Coupling PEG-LZM Polymer Network with Polyphenol Yields Suturable Biohydrogel for Tissue Patching

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Table of content

1. Experimental section

1.1. Fourier Transform Infrared (FTIR) Spectra. The freeze dried PEG-LZM-TA hydrogel was grinded into powder. TA powder was used as the control sample. Fourier transform infrared (FT-IR) spectra of the two samples were obtained using a TENSOR-27 spectrometer (Bruker, German) scanned in the frequency range of 4000-400 cm^{-1} after the progress of the potassium bromide tableting.

1.2. Solution mixing experiment. Firstly, TA and lysozyme (LZM) were separately dissolved in the water at a concentration of 150 mg mL^{-1}. Then, these two solutions were mixed with each other (V/V=1:1), and the change of solution before and after mixing was recorded. Next, TA was firstly dissolved in the DMSO (50 mg mL^{-1}), and then LZM was dissolved by the TA solution a concentration of 150 mg mL^{-1}. Finally, 50% (V/V) water was added to the mixed DMSO solution of TA and LZM，and the change of solution before and after mixing was observed and recorded.

1.3. Cell Cultured and Treatment. THP-1 cells (TIB-202, ATCC, USA) was cultured in medium and was used to evaluate the anti-inflammatory properties of different hydrogels. First, the sterile lysozyme solution reacted with *tetra*-PEG-NHS solution to form hydrogel in a 24 well plate (400 μL per cell, 12 wt %). Then the hydrogel was freeze-dried, and sterile TA solution or PBS was added on the surfaces, after 24h, the unbound TA was washed with sterile PBS. Next, the cells were seeded on the surfaces of these hydrogels at a density of 1.5×10^6 cells per well. After 24 h incubation, 1000 ng mL^{-1} of lipopolysaccharide (LPS) was added to each well. Cells without any treatment acted as controls. After incubation for 4 h, the cells were collected for Real-time RT-PCR.

1.4. Real-time quantitative PCR. Levels of IL-10, IL-13, RelB, HIF-1α, p65 and TNF-α mRNA were detected by RT-qPCR using SYBR Green qPCR Master Mix kit instructions (638320, TakaRa, Beijing, China) in a ABI 7500 fluorescence

quantitative PCR instrument (Applied Biosystems, Maryland, USA), and GAPDH was used as loading control[1, 2]. In addition, the detail of RT-qPCR primer sequence was shown in Table 1.

Table.1 PCR primer

Gene	Primer (5'-3')
IL-10	F:ACGGCGCTGTCATCGATT
	R:TTGGAGCTTATTAAAGGCATTCTTC
IL-13	F:CCTCATGGCGCTTTTGTTGAC
	R:TCTGGTTCTGGGTGATGTTGA
RelB	F:TTTTAACAACCTGGGCATCC
	R:CGCAGCTCTGATGTGTTTGT
HIF-1α	F:ATCCATGTGACCATGAGGAAATG
	R:TCGGCTAGTTAGGGTACACTTC
P65	F:GTGGGGACTACGACCTGAATG
	R:GGGGCACGATTGTCAAAGATG
TNF-α	F:GAGGCCAAGCCCTGGTATG
	R:CGGGCCGATTGATCTCAGC
GAPDH	F:GAAGGTGAAGGTCGG AGTC
	R:GAAGATGGTGATGGGATTTC

2. Supplementary Fig.s

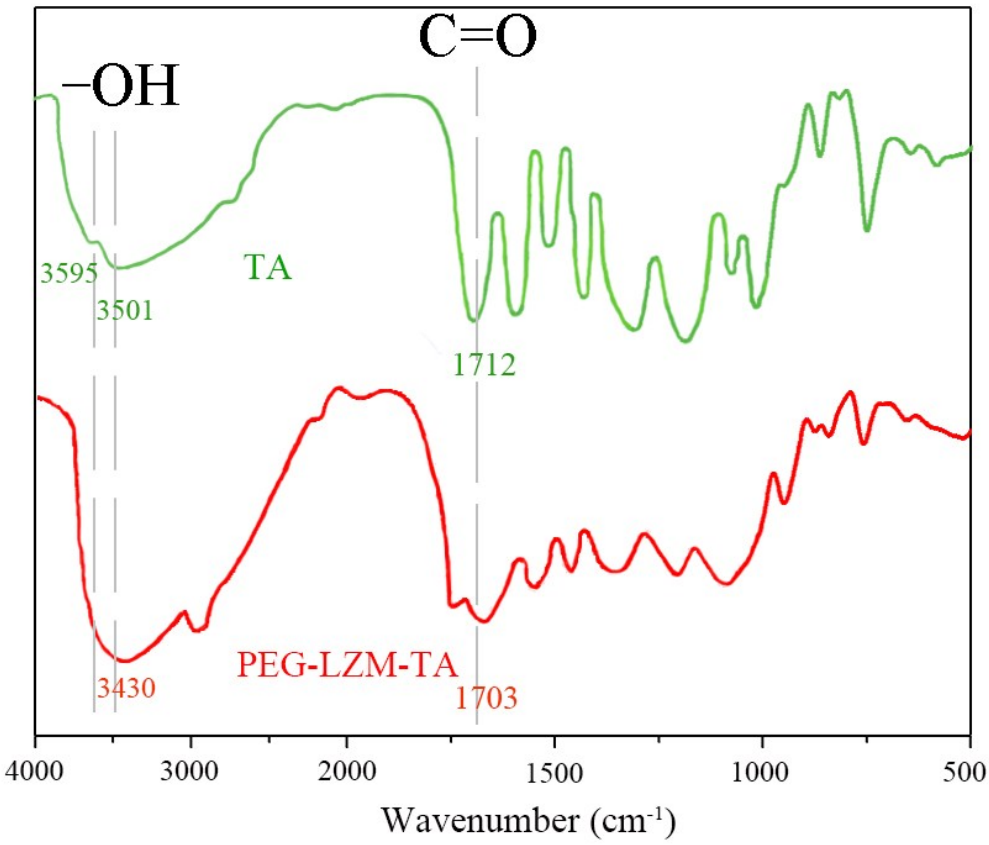

Fig. S1 FTIR spectra of TA molecules and PEG-LZM-TA hydrogel.

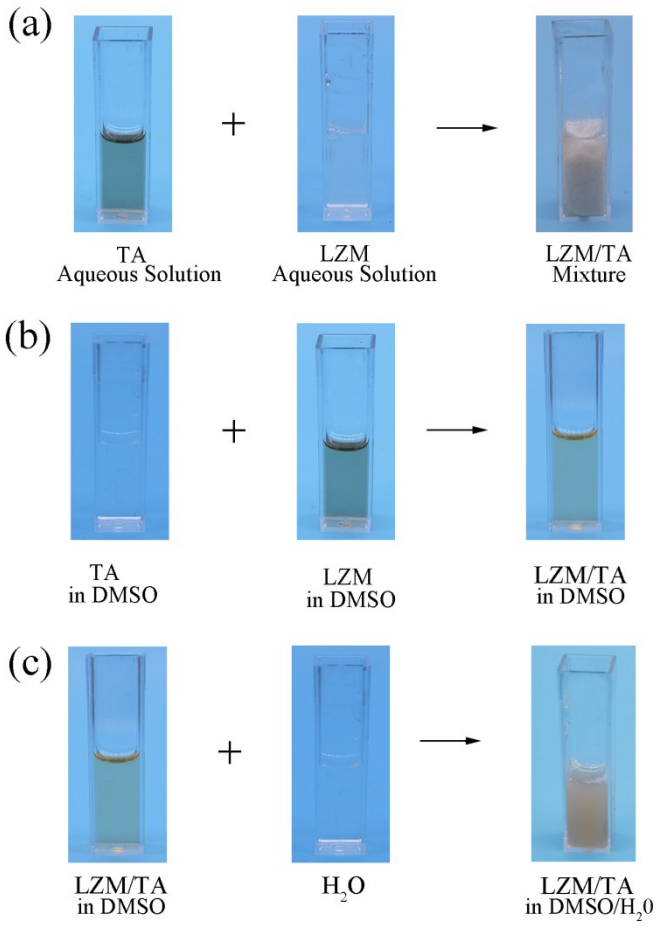

Fig. S2 The experiment of solution mixing between TA and LZM. (a) TA aqueous solution was added to LZM aqueous solution. (b) TA and LZM were dissolved in the DMSO to get a mixed solution, (c) an then water was added to this solution.

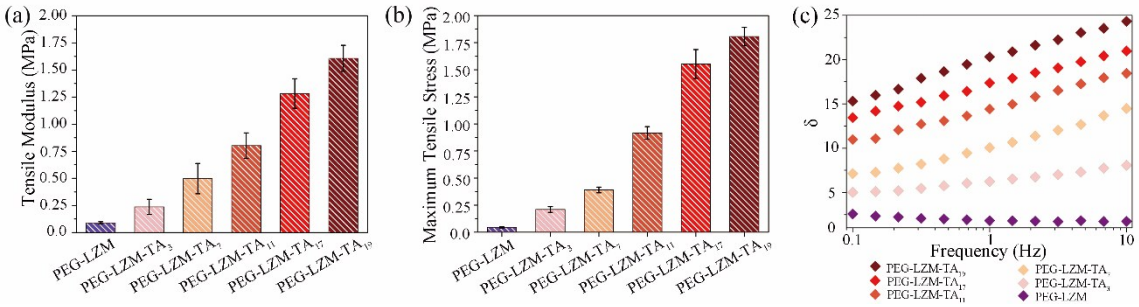

Fig. S3 (a) Initial tensile modulus, (b) maximum tensile strength and (c) loss angle (rheological analysis) of PEG-LZM-TA hydrogels with different TA contents.

3. References

1. T. F. Liu, V. Vachharajani, P. Millet, M. S. Bharadwaj, A. J. Molina and C. E. McCall, *J. Biol. Chem.*, 2015, **290**, 396-408.
2. T. F. Liu, V. T. Vachharajani, B. K. Yoza and C. E. McCall, *J. Biol. Chem.*, 2012, **287**, 25758-25769.