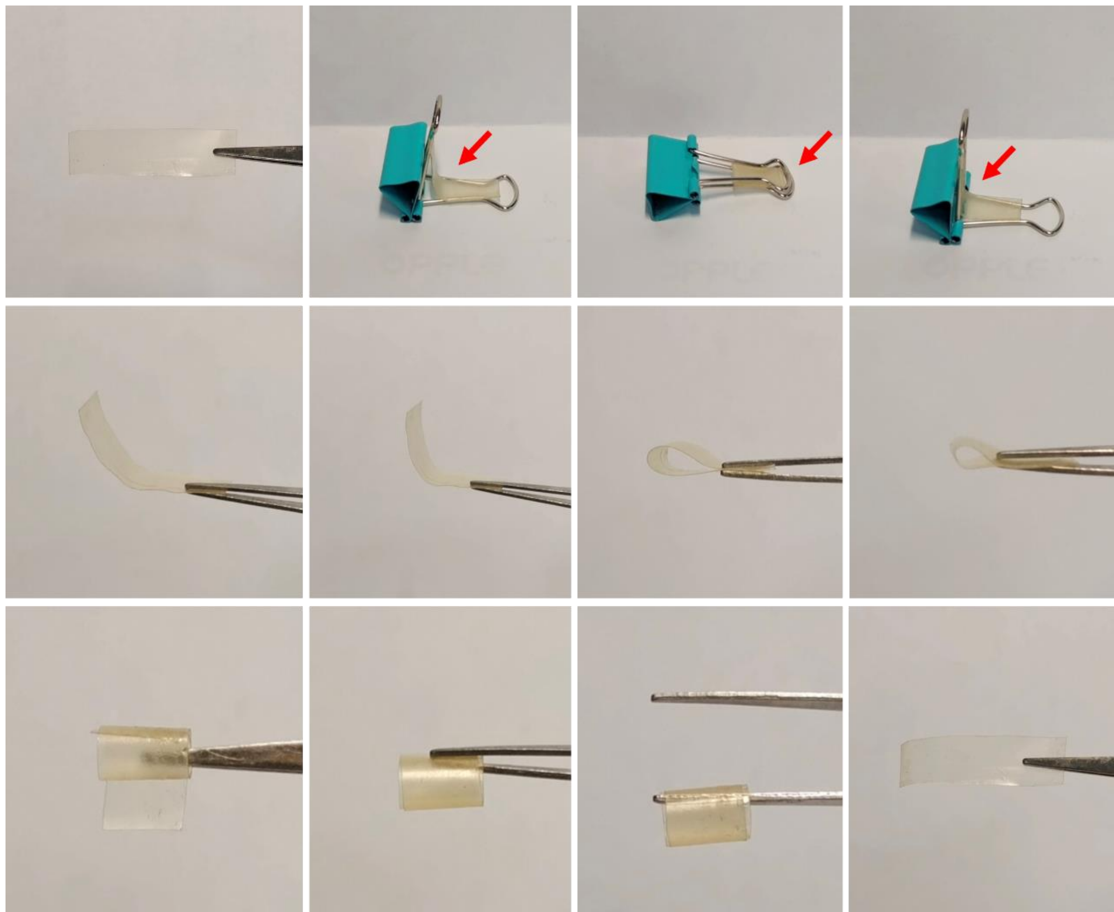

Figure S1. The excellent flexibility of C-P-G film.

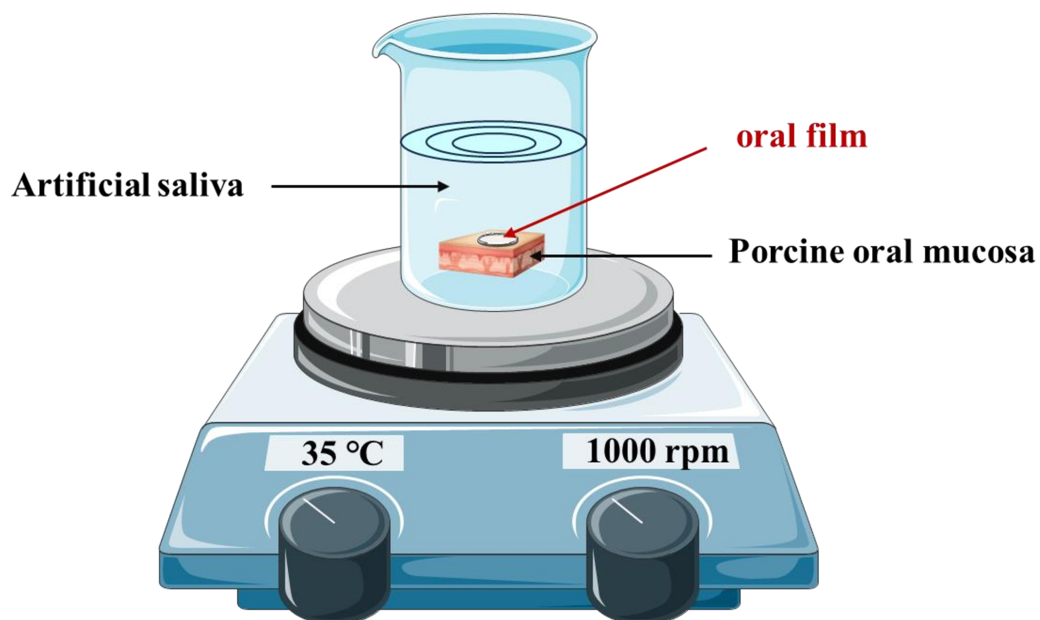

Figure S2. Rotating artificial saliva method. After the round C-P-G film with a diameter of 1 cm was adhered to the porcine oral mucosa, it was placed into a beaker containing 50 mL of artificial saliva, and then a magnetic stirrer was used to rotate the artificial saliva in the beaker at a speed of 1,000 rpm at 35°C, and the time for the oral film to be dislodged from the porcine oral mucosa was recorded.

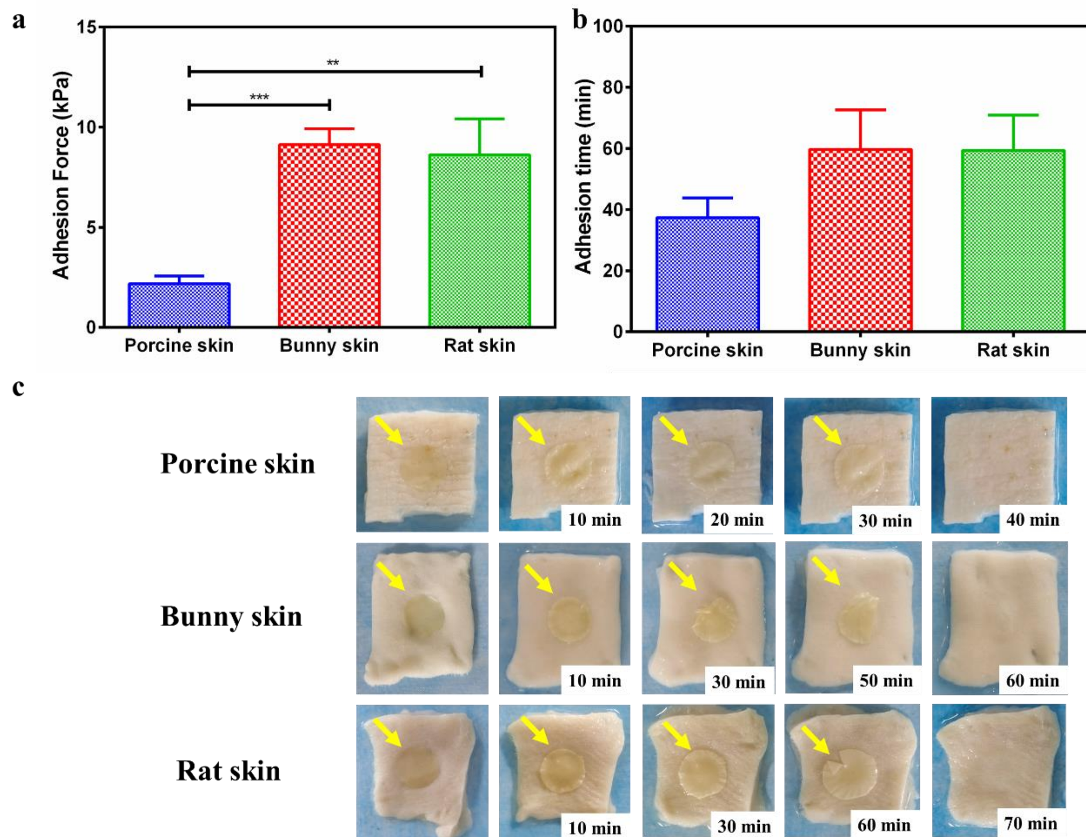

Figure S3. Adhesion performance of C-P-G film to the skin. (a) Adhesion force of C-P-G film to the porcine skin, bunny skin and rat skin (n=3). (b-c) Adhesion time of C-P-G film to the porcine skin, bunny skin and rat skin (n=3).

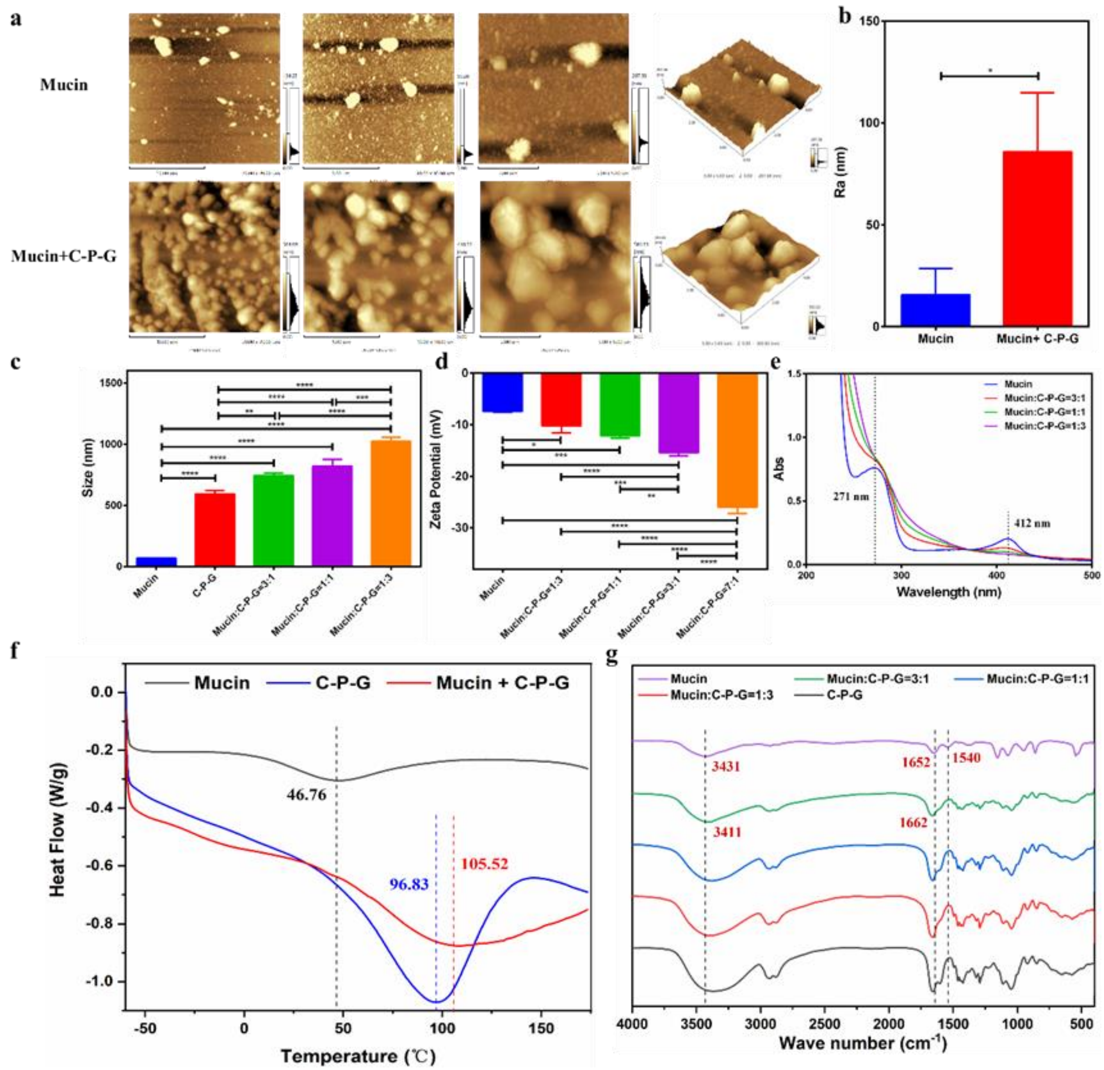

Figure S4. Adhesion mechanism of the adhesive layer. (a) Atomic force microscopy (AFM) images of mucin and mucin + C-P-G. (b) The average surface roughness (Ra) of mucin and mucin + C-P-G (n=3). (c) Variation in the particle size of mucin and mucin + C-P-G (n=3). (d) The zeta potential of mucin and mucin + C-P-G (n=3). (e) UV-vis absorbance spectra of mucin and mucin + C-P-G. (f) Thermodynamic analysis (differential scanning calorimeter, DSC) spectra of mucin and mucin + C-P-G. (g) FTIR spectra of mucin and mucin + C-P-G.

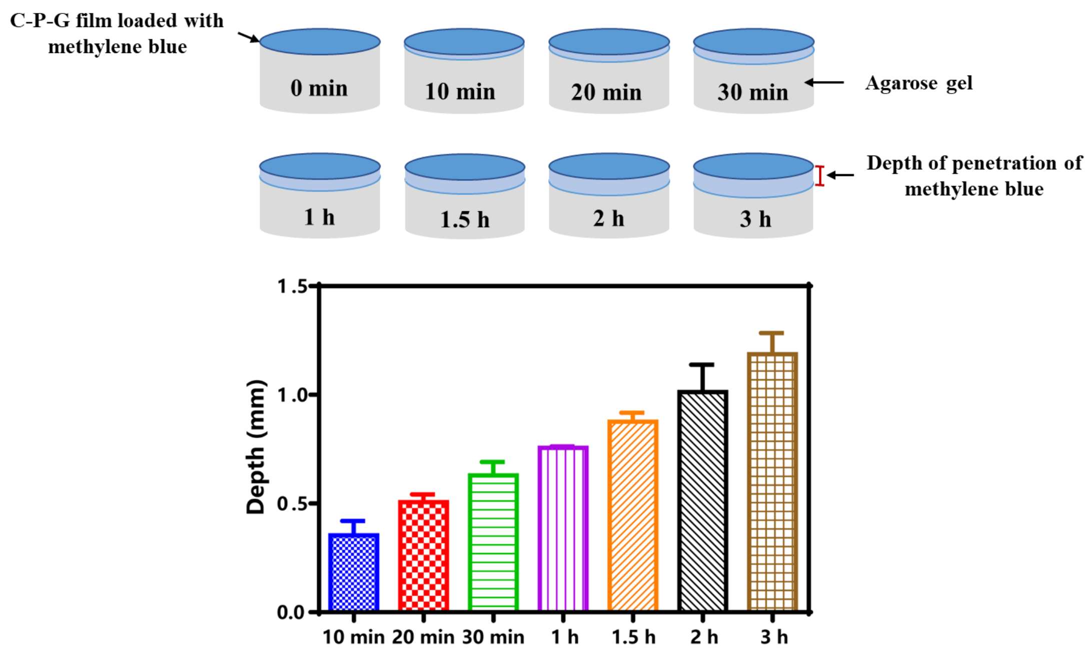

Figure S5. The C-P-G film loaded with methylene blue was adhered to a 2% agarose gel, and the depth of methylene blue into the agarose gel gradually deepened with time (n=3).

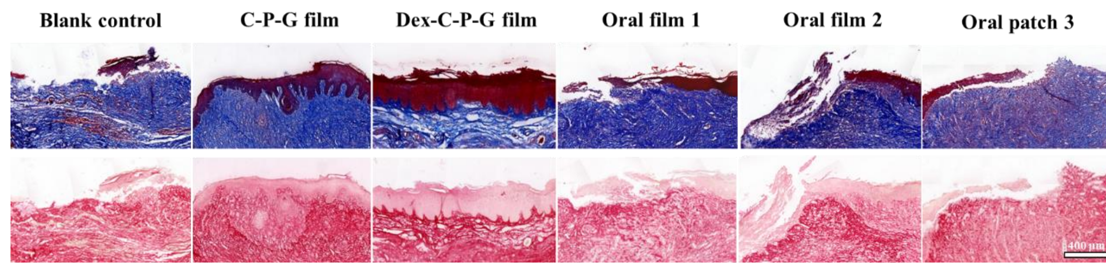

Figure S6. The histological status of the oral mucosa at the ulcers in the different treatment groups was stained by Masson staining and Picrosirius Red staining on Day 8 (scale bar: 400 μm).

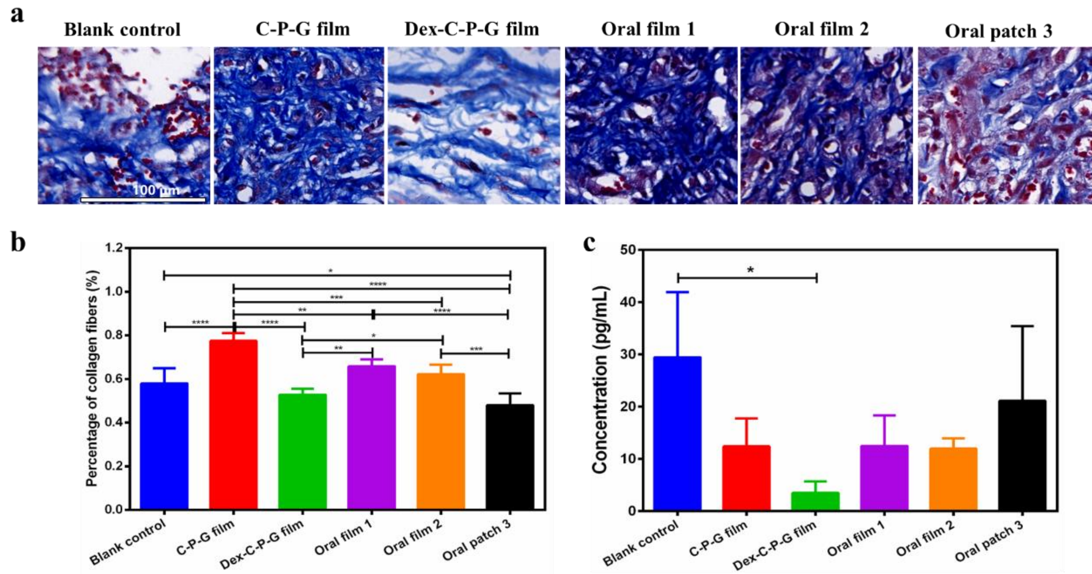

Figure S7. Therapeutic effects in each group. (a) Results of Masson staining: the arrangement of submucosal collagen fibers in the Dex-C-P-G film group was loose, neat and approaching normal, and the C-P-G film group, oral film 1 group, and oral film 2 group were in the period of massive proliferation of collagen fibers, whereas the poorly repaired blank control group and the oral patch 3 group showed adverse proliferation of collagen fibers (scale bar: 100 μm). (b) The percentage of submucosal collagen fibers in each treatment group by Masson staining (n=5). (c) Serum IL-6 levels in each group on Day 8 (n=3).

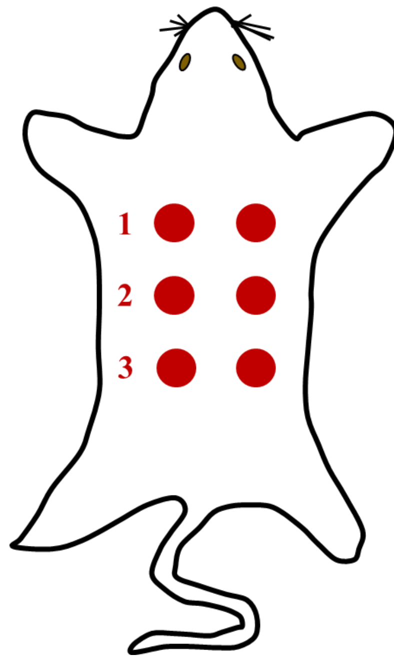

Figure S8. Schematic diagram of intradermal injection sites in allergenic reaction assessment.

Table S1. Filter membrane diffusion test results

Sample	Level	Degree of reaction	Manifestation of reaction area
C-P-G film	0	None	No reaction areas observed around and under the sample
Negative control (PBS solution)	0	None	No reaction areas observed around and under the sample
Positive control (5% phenol solution)	3	Moderate	Reaction area extends beyond the sample size to 1 cm
With cells without sample filter membrane	/	/	No obvious reaction area observed
Cell-free with sample filter membrane	/	/	No obvious reaction area observed

Table S2. Scoring criterion (Magnusson and Kligman grading)

Skin reaction	Grade
No visible change	0
Blotchy or scattered erythema	1
Moderate fused erythema	2
Severe erythema and/or oedema	3

Table S3. Magnusson and Kligman grading after excitation of test and control animals

Group	Animal number	Time	
		24 h	48 h
Polar dip test group	No. 1-10	All Grade 0	All Grade 0
Solvent control group (polar)	No. 11-15	All Grade 0	All Grade 0
Non-polar leaching test group	No. 16-25	All Grade 0	All Grade 0
Solvent control group (non-polar)	No. 26-50	All Grade 0	All Grade 0
Positive control group	No. 31	Grade 2	Grade 2
	No. 32	Grade 2	Grade 2
	No. 33	Grade 1	Grade 1
	No. 34	Grade 1	Grade 1
	No. 35	Grade 1	Grade 1

Table S4. Scoring criteria for oral mucosal irritation response

Response (erythema and crust formation)	Irritation score
No erythema	0
Very slight erythema (barely visible)	1
Clear erythema	2
Moderate erythema	3
Severe erythema (purplish red) to crust formation that interferes with erythema grading	4

Table S5. Buccal sac mucosal tissue microscopic irritation rating scale

Observation site	Epithelial reaction	Leukocyte infiltration	Vascular congestion	Oedema
Sample side				
1-A	1	1	1	0
1-B	1	1	1	0
1-C	1	1	1	1
1-D	1	1	1	1
1-E	1	1	1	2
2-A	1	1	1	1
2-B	2	2	1	1
2-C	2	2	1	1
2-D	1	1	1	1
2-E	1	2	1	2
3-A	1	1	1	1
3-B	1	1	1	1
3-C	1	1	1	2
3-D	1	1	1	2
3-E	1	1	1	2
Control side				
1-A	1	2	2	0
1-B	1	1	2	1
1-C	1	1	2	1
1-D	1	1	1	1
1-E	1	1	1	1
2-A	1	1	1	1
2-B	1	1	1	0
2-C	1	1	1	1
2-D	1	1	1	1
2-E	1	1	1	1
3-A	1	1	1	2
3-B	1	1	1	1
3-C	1	1	1	1
3-D	1	1	1	1
3-E	1	1	1	2

Note: Observation site 1-A in the above table indicates site A of the buccal sac tissue section of animal No. 1, and so on.

Table S6. The physicochemical properties, adhesive properties and therapeutic efficacy of C-P-G films

Item	Mean±SD
Physicochemical properties	
Thickness	0.11 ± 0.01 mm
Mass	9.26 ± 0.99 mg
Surface pH (3 h)	6.926 ± 0.016
Contact Angle	106.1 ± 1.0 °
Adhesive properties	
Adhesive force (on PP25 rotor)	59.04 ± 7.61 kPa
Adhesive force (on porcine Buccal Mucosa)	4.67 ± 0.26 kPa
Adhesive time (*in vitro*)	68.33 ± 11.64 min
Adhesive time (*in vivo*)	152.70 ± 11.12 min
Ulcer size (Dex-C-P-G film)	
Day 1	32.05 ± 7.43 mm^2
Day 3	12.70 ± 7.07 mm^2
Day 6	8.45 ± 7.74 mm^2
Day 8	0.98 ± 1.29 mm^2

Table S7. ***In vitro*** **cytotoxicity evaluation-filter membrane diffusion test reaction grading**

Level	Degree of reaction	Manifestation of reaction area
0	None	No reaction areas observed around and under the sample
1	Very mild	A few malformed or degenerated cells underneath the sample
2	Mild	Reaction area is confined to the underside of the sample
3	Moderate	Reaction area extends beyond the sample size to 1 cm
4	Severe	Reactive area extends more than 1cm beyond the sample

Table S8. Polar leaching operation table

Experiment stage	Leaching ratio	Sample size	Volume of leaching medium	Leaching conditions
Intradermal induction	6 cm^2/mL	85.2 cm^2	14.2 mL	37 ℃, 72 h, 60 rpm
Local induction		315.0 cm^2	52.5 mL	
Excitation		136.0 cm^2	22.7 mL	

Table S9. Non-polar leaching operation table

Experiment stage	Leaching ratio	Sample size	Volume of leaching medium	Leaching conditions
Intradermal induction	6 cm^2/mL	73.8 cm^2	12.3 mL	37 °C, 72 h, 60 rpm
Local induction		315.0 cm^2	52.5 mL	
Excitation		135.0 cm^2	22.7 mL	

Table S10. State of the leachate

Experiment stage	Leaching medium	Leachate	State of the leachate		
			Color	**Clarity or not**	**Particles**
Intradermal induction	Polar	Sample	Colorless	Yes	None
		Solvent control	Colorless	Yes	None
	Non-polar	Sample	Yellow	Yes	None
		Solvent control	Yellow	Yes	None
Local induction	Polar	Sample	Colorless	Yes	None
		Solvent control	Colorless	Yes	None
	Non-polar	Sample	Yellow	Yes	None
		Solvent control	Yellow	Yes	None
Excitation	Polar	Sample	Colorless	Yes	None
		Solvent control	Colorless	Yes	None
	Non-polar	Sample	Yellow	Yes	None
		Solvent control	Yellow	Yes	None

Note: At the end of the leaching, the leachate was used directly in the test without further treatment, such as centrifugation, filtration and so on.

Table S11. Scoring criteria for microscopic examination of oral mucosal tissue response

Response	Score
Epithelium	
Normal, intact	0
Cells degenerate or become flattened	1
Deformation of the tissue	2
Localised erosion	3
Extensive erosion	4
Leukocyte infiltration (per high magnification field of view)	
None	0
Minimal (less than 25)	1
Mild (26-50)	2
Moderate (51-100)	3
Severe (>100)	4
Vascular congestion	
None	0
Very mild	1
Mild	2
Moderate	3
Severe with vascular rupture	4
Oedema	
None	0
Very mild	1
Mild	2
Moderate	3
Severe	4

Table S12. Types of oral mucosal tissue microscopic irritation response

Average score	Degree of irritation response
0	None
1-4	Very mild
5-8	Mild
9-11	Moderate
12-16	Severe

References

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