# Supplementary Information

# A mussel-inspired film for adhesion to wet buccal tissue and efficient buccal drug delivery

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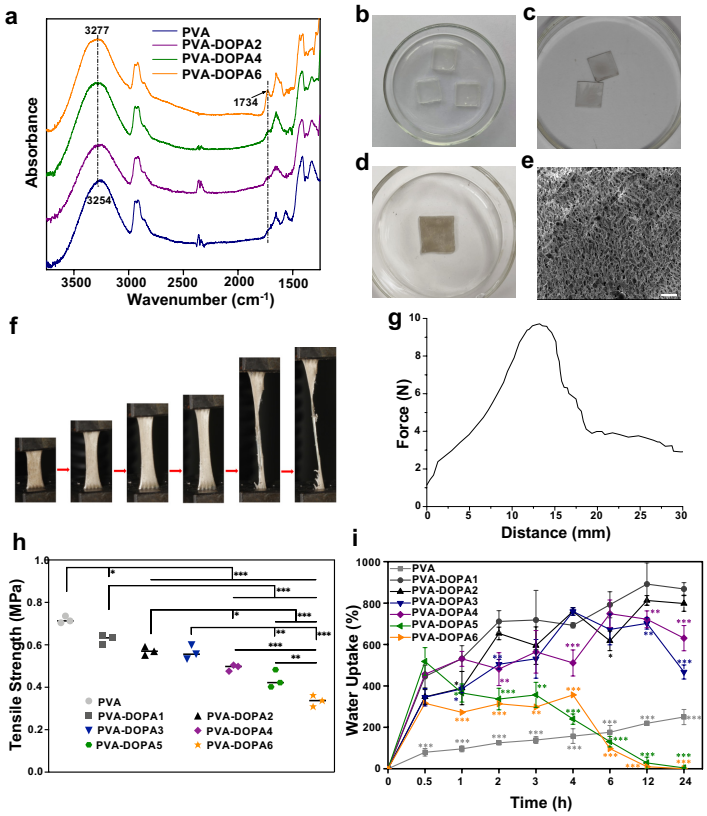
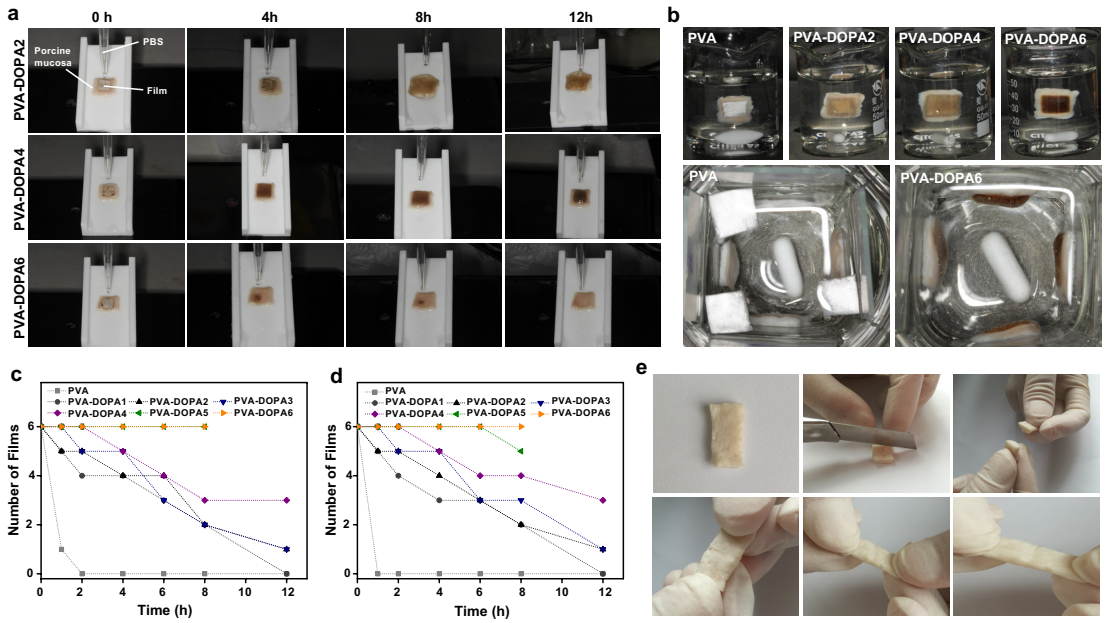
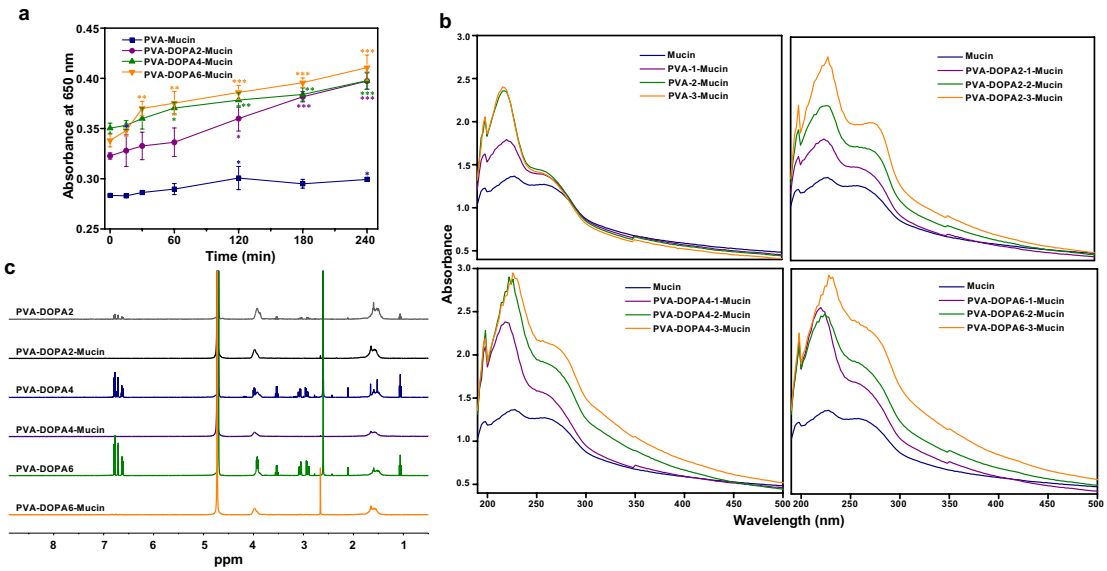
## Supplementary figures



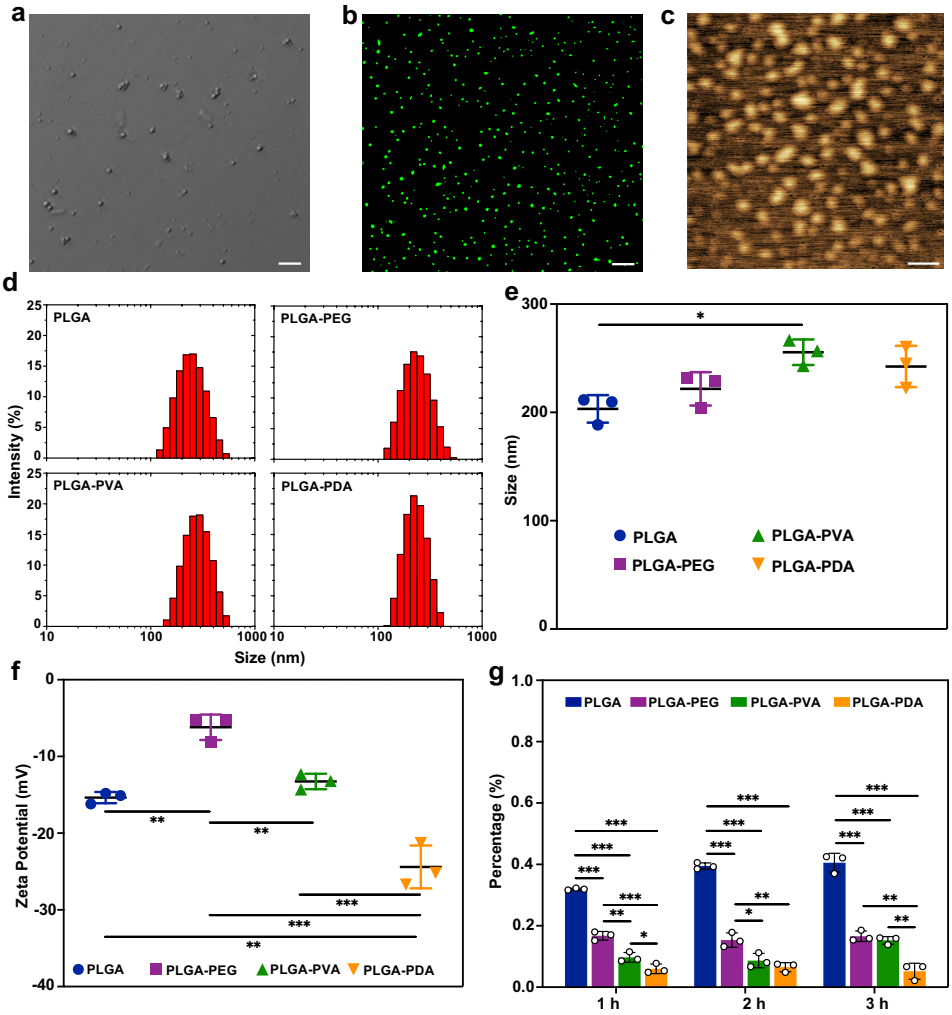

**Supplementary Figure 1. Characterization of PVA-DOPA films.** (a) FTIR spectra of PVA-DOPA polymers with different DOPA contents. PVA: poly(vinyl alcohol), DOPA: 3,4-dihydroxy-D-phenylalanine. (b) Photograph of ethyl cellulose protective cap. (c) Photograph of dry PVA-DOPA mucoadhesive film. (d) Photograph of PVA-DOPA mucoadhesive film after hydration. (e) SEM image of PVA-DOPA film. Scale bar: 200 $\mu$m (f) Tensile strength testing of PVA-DOPA3 film. (g) Representation of the stress-distance curve, during the tensile tests, for PVA-DOPA3 film. (h) Tensile strength of PVA-DOPA films with different DOPA contents. $n = 3$ independent samples per group; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. (i) Swelling behavior of PVA-DOPA films different DOPA contents as a function of time. $n = 3$ independent samples per group; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs PVA-DOPA1 group. All data are Mean ± S.D. Statistics was calculated by one-way ANOVA followed by Tukey's post-test. Exact $P$ values are given in the Source Data file. Source data are provided as a Source data file.



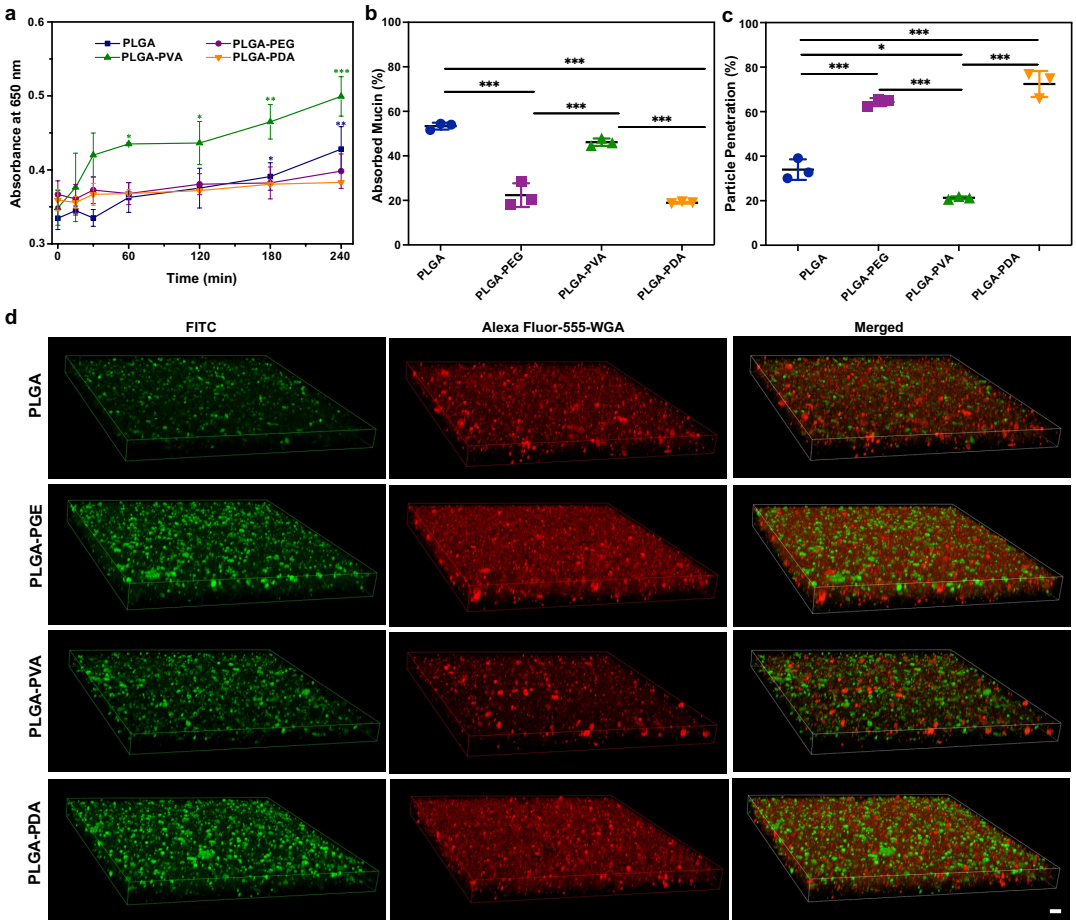

**Supplementary Figure 2. Residence time and self-healing properties of PVA-DOPA films.** (a) Experimental set-up for in vitro residence time measurement using the flow-through method. PVA: poly(vinyl alcohol), DOPA: 3,4-dihydroxy-D-phenylalanine. (b) Experimental set-up for in vitro residence time measurement using the rotating disc method. (c) The number of different PVA-DOPA films left on the porcine buccal mucosa as a function of time using the flow-through method. (d) The number of different PVA-DOPA films left on the porcine buccal mucosa as a function of time using the rotating disc method. (e) Photographs of self-healing properties of PVA-DOPA film.



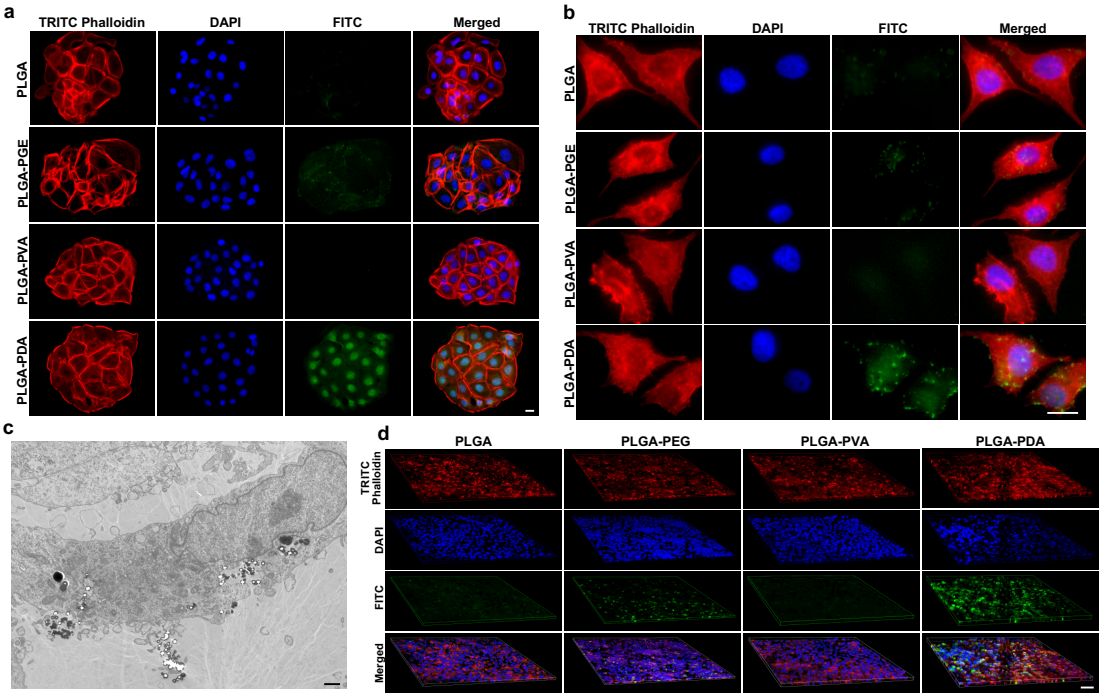

**Supplementary Figure 3. Interactions of PVA-DOPA films with mucin.** (a) Variation of turbidity of different PVA-DOPA-Mucin mixtures as a function of time. PVA: poly(vinyl alcohol), DOPA: 3,4-dihydroxy-D-phenylalanine. $n = 3$ independent samples per group; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs value at 0 h. All data are Mean ± S.D. Statistics was calculated by one-way ANOVA followed by Tukey's post-test. Exact $P$ values are given in the Source Data file. (b) UV-vis absorbance spectra of different concentrations (0.05, 0.10, and 0.15 mg/ml) of PVA, PVA-DOPA2, PVA-DOPA4, and PVA-DOPA6 after mixed with mucin suspension. (c) Comparison of $^{1}$H-NMR spectra of different PVA-DOPA polymers before and after mixed with mucin suspension. Source data are provided as a Source data file.



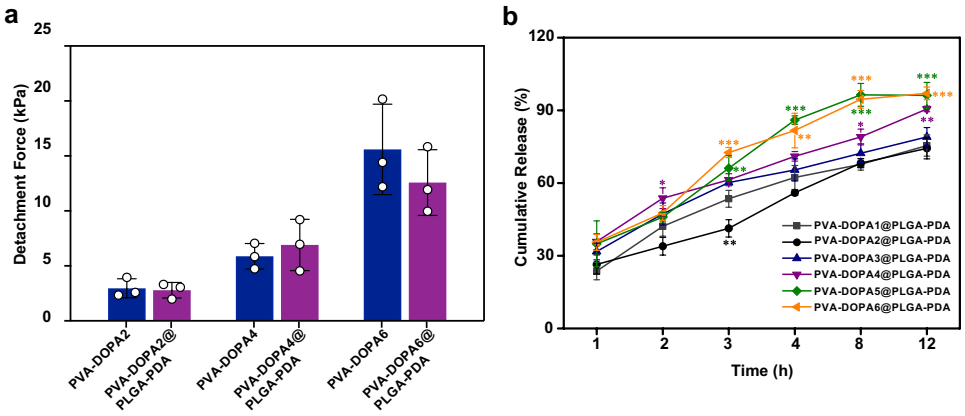

**Supplementary Figure 4. Characterization of different PLGA NPs.** (a) Optical micrography of PLGA NPs. Sale bar: 2 $\mu$m. (b) Fluorescence image of PLGA NPs. Sale bar: 2 $\mu$m. (c) AFM image of PLGA NPs. Sale bar: 200 nm. (d) Size distribution of PLGA, PLGA-PEG, PLGA-PVA, and PLGA-PDA NPs. PLGA: poly(lactic-co-glycolic acid), PEG: poly(ethylene glycol), PVA: poly(vinyl alcohol), PDA: polydopamine. (e) Particle size of PLGA, PLGA-PEG, PLGA-PVA, and PLGA-PDA NPs. *$P$ = 0.012. (f) Zeta-potential of PLGA, PLGA-PEG, PLGA-PVA, and PLGA-PDA NPs. **$P$ < 0.01; ***$P$ < 0.001. (g) Percentage of PLGA, PLGA-PEG, PLGA-PVA, and PLGA-PDA NPs interacted with rose bengal solution. *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001. All data are Mean ± S.D. $n$ = 3 independent samples per group. Statistics was calculated by one-way ANOVA followed by Tukey's post-test. Exact $P$ values are given in the Source Data file. Source data are provided as a Source data file.



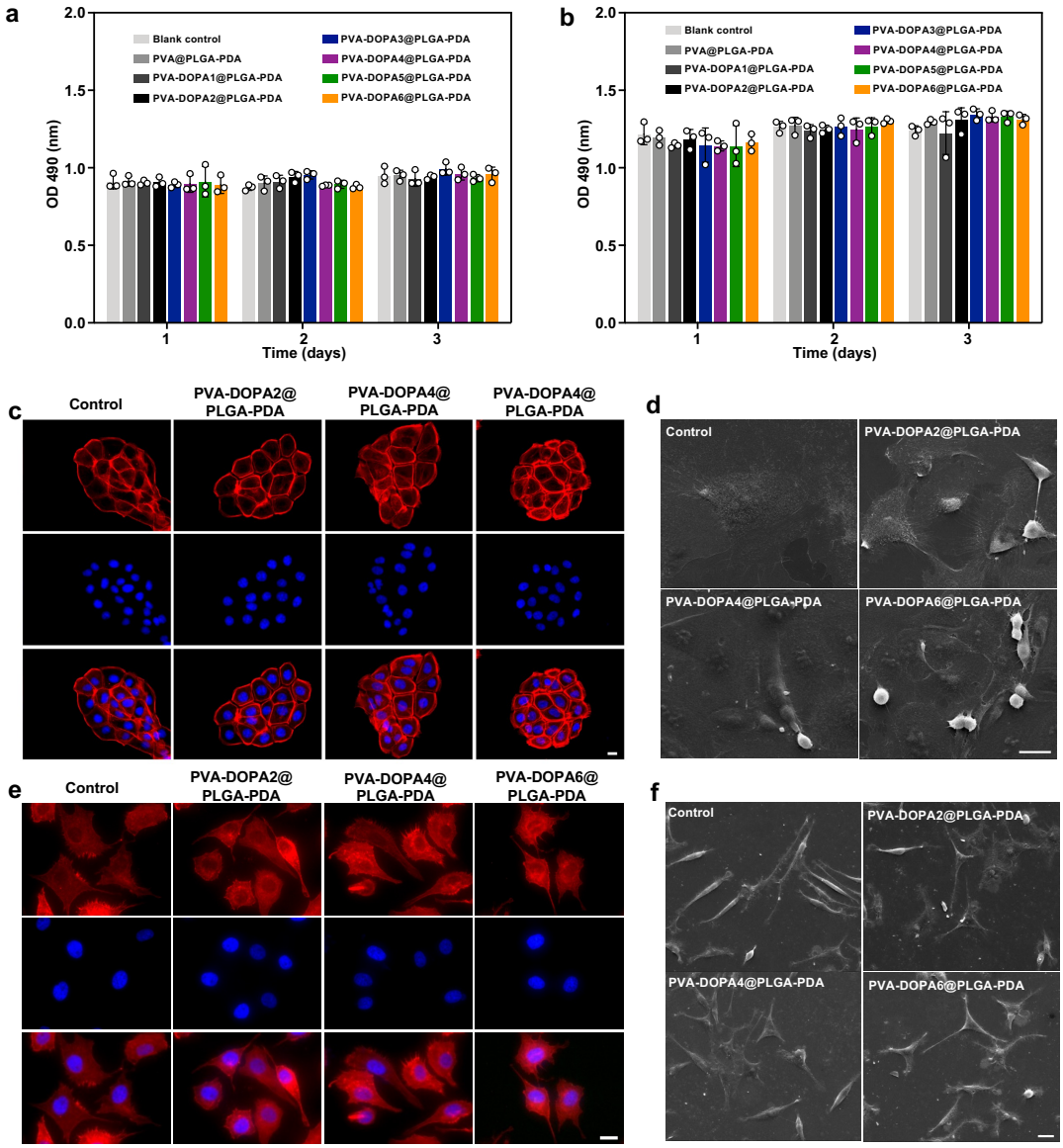

**Supplementary Figure 5. Mucus-penetrating properties of different PLGA NPs.** (a) Variation of turbidity of different NPs-Mucin mixtures as a function of time. PLGA: poly(lactic-co-glycolic acid), PEG: poly(ethylene glycol), PVA: poly(vinyl alcohol), PDA: polydopamine. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs value at 0 h. (b) Percentage of absorbed PLGA, PLGA-PEG, PLGA-PVA, and PLGA-PDA NPs onto mucin particles. ***$P < 0.001$. (c) Percentage of penetrated NPs across mucus layer in an agarose gel assay after 6 h. *$P = 0.016$; ***$P < 0.001$ (d) *Z*-stacks of PLGA, PLGA-PEG, PLGA-PVA, and PLGA-PDA NPs diffusion (green) in mucin suspension (red). Scale bar: 50 μm. All data are Mean ± S.D. $n = 3$ independent samples per group. Statistics was calculated by one-way ANOVA followed by Tukey's post-test. Exact *P* values are given in the Source Data file. Source data are provided as a Source data file.



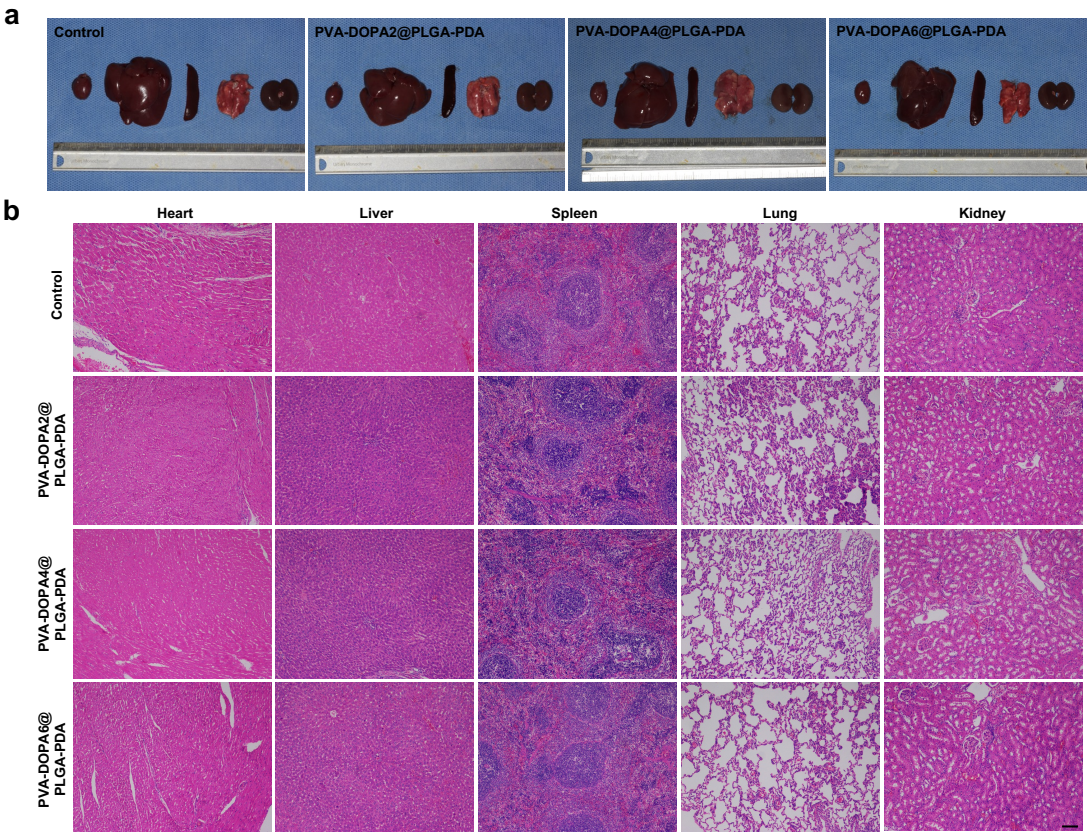

**Supplementary Figure 6. Cellular uptake of different PLGA NPs in vitro.** (a) Fluorescence image of cellular uptake of different NPs in HOK after incubation for 2 h. Scale bar: 20 μm. PLGA: poly(lactic-co-glycolic acid), PEG: poly(ethylene glycol), PVA: poly(vinyl alcohol), PDA: polydopamine. (b) Fluorescence image of cellular uptake of different NPs in HGECs after incubation for 2 h. Scale bar: 20 μm. (c)TEM images of cellular internalization process of PLGA-PDA NPs in HOK after incubation for 2 h. Scale bar: 1 μm. (d) 3D images of the cellular transport of NPs in the TR146 cell monolayer. Scale bar: 100 μm. $n$ = 3 independent cells per group.



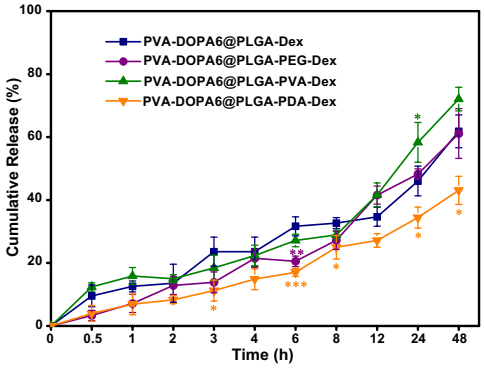

**Supplementary Figure 7. Detachment force and release profile of PVA-DOPA@NPs films after incorporated with PLGA-PDA NPs.** (a) Comparison of detachment force of different PVA-DOPA films before and after incorporated with NPs. PVA: poly(vinyl alcohol), DOPA: 3,4-dihydroxy-D-phenylalanine, PLGA: poly(lactic-co-glycolic acid), PDA: polydopamine. (b) Release profile of NPs from PVA-DOPA@PLGA-PDA films with different DOPA contents as a function of time. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs PVA-DOPA1@PLGA-PDA group. All data are Mean ± S.D. $n = 3$ independent samples per group. Statistics was calculated by one-way ANOVA followed by Tukey's post-test. Exact $P$ values are given in the Source Data file. Source data are provided as a Source data file.



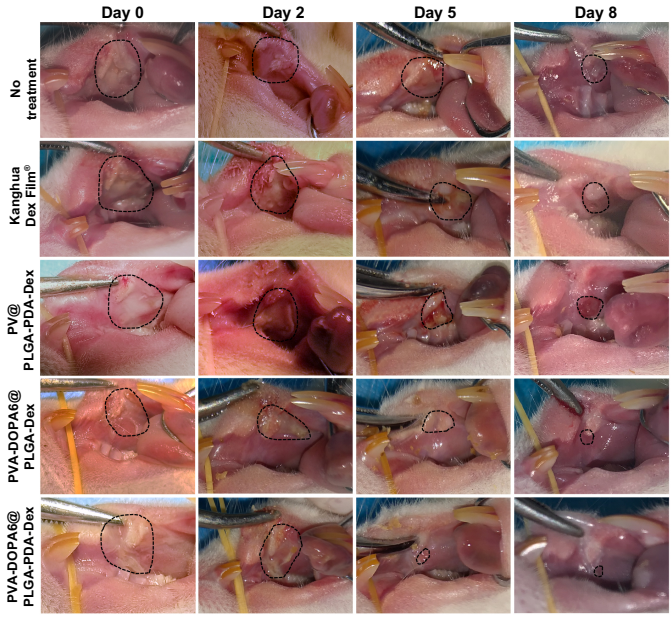

**Supplementary Figure 8. In vitro biosafety evaluation of PVA-DOPA@PLGA-PDA film.** (a) (b) CCK-8 assay of HOK and HGECs after incubated with different films for 1, 2, and 3 days, respectively. PVA: poly(vinyl alcohol), DOPA: 3,4-dihydroxy-D-phenylalanine, PLGA: poly(lactic-co-glycolic acid), PDA: polydopamine. (c) Fluorescence image of cell attachment of HOK after incubated with different PVA-DOPA@NPs films for 24 h. Scale bar: 20 μm. (d) SEM image of cell attachment of HOK after incubated with different PVA-DOPA@NPs films for 24 h. Scale bar: 20 μm. (e) Fluorescence image of cell attachment of HGECs after incubated with different PVA-DOPA@NPs films for 24 h. Scale bar: 20 μm. (f) SEM image of cell attachment of HGECs after incubated with different PVA-DOPA@NPs films for 24 h. Scale bar: 20 μm. All data are Mean ± S.D. $n$ = 3 independent cells per group. Statistics was calculated by one-way ANOVA followed by Tukey's post-test. Source data are provided as a Source data file.



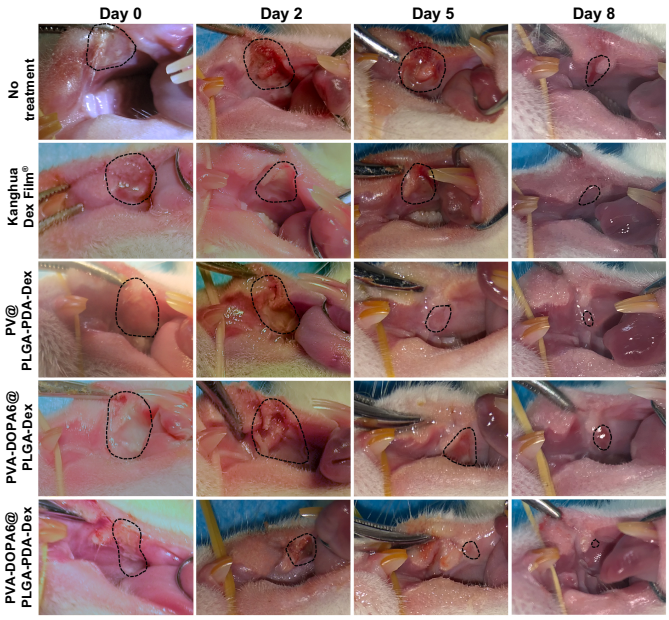

**Supplementary Figure 9. In vivo biosafety evaluation of PVA-DOPA@PLGA-PDA film.** (a) Photographs of rat major organs (heart, liver, spleen, lung, and kidney) after subcutaneously implanted with different films in the backs of SD rats for 7 days. PVA: poly(vinyl alcohol), DOPA: 3,4-dihydroxy-D-phenylalanine, PLGA: poly(lactic-co-glycolic acid), PDA: polydopamine. (b) H&E staining of major organs (heart, liver, spleen, lung, and kidney) after subcutaneously implanted with different films in the backs of SD rats for 7 days. Scale bars: 100 μm. $n$ = 3 animals per group.



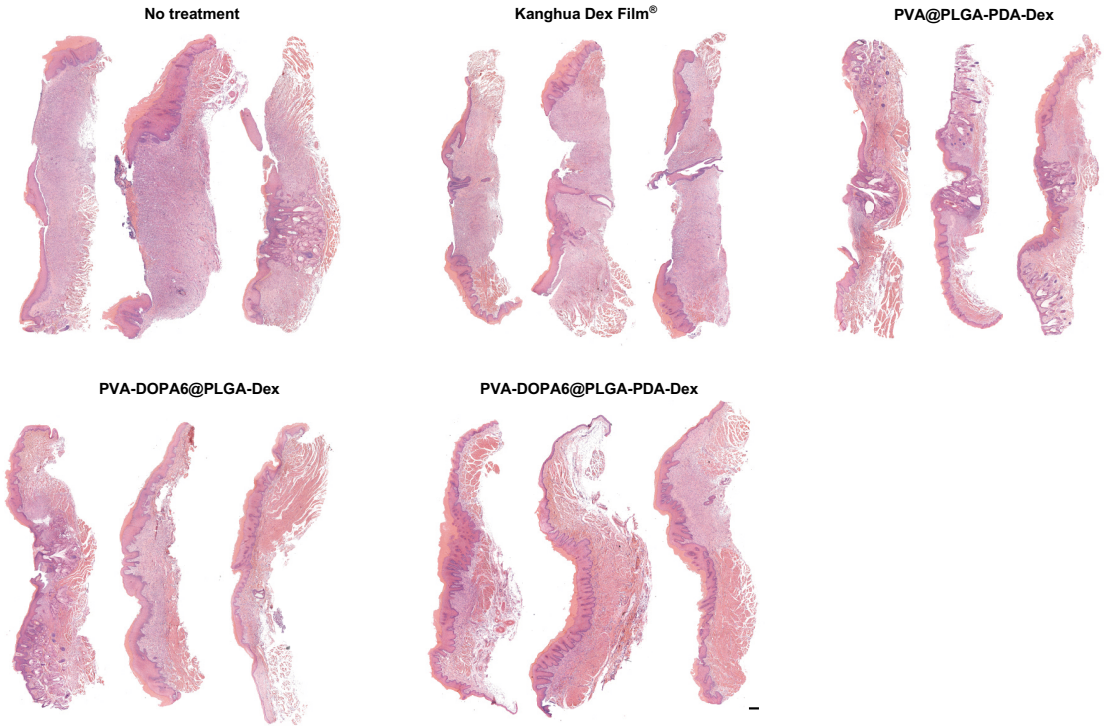

**Supplementary Figure 10.** In vitro release profile of Dex from PVA-DOPA6@NPs film incorporated with different NPs as a function of time. PVA: poly(vinyl alcohol), DOPA: 3,4-dihydroxy-D-phenylalanine, PLGA: poly(lactic-co-glycolic acid), PEG: poly(ethylene glycol), PVA: poly(vinyl alcohol), PDA: polydopamine. *$P$ < 0.05; **$P$ = 0.001; ***$P$ < 0.001 vs PVA-DOPA6@PLGA-Dex group. All data are Mean ± S.D. $n$ = 3 independent samples per group. Statistics was calculated by one-way ANOVA followed by Tukey's post-test. Exact $P$ values are given in the Source Data file. Source data are provided as a Source data file.



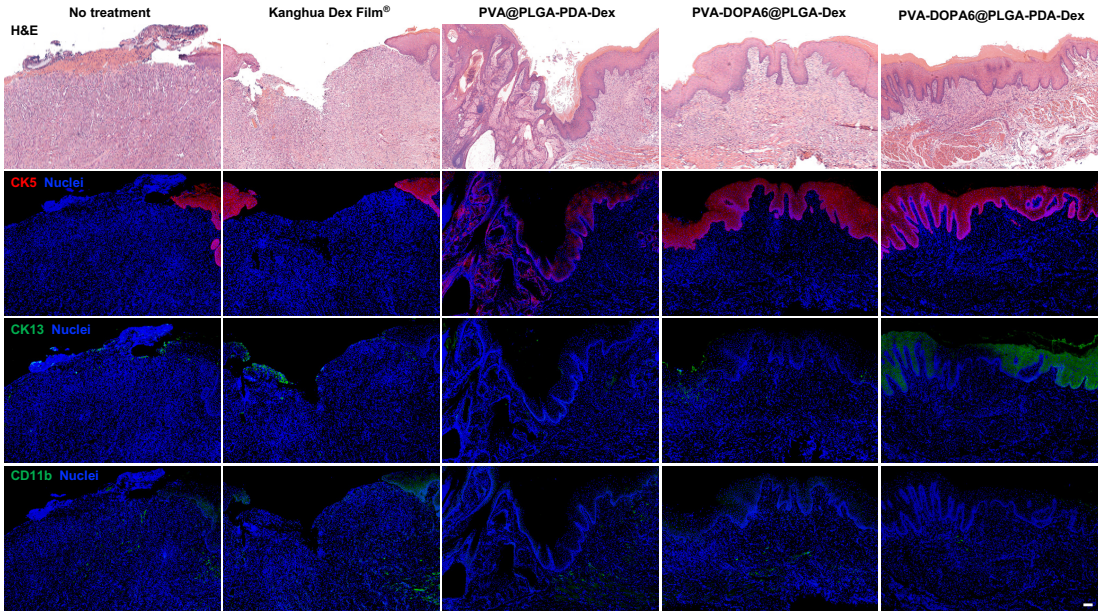

**Supplementary Figure 11.** Gross inspection of buccal mucosa ulcers in SD rats treated with Kanghua Dex Film®, PVA@PLGA-PDA-Dex, PVA-DOPA6@PLGA-Dex, PVA-DOPA6@PLGA-PDA-Dex film, and no treatment at day 0, 2, 5 and 8. Dex: dexamethasone, PVA: poly(vinyl alcohol), DOPA: 3,4-dihydroxy-D-phenylalanine, PLGA: poly(lactic-co-glycolic acid), PDA: polydopamine. $n$ = 3 animals per group. (group 2)



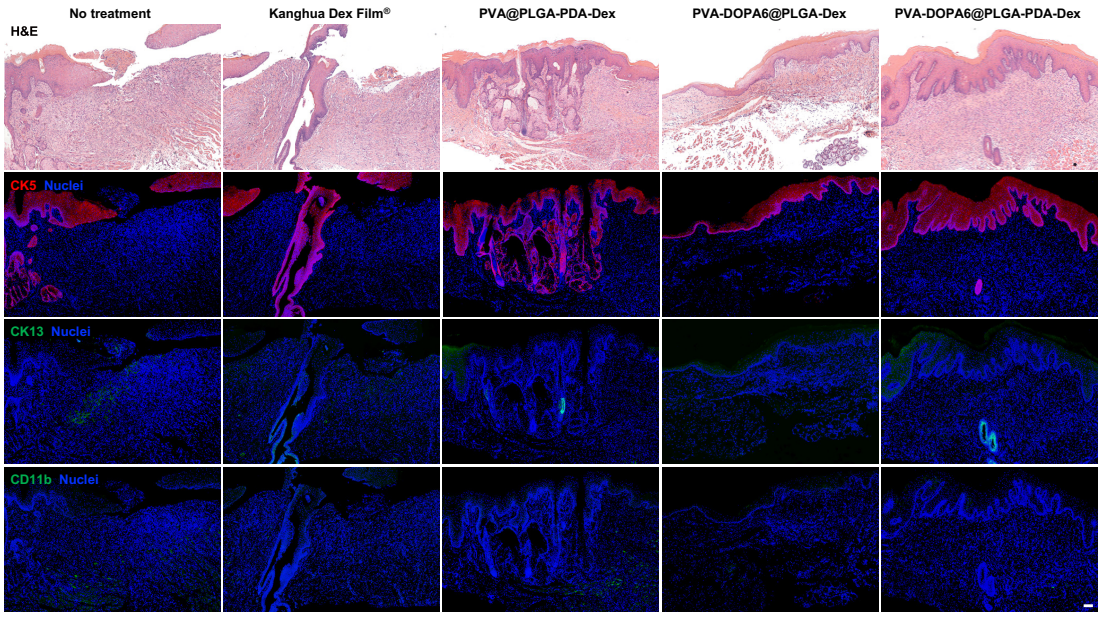

**Supplementary Figure 12.** Gross inspection of buccal mucosa ulcers in SD rats treated with Kanghua Dex Film®, PVA@PLGA-PDA-Dex, PVA-DOPA6@PLGA-Dex, PVA-DOPA6@PLGA-PDA-Dex film, and no treatment at day 0, 2, 5 and 8. Dex: dexamethasone, PVA: poly(vinyl alcohol), DOPA: 3,4-dihydroxy-D-phenylalanine, PLGA: poly(lactic-co-glycolic acid), PDA: polydopamine. $n$ = 3 animals per group. (group 3)




**Supplementary Figure 13.** H&E staining images of cross-section of regenerated oral ulcer in Sprague Dawley rats treated with Kanghua Dex Film®, PVA@PLGA-PDA-Dex, PVA-DOPA6@PLGA-Dex, PVA-DOPA6@PLGA-PDA-Dex film, and no treatment at day 8. Scale bar: 200 μm. $n$ = 3 animals per group. (group 1-3)




**Supplementary Figure 14.** H&E staining and immunohistochemistry staining of anti-keratin5 (CK5, red), anti-keratin13 (CK13, green), and anti-CD11b (CD11b, green) of regenerated oral ulcer at day 8. Nuclei (blue) was stained with DAPI. Scale bar: 100 μm. *n* = 3 animals per group. (group 2)




**Supplementary Figure 15.** H&E staining and immunohistochemistry staining of anti-keratin5 (CK5, red), anti-keratin13 (CK13, green), and anti-CD11b (CD11b, green) of regenerated oral ulcer at day 8. Nuclei (blue) was stained with DAPI. Scale bar: 100 μm. *n* = 3 animals per group. (group 3)

## Supplementary Tables

**Supplementary Table 1.** Degree of substitution of catechol and mass fraction of catechol in PVA-DOPA conjugates calculated from the results of $^{1}$H-NMR and UV-vis spectra.

| Samples | Molar ratio of PVA/DOPA | Degree of substitution of catechol calculated from $^{1}$H-NMR (%) | Mass fraction of catechol calculated from UV-vis (wt%) |
|---|---|---|---|
| PVA-DOPA1 | 6:1 | 4.7 | 16.0 |
| PVA-DOPA2 | 6:2 | 9.3 | 27.9 |
| PVA-DOPA3 | 6:3 | 28.0 | 35.0 |
| PVA-DOPA4 | 6:4 | 41.3 | 42.2 |
| PVA-DOPA5 | 6:5 | 57.3 | 61.5 |
| PVA-DOPA6 | 6:6 | 64.6 | 72.0 |

**Supplementary Table 2.** Thickness and surface pH of different PVA-DOPA films. Data are presented as the means ± standard deviations (SDs). ($n$ = 3 independent samples per group)

| Samples | Thickness（mm） | Surface pH |
|---|---|---|
| PVA | 1.06±0.03 | 6.8±0.06 |
| PVA-DOPA1 | 1.04±0.03 | 6.6±0.08 |
| PVA-DOPA2 | 1.00±0.04 | 6.6±0.05 |
| PVA-DOPA3 | 0.99±0.05 | 6.7±0.05 |
| PVA-DOPA4 | 1.00±0.06 | 6.7±0.08 |
| PVA-DOPA5 | 1.04±0.06 | 6.6±0.08 |
| PVA-DOPA6 | 1.03±0.06 | 6.7±0.12 |

**Supplementary Table 3.** Heat of fusion ($\Delta H_m$) of different PVA-DOPA after interacted with mucin.

| Samples | $\Delta H_m$/Jg$^{-1}$ |
|---|---|
| PVA-Mucin | 4.26 |
| PVA-DOPA1-Mucin | 26.80 |
| PVA-DOPA2-Mucin | 35.69 |
| PVA-DOPA3-Mucin | 46.09 |
| PVA-DOPA4-Mucin | 59.18 |
| PVA-DOPA5-Mucin | 73.58 |
| PVA-DOPA6-Mucin | 123.37 |

**Supplementary Table 4.** Pharmacokinetic parameters of Dex after administrated with different formulations of Dex via oral or buccal route in Sprague Dawley rats. Data are presented as the means ± standard deviations (SDs). ($n$ = 3 animals)

| Samples | $T_{max}$ (h) | $C_{max}$ (ng/ml) | $T_{1/2}$ (h) | $AUC_{0-24}$ (ng/ml*h) |
|---|---|---|---|---|
| Dex (oral) | 1.00 | 13.43±4.12 | 2.1±0.2 | 45.18±11.48 |
| PLGA-PDA-Dex NPs (oral) | 1.00 | 6.94±0.68 | 4.1±2.7 | 64.70±1.52 |
| PVA-DOPA@PLGA-Dex film (buccal) | 5.3±2.3 | 9.01±2.38 | 8.9±2.7 | 78.12±8.23 |
| PVA-DOPA@PLGA-PDA-Dex film (buccal) | 12.0 | 11.62±3.36 | 20.7±0.7 | 160.17±43.86 |

$T_{max}$: time at which $C_{max}$ is attained; $C_{max}$: maximum plasma concentration; $T_{1/2}$: elimination half-life; AUC: area under concentration-time curve.