Supplementary Materials for

Dual-functional guanosine-based hydrogel: high-efficiency protection in radiation-induced oral mucositis

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Materials and Methods

1. Materials

All chemicals and solvents used in this study were commercially available, of analytical grade, and employed without further purification.Deionised water (DI water) was purified using a Milli-Q purifier. Cells or animals were precisely irradiated using The RS 2000 Biological Irradiator (Rad Source Technologies, USA). The operating parameters were a voltage of 160 kV and a current of 25 mA. Microscopic images were scanned using the SLIDEVIEW VS200 research slide scanner (Olympus, Japan).

Human Oral Keratinocytes (HOK) and NCTC clone929 (L-929) were used in this study. HOK was cultured in Keratinocyte SFM medium (K-SFM; Gibco, USA). L-929 was cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) with 10% fental bovine serum (Gibco, USA). Both KSFM and DMEM medium were supplemented with 100 IU/mL penicillin-streptomycin (Cellgro, USA). They were cultured in a humidified incubator containing 5% CO_2 and 95% air at 37 °C.

Male BALB/c mice (6-8 weeks of age, 22.5-26.8 g) and Sprague-Dawley rats（7 weeks of age, 170-220 g）were purchased from the Animal Center of Sichuan University (China). All animal experiments were approved by the Ethics Committee of West China Hospital of Stomatology Sichuan University (China). The animals were housed in a temperature-controlled environment (22 ± 1°C) with a 12-hour light/dark cycle, where they had unrestricted access to sterilized water and food. They underwent a one-week adaptive feeding period under these conditions before the experiments.

2. Construction of hydrogels

G-PVA hydrogel was synthesized via a one-pot method. First, we precisely weighed G (0.84-2.54 wt%) was dissolved in a specific volume of a mixed solution

containing boric acid (0.05 M), lithium hydroxide (0.05 M), and deionized water. The molar concentration ratios of G, boric acid, and lithium hydroxide were maintained at 1:1:1. This mixture was heated to form a transparent liquid. Afterward, a proportional amount of PVA powder (with a degree of polymerization ranging from 3318 to 4227) was added and stirred until completely dissolved. A certain volume of glycerol (20%) was also added and mixed evenly. After cooling to room temperature, the formation of the hydrogel was confirmed by the vial inversion test, which showed no liquid flow, and photographs of the sample were taken.

G hydrogel was prepared as follows: G (1.98 wt%) was accurately weighed and dissolved in deionized water containing boric acid and lithium hydroxide, also maintaining a 1:1:1 molar ratio. The solution was heated until clear. After cooling to room temperature, the formation of the hydrogel was confirmed using the vial inversion test.

3. Scanning electron microscopy (SEM)

G-PVA hydrogel (1.98 wt%) was lyophilized. The cross-section of the dried gel was mounted onto a silica wafer and coated with gold. The microstructure of the hydrogel was observed using a high-resolution INSPECT F50 (FEI, USA). To confirm the tight adhesion of G-PVA to pig skin tissue, the pig skin with adhered gel was lyophilized, followed by SEM imaging using the same procedure.

4. Atomic force microscopy (AFM)

Diluted G-PVA hydrogel (1.98 wt%) in deionized water was applied to freshly cleaved mica substrates. After the samples dried, they were scanned using an SPM-9700 scanning probe microscope (Shimadzu, Japan).

5. In vitro adhesion tests

Firstly, the adhesive strength of the G-PVA hydrogel on metal surfaces was assessed using the tack mode of a modular smart rotational rheometer MCR302 (Anton Paar, Austria). The hydrogel was uniformly applied to a base using PP25 parallel plates (25 mm in diameter), with a gap set at 0.5 mm or 0.75 mm. The PP25 plate rose at a speed of 100 μm/s. The absolute value of the minimum negative normal force during the rise was recorded as the separation force during the adhesion process.

Additionally, the adhesion strength of G-PVA hydrogel to pig skin was measured in the tensile mode using a universal testing machine (#5565, Instron, USA). The hydrogel, adhered to pig skin over an area of 3 cm by 1 cm, was secured to the device. The G-PVA hydrogel was adhered to pig skin tissue (with a bond length of 3 cm and width of 1 cm) and secured on the device. The crosshead was moved downward, adhering the hydrogel between two pieces of pig skin tissue. After stabilizing the bond, the test was conducted at a speed of 10 mm/min. The peak load (separation force) was determined from the mechanical curve. The adhesive strength of the materials were calculated using the formula:

$$p=F/S$$

where p is the adhesive strength in Pa, F is the force of separation in N, and S is the area of adhesion in m^2.

6. Hydrogel water retention test

A specific quantity of G-PVA hydrogel was placed in a petri dish, and its initial mass (W_f) was recorded. The dish was exposed to room temperature air. At specified time intervals, the mass of the hydrogel (Wt) was recorded. The rate of water loss was calculated using the following equation:

$$\text{Water loss rate (\%)} = (W_f - W_t) / W_t \times 100\%$$

7. Powder X-ray diffractometry (PXRD)

G hydrogel, G-PVA hydrogel, and PVA solution were dehydrated and lyophilized, and their diffraction patterns were obtained at room temperature using an X-ray powder diffractometer (PANalytical-Empyrean, Netherlands).

In the PXRD analysis, the interplanar spacing (d) can be calculated using the following equation:

$$n\lambda=2d\sin(\theta)$$

where n represents the diffraction order (typically set to 1), λ is the wavelength of the X-ray, d is the interplanar spacing, and θ is half of the diffraction angle.

8. Circular dichroism (CD)

As previously described, G gel and G-PVA hydrogel were prepared and diluted with deionized water to a 1:100 ratio. The ellipticity change curves of each sample group in the 200-400 nm wavelength range were recorded at 25°C using a J-810 spectrometer (JASCO, Japan).The scans were conducted at a rate of 50 nm/min with a bandwidth of 2.0 nm. The CD spectra showed the average data from three measurements and were zero-corrected.

9. Fourier transform infrared (FT-IR) spectroscopy

G, boric acid, PVA powder, and lyophilized G-PVA hydrogel were individually combined with potassium bromide powder and compressed into pellets. Infrared spectra were then captured from 400 to 4000 cm^{-1} using a Nicolet 6700 Fourier transform infrared spectrometer (Thermo Electron Corporation, USA).

10. Nuclear magnetic resonance (NMR)

Solutions of $LiB(OH)_4$, G hydrogel, and G-PVA hydrogel, after lyophilization, were dissolved in heavy water (D_2O). After complete dissolution, the solutions were transferred to NMR tubes. After cooling at room temperature, ^{11}B NMR spectra were recorded at 600 MHz using AV II spectrometers (Bruker, Germany).

11. Rheological experiments

Rheological properties of G-PVA hydrogel were analyzed using an MCR302 rheometer, with storage modulus (G') and loss modulus (G'') recorded. G-PVA hydrogel (1.98 wt%) was prepared following standard protocols, heated to a fluid state, and transferred to a rheometer plate preheated to 80°C. PP25 was lowered to a 0.5 mm gap with the hydrogel, and excess sample was scraped off. The temperature was then lowered to 25°C for a time sweep. The strain was fixed at 1%, and the angular frequency ranged from 1 to 100 rad/s for frequency sweeping. The strain ranged from 0.1% to 100% at a fixed angular frequency of 10 rad/s for strain sweeping. In self-healing assessments, the strain was set in two stages (0.1% and 1000%), with a constant angular frequency of 10 rad/s. The high strain phase of 1000% lasted 60 seconds, and the low strain recovery phase of 0.1% lasted 200 seconds, cycled three times. Additionally, viscosity changes of the G-PVA hydrogel with increasing shear rates were also recorded. All tests were repeated three times.

12. Wet adhesion performance

Firstly, in vitro adhesion time of G-PVA hydrogel was assessed using the rotating artificial saliva method, applying the hydrogel and comparative commercial products to pig skin and buccal mucosa (1 cm × 1 cm).These samples were submerged in beakers with artificial saliva, stirred at 450 rpm at 37°C. The residue on the tissues was observed and recorded at specific intervals. Additionally, the rat tongue model was used, where G-PVA hydrogel and Triamcinolone Acetonide Oral Paste (Ningzhizhu®, China), mixed with 1% chlorophyll, were applied to the tongues of rats anesthetized with isoflurane. A burette filled with PBS simulated natural saliva flow at 3 mL/min. A dye group was set up to demonstrate the non-adhesive properties of chlorophyll. Lastly, anesthetized with pentobarbital sodium (40 mg/kg, intraperitoneal), G-PVA hydrogel and controls were applied to rat tongues, and in vivo adhesion time and residue were recorded.

13. Adhesion mechanisms

Mucin from pig stomach was dissolved in deionized water to a final concentration of 1 mg/mL. PVA, G, and G-PVA hydrogel were subsequently dissolved in the mucin solution and thoroughly mixed. First, UV-Vis absorption tests were started by diluting the samples and placing them in the 1 cm quartz cuvettes, using a UV–Vis spectrophotometer (Cary Series; Agilent Technologies, USA). Afterwards, the samples were lyophilized for infrared analysis. Lastly, the zeta potential changes in the G-PVA hydrogel-mucin mixtures over time were measured using a Nanoparticle Zeta Potential Analyzer (Zetasizer Nano ZS; Malvern Panalytical, UK).

14. Atomic absorption spectroscopy (AAS)

6 mL of G-PVA hydrogel was prepared, and 12 mL of artificial saliva was placed on top of the hydrogel. At predetermined time points, 6 mL of the release medium was collected, and an equal volume of artificial saliva was added to replenish the system . This release medium was then treated with nitric acid for digestion, followed by microwave digestion. The release of Li^+ from the G-PVA hydrogel in artificial saliva was detected using the Atomic Absorption Spectroscopy (SpectrAA 220FS; VARIAN, USA). The AAS experiments were repeated three times. The mass of Li^+ released from G-PVA hydrogel at different time points was calculated using the following equation:

$$Mt = C_t \text{ x } V_a + \sum \left(C_{t-1} \times V_b\right)$$

Where C_t is the concentration of Li^+ recorded at time. V_a is the total volume, and V_b is the volume removed from the system each time.

15. Antioxidant properties

The $ABTS^+$ radical scavenging capabilities of G-PVA hydrogel, G hydrogel, and PVA solution were initially evaluated using the Total Antioxidant Capacity Assay Kit (Beyotime, China). Under the influence of antioxidants, ABTS+ is reduced to colorless ABTS, and the absorbance of $ABTS^+$ at 734 nm was measured to determine and calculate the total antioxidant capacity of the samples. Trolox standard solutions from 0 to 0.9 mM were used to establish a standard curve. The Trolox equivalent antioxidant capacity (TEAC) values were calculated as the final index.

Additionally, Electron Paramagnetic Resonance (EPR) was used to detect the generation of free radicals after X-ray irradiation. HOK cells were plated in six-well plates (2×10^5 cells/well), irradiated at 12 Gy, while the control group was not irradiated. Supernatants were collected, and EPR spectra were captured using the EMXplus X-band EPR Spectrometer A300 (Bruker, USA), with DMPO (5,5-dimethyl-1-pyrroline N-oxide) serving as the radical scavenger. Finally, the scavenging abilities of G-PVA hydrogel and its components against superoxide anion and hydroxyl radicals were assessed using the Superoxide Anion Assay Kit (Grace Biotechnology, China) and Hydroxyl Radical Scavenging Assay Kit (Yuanye Bio-Technology, China), respectively.

16. ROS scavenging assay

The levels of reactive oxygen species (ROS) in HOK cells after X-ray exposure were assessed using the Reactive Oxygen Species Assay Kit (Beyotime, China). Cells were incubated with the 2',7'-dichlorofluorescin diacetate (DCFH-DA, 1/1000) probe in the dark for 30 minutes. After washing off the unentered probes, ROS levels were assessed by the intensity of green fluorescence using a fluorescence microscope.

Additionally, HOK cells were seeded in six-well plates (2×10^5 cells/well). After 6 Gy of irradiation, cells were again incubated with the DCFH-DA probe for 30 minutes and analyzed with a flow cytometer (NovoExpress; Agilent Technologies,

USA) using an excitation wavelength of 488 nm. FlowJo software was used for data analysis.

17. Mitochondrial membrane potential (MMP) detection

HOK cells were seeded in confocal culture dishes (2.5×10^5 cells/well) and irradiated with 6 Gy after 24 hours. One hour post-irradiation, the mitochondrial membrane potential of the cells was assessed using a Mitochondrial membrane potential assay kit with JC-1 (Beyotime, China). The fluorescence changes were observed with JC-1 staining solution incubated with HOK cells at 37°C for 25 minutes. We captured the fluorescence images using a confocal microscope (N-SIM; Nikon, Tokyo, Japan). JC-1 monomers were excited at 488 nm and aggregates at 561 nm.

18. Western blot

Initially, the expression levels of γH2AX protein in HOK cells after 6 Gy irradiation at various time points (0, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 24 h) were quantified using protein blotting to reflect DNA damage. The expression levels of γ-H2AX protein in HOK cells treated with G-PVA hydrogel or Trolox after irradiation were also quantified.

These experiments were conducted as follows: HOK cells were seeded in six-well plates (2×10^5 cells/well), and proteins were harvested using RIPA buffer with protease inhibitors. Protein concentrations were determined by a BCA protein assay kit (Thermo Scientific, USA). Proteins were then subjected to 12% SDS-PAGE at 120 V for one hour, transferred onto PVDF membranes using a semi-dry transfer system (Bio-Rad Laboratories) at 300 mA for 1 h. The PVDF membrane was blocked at room temperature with 5% non-fat milk in TBST for one hour. The membrane strips carrying the respective proteins were incubated in primary antibody solutions

overnight at 4°C. Primary antibodies were Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (1/1000, CST, USA) and alpha Tubulin Rabbit mAb (1/2000, zen-bio, China). After washing off the primary antibodies, the membranes were incubated with IgG(H+L)HRP-labeled Goat Anti-Rabbit secondary antibody (ZB-2306; 1/4000, ZSGB-BIO, China) at 37°C for one hour. Bands were visualized using an Amersham Imager 600 chemiluminescence imaging system (GE Healthcare, USA).

19. Immunofluorescence

Sterile slides were placed in 24-well plates, and HOK cells were seeded at 1 × 10^5 cells per well. After X-ray irradiation, cells were fixed with 4% paraformaldehyde (Biosharp, China) for 15 minutes post one hour irradiation (6 Gy). After three washes with PBS, cells were treated with 0.5% Triton-X100 for 10 minutes at room temperature, followed by blocking with 5% BSA solution for one hour. After blocking, cells were incubated with Phospho-Histone H2A.X (Ser139) Mouse mAb (sc-517348; 1/500, Santa Cruz Biotechnology, USA) overnight at 4°C. After washing with PBS and light protection, cells were incubated with a Goat anti-Mouse IgG (H+L) Secondary Antibody, Cy3 (#30010; 1:400, Thermo Scientific, USA) for one hour and DAPI Staining Reagent (Servicebio, China) for 5 minutes at room temperature. Slides were dried and mounted using Prolong live antifade reagent (Thermo Scientific, USA) and subsequently scanned with SLIDEVIEW VS200 (Olympus, Japan).

20. EDU assay

HOK cells were seeded in 12-well plates (2×10^5 cells/well) and irradiated (6 Gy) 12 hours later. One hour post-irradiation, cell proliferation in the various groups was assessed using the BeyoClick EdU Cell Proliferation Kit with Alexa Fluor 594 (Beyotime, China). EDU working solution (20 μM) was added to the cell culture medium and incubated for four hours, followed by fixing with 4% paraformaldehyde for 15 minutes. After washing with PBS, cells were treated with 0.3% Triton X-100

(Beyotime, China) for 15 minutes. After another wash, Click reaction solution was added to the cells and incubated in the dark at room temperature for 30 minutes. Finally, we used Hoechst 33342 for nuclear staining. Fluorescence microscopy was used to observe and record the intensity of red fluorescence, indicative of proliferating cells.

21. Quantitative real-time PCR (qPCR)

HOK cells were seeded into six-well platess (2×10^5 cells/well) and were irradiated with 6 Gy of X-ray. Total RNA from the treated HOK cells was extracted using the Cell Total RNA Isolation Kit (Foregene, China). The RNA was reverse transcribed into cDNA using an RT-PCR system (Bio-Rad, USA). The qPCR process was conducted using ChamQ Universal SYBR qPCR Master Mix (Vazyme, China) on a real-time PCR system (H7900; Applied Biosystems, USA). The cycling conditions were: 95°C for 10 minutes (1 cycle); 95°C for 15 seconds and 60°C for 60 seconds (40 cycles); 95°C for 15 seconds, 60°C for 15 seconds, and 95°C for 15 seconds (1 cycle). The expressions of IL-6, IL-18, TNF-α, CXCL9, TGF-β, IFN-α, and IL-1β were quantified in the cells. The primer sequences are provided in the Table S1.

22. Biocompatibility

Firstly, the cell viability of HOK and L929 cells treated with G-PVA hydrogel was assessed using the CCK-8 assay. A hydrogel solution (0.05 g/mL) was prepared and diluted with KSFM and DMEM medium. HOK and L929 cells were then seeded in 96-well plates ($2.5x10^4$ cells/well) containing the hydrogel solution, with normal media serving as the control. After 48 hours, the media was replaced with a CCK-8 solution mixed with normal media (1:9), and the absorbance at 450 nm was measured after 2 hours using a Varioskan LUX multimode microplate reader (Thermo Scientific, USA). The relative cell viability (%) was calculated using the equation:

$$\text{cell viability} = (OD_{treat} - OD_{blank})/(OD_{control} - OD_{blank}) \times 100\%$$

Secondly, HOK and L929 cells seeded in 24-well plates (0.85×10^4 cells/well) were incubated with the diluted hydrogel solution for 48 hours, followed by staining with the Calcein/PI Live/Dead Viability/Cytotoxicity Assay Kit (Beyotime, China). Cell survival rates after treatment with G-PVA hydrogel were observed and calculated under a fluorescence microscope. Lastly, G-PVA hydrogel was consistently applied to the tongues of mice over a week, followed by histological examination of their vital organs using H&E staining.

23. Efficacy in a RIOM mouse model

BALB/c mice (total 18), after acclimating for one week, were randomly divided into three groups (N=6): a control group (non-irradiated), an irradiated group, and an irradiated group treated with G-PVA hydrogel. To control for the impact of leaving their housing on the mice, we also took the control group to the radiation room but did not expose them to radiation. In the treated group, G-PVA hydrogel was uniformly applied to the anterior dorsal surface of the mouse tongues before irradiation. To prevent traumatic ulcers, we avoid overstretching the tongue, which however limits the area of application. Mice were then secured in the irradiator, protected below the neck with a custom-made lead shield. During the modeling period, the body weight of the mice in each group was recorded at fixed times daily. Irradiation was administered for five days, followed by a two-day break, continuing over two weeks, with an additional dose of 12 Gy given on the first day of the third week. The mice were euthanized on day 21, and tissues were collected. Tongues were stained with 0.5% toluidine blue to highlight ulcer areas. If ulcerative lesions appear in the irradiated and treated groups after staining, it indicates successful establishment of the RIOM mouse model, and mice without any tongue damage will subsequently be excluded. If lesions appear in the control group, these will also be excluded. Tissues were fixed in 4% paraformaldehyde for 48 hours, dehydrated, embedded in paraffin, and cut into 4-

micron sections for H&E staining, TUNEL assay, and histopathological analysis. Only the experiment operators were aware of the groupings, while those involved in data collection and analysis were not informed of the specific animal group assignments. Images were scanned using the SLIDEVIEW VS200 research slide scanner (Olympus, Japan).

24. TUNEL staining

Cell apoptosis in the mucosal tissues of the mice was assessed using the In Situ Cell Death Detection Kit, Fluorescein (Roche, Switzerland). Tissue sections were dewaxed, hydrated, and fixed before application of the TUNEL mixture. The sections were incubated at 37°C in the dark for one hour. Apoptotic cells were identified by their green fluorescence in the TUNEL assay.

25. Immunohistochemistry

Immunohistochemistry was used to assess the expression of pro-inflammatory markers in tongue tissues from different groups after irradiation. After dewaxing, rehydration, antigen retrieval, and blocking, primary antibodies were applied to the sections and incubated overnight at 4°C. The primary antibodies used were Anti-IL-6 antibody (#9324; 1 μg/mL, Abcam, UK) and Anti-IL-1β antibody (#283818; 1/500, Abcam, UK). After the primary antibodies removal, two-step universal detection kits (PV-6001/6002; ZSGB-BIO, China) were used to incubate with secondary antibodies at 37°C for 1 hour. Subsequent processes included DAB staining, hematoxylin staining, and sealing with neutral balsam. The mean percentages of IL-6 and IL-1β positive cells were calculated for each group.

26. Statistical analysis

All quantitative experiments were repeated at least three times.Unless otherwise noted, data are presented as mean ± S.E.M.(standard error of the mean). Data analysis

was conducted using GraphPad Prism 9.0 software. Two samples were analyzed using Student's test and multiple sample comparisons were performed using one-way ANOVA with Tukey's multiple comparisons test. Results were considered statistically significant when $p < 0.05$. Significance levels were marked as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$P < 0.0001$.

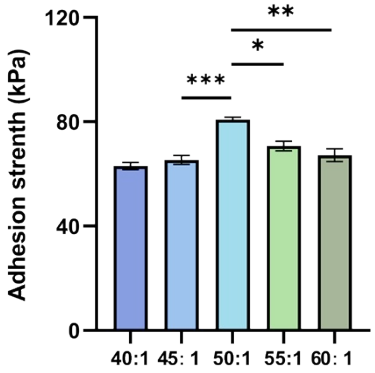


Figure S1. The adhesion strengths of hydrogels at different concentrations of PVA was measured with rheology adhesion tests (guanosine: 0.07 M). *$P<0.05$, **$p < 0.01$, ***$P < 0.001$

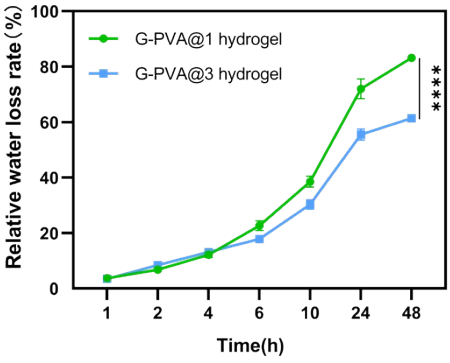
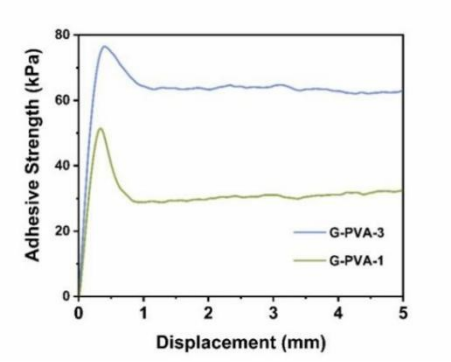
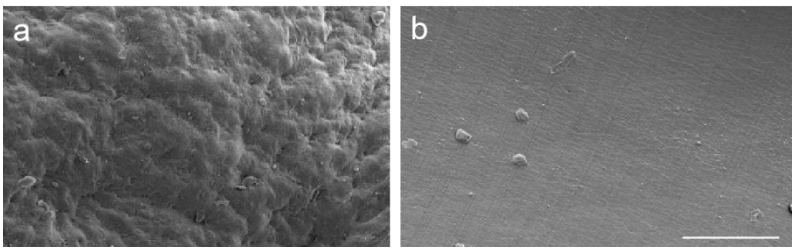


Figure S2. Relative water loss rate of G-PVA@1 hydrogel and G-PVA@3 hydrogel over 48h. ****$P < 0.0001$



340

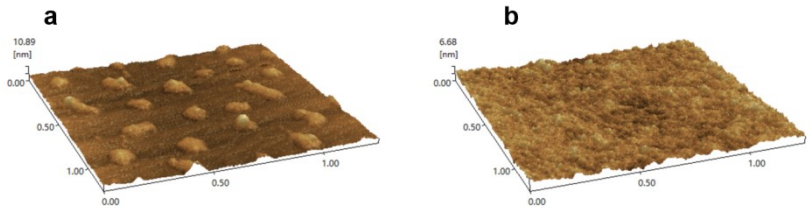
341 **Figure S3.** The adhesion strength-displacement curve of G-PVA@1/3 hydrogel.



342

343 **Figure S4.** Scanning electron microscopy images of G-PVA@1/3 hydrogel. a: G-

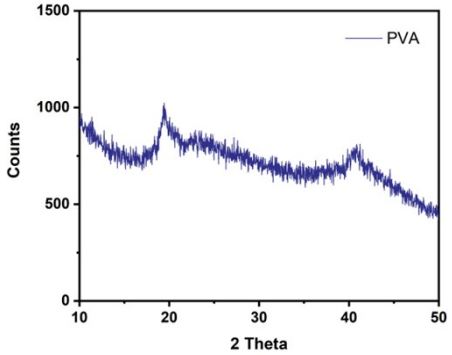
344 PVA@1; b: G-PVA@3. Scale bar: 200 μm.



345

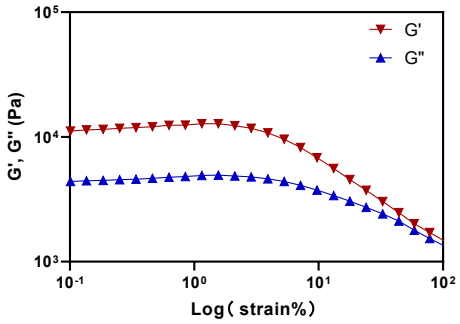
346 **Figure S5.** Atomic force microscopy images of G-PVA@1/3 hydrogel. Scale bar: 500

347 nm.



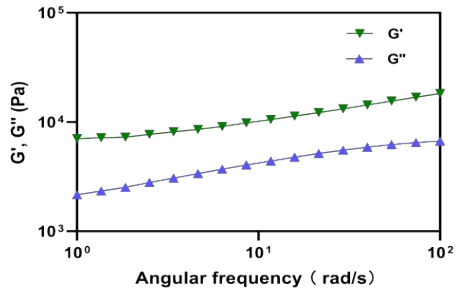
348

349 **Figure S6.** Powder X-ray diffractometry spectrum of the PVA powder.



350

351 **Figure S7.** G' and G" values were recorded when the G-PVA hydrogel was subjected
352 to strain changes from 0.1% to 100%.

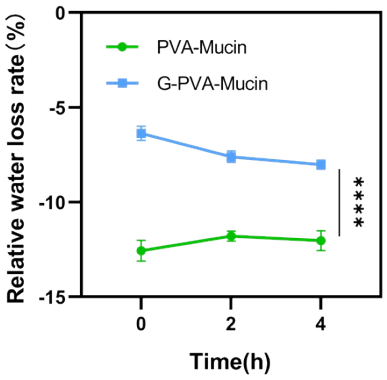


353

354 **Figure S8.** Frequency scans of G-PVA hydrogel at a constant strain of 100%.

355

356 **Figure S9.** The films were removed by water within 15 minutes, utilizing the
357 oscillation method. a: compound chlorhexidine and dexamethasone pellicle; b:
358 propolis oral film.



359

360 **Figure S10.** Variation in the zeta potential of PVA-mucin and G-PVA-mucin
361 mixtures. As the mixing time increased, the zeta potential of the G-PVA and mucin

mixture shifted progressively to more negative values. ****P < 0.0001

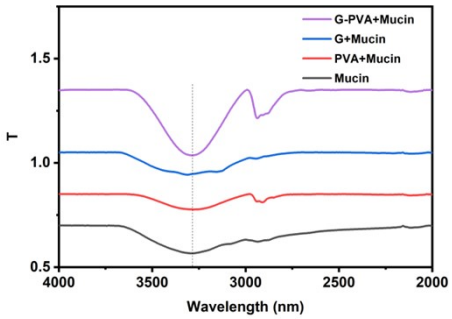


Figure S11. Fourier transform infrared spectroscopy of G-PVA-mucin, G-mucin, PVA-mucin mixtures, and mucin.

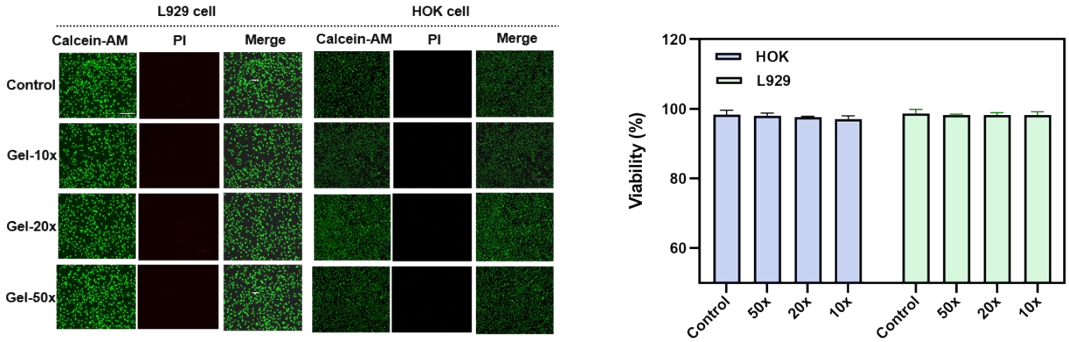


Figure S12. Live/Dead staining of HOK cells and L929 cells cultured in G-PVA hydrogel for 48h. Scale bar: 40 μm.

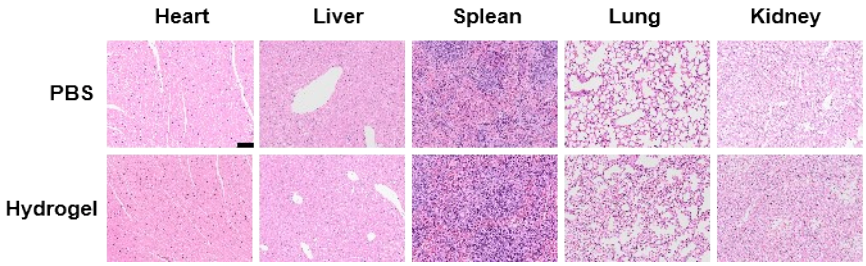
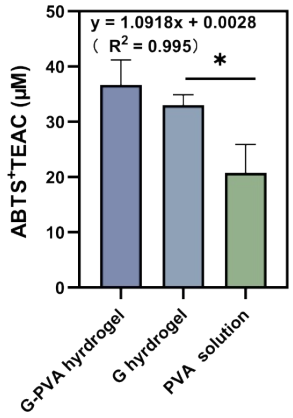
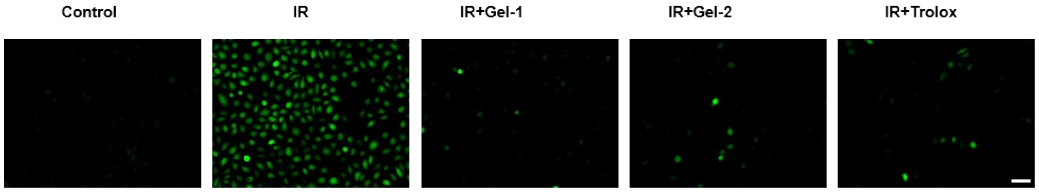


Figure S13. H&E staining of major organs from mice 7 days after smearing their tongues with G-PVA hydrogel or PBS. Scale bar: 100 μm.



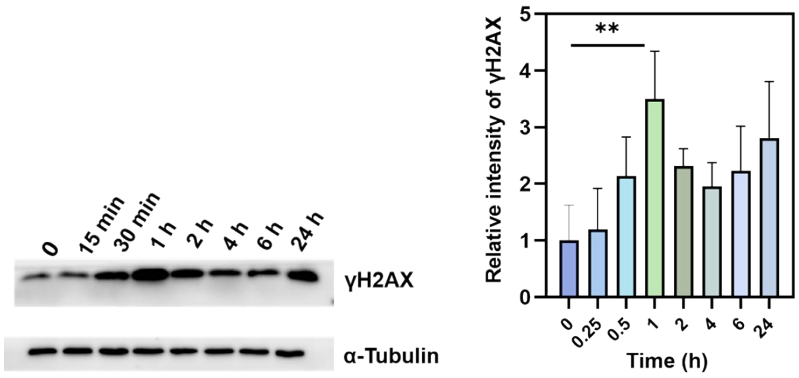
373

374 **Figure S14.** The total antioxidant capacity of the G-PVA hydrogel was assessed using
375 the $ABTS^{+}$ radical scavenging assay, and a standard curve was established using a
376 Trolox standard solution. *P＜0.05



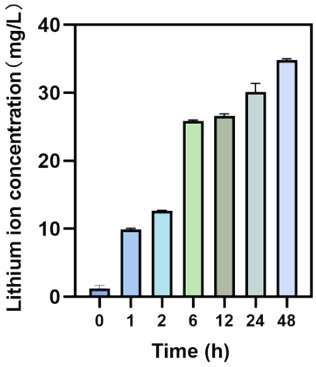
377

378 **Figure S15.** Reactive oxygen species (ROS) levels in HOK cells, stained with 2,7-
379 dichlorodihydrofluorescein diacetate (DCFH-DA), were monitored post-irradiation.
380 Scale bar: 20 μm.



381

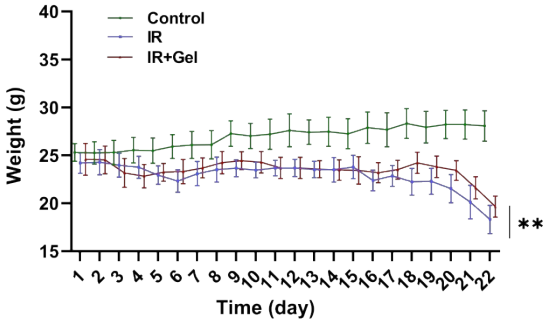
382 **Figure S16.** Western blot analysis quantitatively assessed the expression levels of γ-
383 H2AX at various time points after X-ray irradiation. **P < 0.01



384

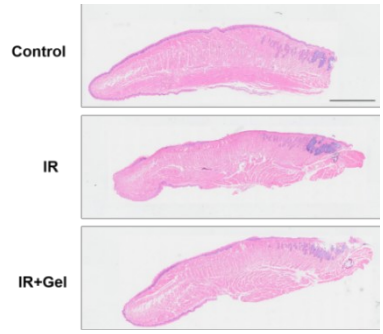
385 **Figure S17.** A certain concentration of Li^+ions can be released from the G-PVA

386 hydrogel into artificial saliva within 48 h.



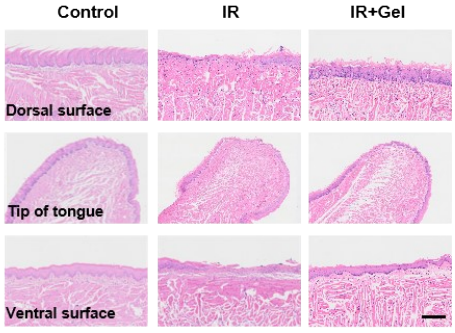
387

388 **Figure S18.** Daily weight recordings of mice.



389

390 **Figure S19.** H&E-stained images of the entire tongues. Scale bar: 2 mm.



391

392 **Figure S20.** H&E-stained images of tongue epidermis. Scale bar: 200 μm.

Table S1. Primers for quantitative real-time PCR.

Primer	Sequence (5' to 3')
IL-6-F	*ACTCACCTCTTCAGAACGAATTG*
IL-6-R	*CCATCTTTGGAAGGTTCAGGTTG*
IL-18-F	*TCTTCATTGACCAAGGAAATCGG*
IL-18-R	*TCCGGGGTGCATTATCTCTAC*
TNF-α-F	*CCTCTCTCTAATCAGCCCTCTG*
TNF-α-R	*GAGGACCTGGGAGTAGATGAG*
CXCL9-F	*CCAGTAGTGAGAAAGGGTCGC*
CXCL9-R	*AGGGCTTGGGGCAAATTGTT*
TGF-β-F	*GGCCAGATCCTGTCCAAGC*
TGF-β-R	*GTGGGTTTCCACCATTAGCAC*
IFN-α-F	*TCGGTAACTGACTTGAATGTCCA*
IFN-α-R	*TCGCTTCCCTGTTTTAGCTGC*
IL-1β-F	*TTCGACACATGGGATAACGAGG*
IL-1β-R	*TTTTTGCTGTGAGTCCCGGAG*

Table S2. The oral mucositis index was statistically evaluated using Parkin's scoring system. The final score is the sum of the edema score and the other score.

Score	Condition	Description
0.5	Edema	Doubtful if any swelling
1		Slight but definite swelling
2		Severe swelling
0.5	Other	Doubtful if abnormally pink
1		Slight but definite reddening
2		Severe reddening
3		Focal desquamation
4		Exudate or crusting involving about half of lip area
5		Exudate or crusting involving more than half of lip area