Supplementary Information

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

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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 μm (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. Scale bar: 2 μm. (b) Fluorescence image of PLGA NPs. Scale bar: 2 μm. (c) AFM image of PLGA NPs. Scale 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 \( \mu \text{m} \). \( n = 3 \) 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                                      | $T_{\text{max}}$ (h) | $C_{\text{max}}$ (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           |

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