Electronic Supplementary Material (ESI) for Biomaterials Science.

Mucoadhesive chitosan-methylcellulose oral patches for the treatment of local mouth bacterial infections

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Supporting Info 1: Artificial saliva preparation

Artificial saliva was prepared according to the previous literature [1,2]. Briefly, the reagents reported in Table S 1 were dissolved in deionized water (dH_2O) at 37 °C. If necessary, the final pH was adjusted to 6.8 through the addition of 0.1 M HCl solution.

Table S 1 - Artificial saliva composition

Reagent	**Molecular Weight (g/mol)**	**Concentration (mg/L)**
Calcium chloride ($CaCl_2$)	110.98	172.0
Potassium chloride (KCl)	74.55	963.9
Potassium dihydrogen phosphate (KH_2PO_4)	136.09	654.5
Potassium thiocyanate (KSCN)	97.18	189.2
Sodium chloride (NaCl)	58.44	125.6
Sodium bicarbonate ($NaHCO_3$)	84.01	630.8
Sodium sulphate (Na_2SO_4)	142.04	336.5
Ammonium chloride (NH_4Cl)	53.49	178.0
Urea ($CO(NH_2)_2$)	60.06	200.0

Supporting Info 2: GS calibration curve

A standard calibration curve was first obtained for GS in PBS. GS was prepared at different concentrations ranging from 0.1 to 1,000 µg/mL. Next, 80 µL of GS in PBS solution at different concentrations were taken from each well, mixed with 40 µL of the TNBSA solution (1:500 in 0.1 M $NaHCO_3$), and incubated at 37 °C for 2 h in a 96-well culture plate. The absorbance was measured at λ = 364 nm with a Synergy H1 spectrophotometer (BioTek, Italy) and plotted, after blank (fresh PBS mixed with the TNBSA solution) subtraction, as a function of GS concentration. Tests were carried out in triplicate (n = 3). A linear relationship (R^2 ≥ 0.99) between the absorbance and the GS concentration was detected in the 0.1 – 100 µg/mL range (Figure S 1). The resulting curve was used to quantify the GS in CS-MC samples.

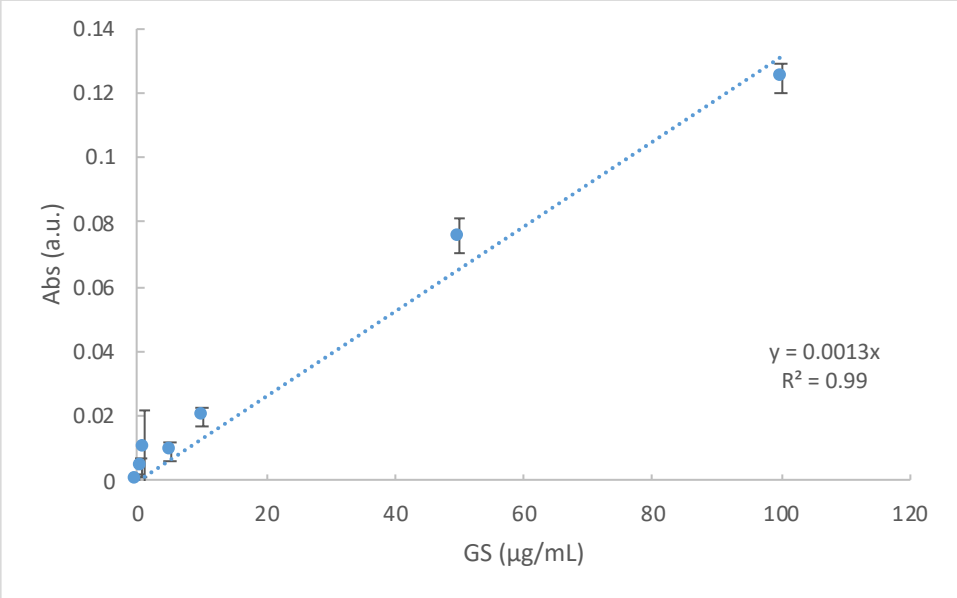

Figure S 1 – Calibration curve obtained for GS in the 0.1 – 100 µg/mL range.

Supporting

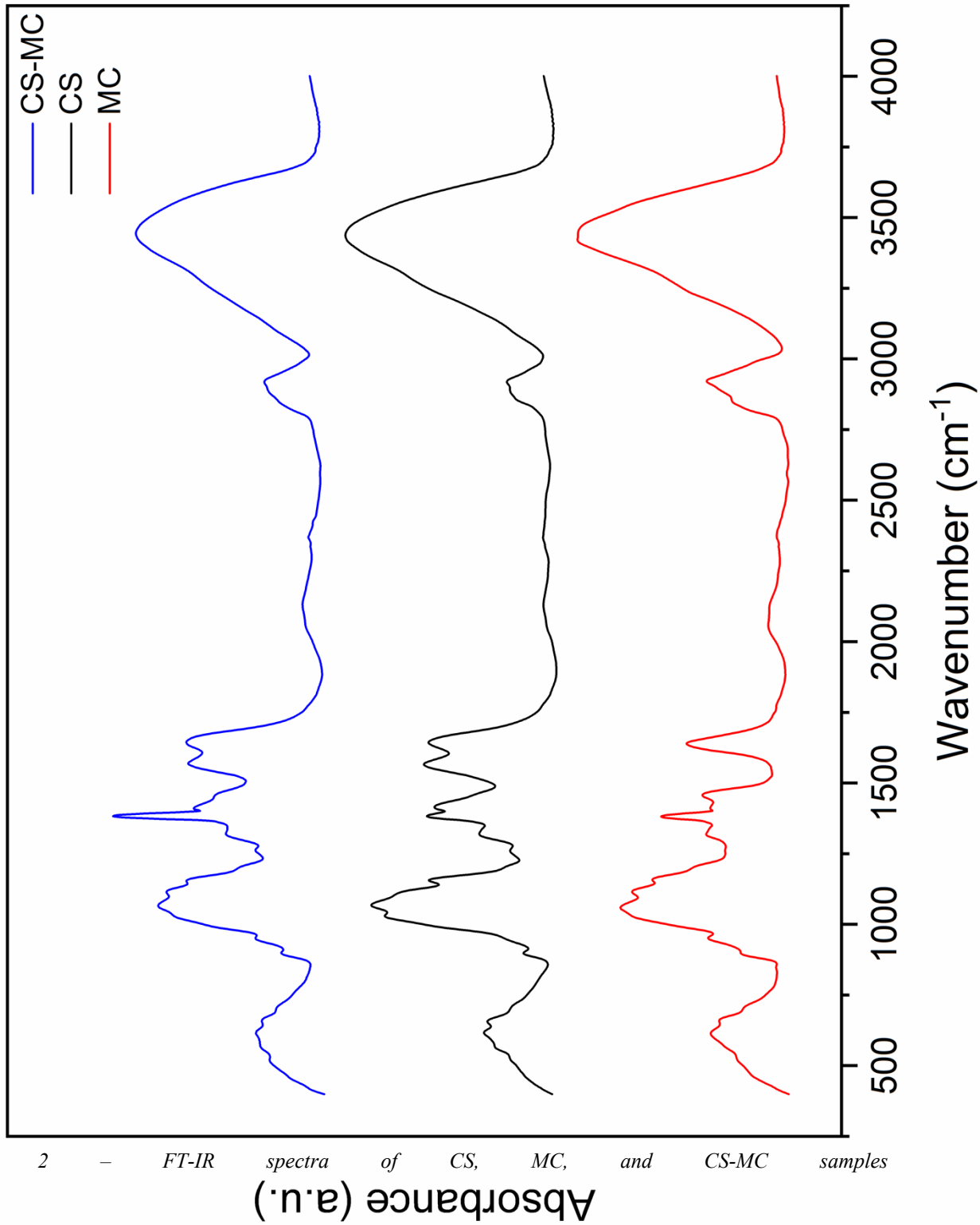

Figure S 2 – FT-IR spectra of CS, MC, and CS-MC samples

Supporting Info 4: Ninhydrin assay for CS quantification

CS fraction in CS-MC patches was assessed by ninhydrin assay [3], determining the primary amino groups of CS. Two solutions were prepared as follows:

Solution I: 1.05 g of citric acid, 10 mL of a 1.0 M aqueous NaOH solution, and 0.04 g $SnCl_2 \cdot 2H_2O$ were mixed, then deionized water (dH_2O) was added until 25 mL;

Solution II: 1 g of ninhydrin was added to 25 mL ethylene glycol monomethyl ether.

Solution I and II were stirred for 45 min to give the final solution (NHN solution), which was stored in the dark until use. For the assay, CS and CS-MC specimens were soaked in 0.25 M acetic acid (AA) (1 mg sample: 670 µL AA) and allowed to dissolve overnight (ON) at room temperature (r.t.). 900 µL of NHN solution was next added, and the samples were heated at 100 °C for 30 min. The solution was then cooled down in an ice bath, then diluted with 2 mL of a mixture of 50 % isopropanol in dH_2O. A blank was also prepared using water, 0.25 M AA, and the NHN solution. The absorbance of the solutions was read at $\lambda = 570$ nm with a Synergy H1 spectrophotometer.

CS fraction in CS-MC samples was calculated as follows (Eq. S0):

$$CS\ (\%) = \left[\frac{RFU_{CS-MC} - RFU_{BLK}}{RFU_{CS} - RFU_{BLK}}\right] \times 100 \qquad \text{(S0)}$$

where RFU_{CS-MC}, RFU_{CS}, and RFU_{BLK} are the fluorescence of CS-MC, CS, and blank solutions, respectively.

The mean CS content in the CS-MC patches was 66.2 %, in accordance with the results of the dissolution tests (please refer to the main text).

References

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2. Gal, J. About a synthetic saliva for in vitro studies. *Talanta* **2001**, *53*, 1103–1115.
3. Cui, L.; Jia, J.; Guo, Y.; Liu, Y.; Zhu, P. Preparation and characterization of IPN hydrogels composed of chitosan and gelatin cross-linked by genipin. *Carbohydr. Polym.* **2014**, *99*, 31–38.