Supplementary Material

STS load PCL- MECM based hydrogel hybrid scaffold promote meniscal regeneration via modulating macrophage phenotype polarization

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Methods

Preparation of inner MECM and outer MECM

Pig knee meniscus (n = 40) was purchased from abattoir. The meniscus was placed in sterile phosphate buffered saline (PBS) at pH 7.6. The meniscus is cut into the medial and lateral parts（Figure S1）. Cut the inner and outer of the meniscus into pieces about 1 mm in size. The fragments were decellularized using the method described in the previous literature[1]. Briefly, the tissue sections were placed into a hypotonic Tris-HCL buffer solution (10 mM Tris–HCL, pH 8.0) and 6 cycles of freezing (at-80℃) and thawing (at 37℃) were conducted. The tissue sections slurry was homogenized and treated with 0.25% trypsin (Gibco, USA) in PBS for 24 h at 37℃ with vigorous agitation. The trypsin solution was replaced with the fresh one at every 4 h. Trypsinized tissue sections slurry was washed with a hypertonic buffer solution (1.5 M NaCl in 50 mM Tris-HCL, pH 7.6) and treated with nuclease solution (50 U ml-1 DNAse (Sigma) and 1 U ml-1 RNAse A (Sigma) in 10 mM Tris–HCL, pH 7.5) with gentle agitation at 37℃ for 4 h. To remove all the enzymes, the enzyme-treated tissue sections slurry was washed with the hypotonic Tris–HCL solution for 20 h following treatment with 1% Triton X-100 solution(J.T.BAKER) for 24 h. The decellularized meniscus tissue was washed at least for 3 days to remove all the detergent. Inner MECM and outer MECM are stored in sterile glassware and stored at 4℃. Safranin O and TB staining of the outer MECM, inner MECM, and the outer and inner of the native meniscus. The specific operation method shall be carried out according to the manufacturer's plan.

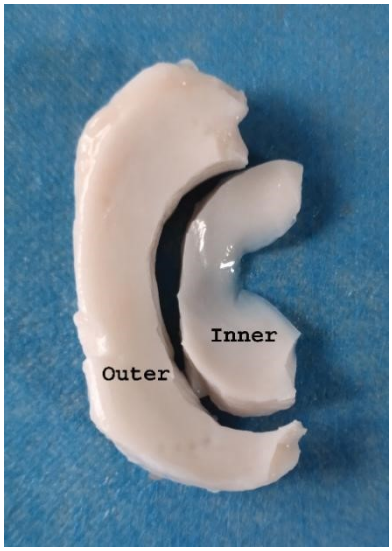


Figure S1: Macroscopic view of the inner and outer of native meniscus.

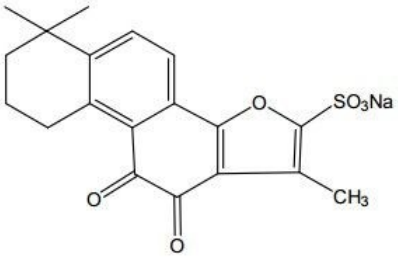


Figure S2: The molecular structure of STS.

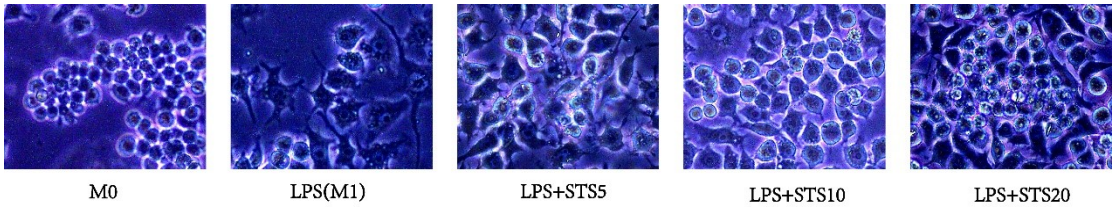


Figure S3: The RAW264.7 macrophages morphology changes after STS treatment

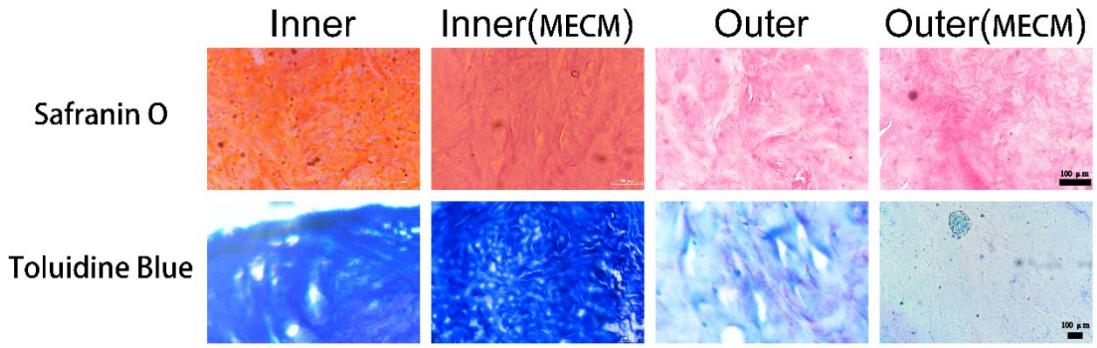


Figure S4: Safranin O and TB staining of the outer MECM, inner MECM, and the outer and inner of the native meniscus.

Gene	Direction of primer	The primer sequences(5′-3′)
IL-1β（mouse）	FORWARD	TACAGGCTCCGAGATGAACA
	REVERSE	AGGCCACAGGTATTTTGTCG
iNOS(mouse)	FORWARD	GTTCTCAGCCCAACAATACAAGA
	REVERSE	GTGGACGGGTCGATGTCAC
CD206(mouse)	FORWARD	ATGGATGTTGATGGCTACTGG
	REVERSE	TTCTGACTCTGGACACTTGC
Retnal (mouse)	FORWARD	TTGCAACTGCCTGTGCTTAC
	REVERSE	CTGGGTTCTCCACCTCTTCA
Gapdh（mouse）	FORWARD	CTTTGTCAAGCTCATTTCCTGG
	REVERSE	TCTTGCTCAGTGTCCTTGC
IL-1β（rabbit）	FORWARD	TAC AAC AAG AGC TTC CGG CA
	REVERSE	GGC CAC AGG TAT CTT GTC GT
MMP-13(rabbit)	FORWARD	AGGAAGACCTCCAGTTTGCAGAG
	REVERSE	GCTGCATTCTCCTTCAGGATTC
BCL-2(Rabbit)	FORWARD	CGGAAGGGACTGGACCAGAGA
	REVERSE	GCTGTCATGGGGATCACCTCC
Caspase-3(Rabbit)	FORWARD	AAGCCACGGTGATGAAGGAGT
	REVERSE	TCGGCAAGCCTGAATAATGAA
SOD-1(Rabbit)	FORWARD	GCACGGATTCCATGTCCACCA
	REVERSE	TCACATTACCCAGGTCGCCCA

Gapdh（rabbit）	FORWARD	TTGTCGCCATCAATGATCCAT
	REVERSE	GATGACCAGCTTCCCGTTCTC

Table S1: The primer sequences

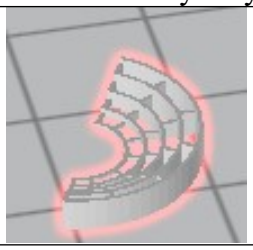
Project	parameter
3D Bioprinter	PanoSpace BioPro
Nozzle 1	pcl
Moving speed	5 mm/s
Temperature	130℃
Air pressure	470kpa
Platform temperature:	room temperature
FillStyle	Cross lines layer by layer
Effect picture	

Table S2: Parameters of the designed 3D-printed constructs

1. Pati, F., et al., *Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink.* Nat Commun, 2014. **5**: p. 3935.