Supporting Information

PCL-MECM Based Hydrogel Hybrid Scaffolds and Meniscal Fibrochondrocytes Promote Whole Meniscus Regeneration in a Rabbit Meniscectomy Model

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1. MATERIALS AND METHODS

1.1 Preparation of decellularized meniscus extracellular matrix (MECM)

An improved method was used to prepare MECM based on our previous study[1]. All the menisci were harvested from adult swine knee joints of freshly slaughtered. After removing the surrounding synovial tissue, the menisci were cut into slices by scalpel and washed with Phosphate Buffer Solution (PBS; Sigma-Aldrich Ltd., St. Louis, MO, USA) three times. The slices were transferred to a sterile jar and soaked in a 30% hydrogen peroxide solution for 2 hours in swing bed at 37℃, then washed with sterile deionized water ten times to remove residual hydrogen peroxide solution. A slice and some rinse solution at the last time were took for bacterial culture. After confirming the sterility, meniscus slices were homogenized to using a tissue pulverizer at 4℃ under aseptic conditions. 0.25% trypsin and acetic acid were added to the tissue slurry with stirring for digestion 12 h at 37 ℃. A differential centrifugation method was used to prepare MECM, as follows: Meniscal slurry was centrifuged for 10 min at 1,000 rpm. Precipitate was removed and supernatant was centrifuged for another 20min at 3,000 rpm, then the new supernatant was centrifuged for 30min at 6,000 rpm and 60min at 10,000 rpm again. Finally, the supernatant was removed and the precipitate was collected. MECM was frozen and lyophilized, and then shattered into powder.

1.2 Identification of decellularized MECM

The prepared MECM was evaluated by histological staining and biochemical assays for DNA, glycosaminoglycan (GAG) and collagen compared with a native meniscus. Hoechst 33258 and hematoxylin and eosin (H&E) staining were used to detect nucleic acids. Toluidine blue (TB) and safranin O staining were used to evaluate the GAG content. The collagen distribution in the sample was identified by Sirius red staining. DNA, GAG and collagen contents were further analyzed quantitatively,

and all biochemical assays were performed according to the manufacturer's protocol.

1.3 Rabbit meniscal fibrochondrocytes isolation and expansion

Ethical approval for the isolation of rabbit meniscal fibrochondrocytes was obtained from Institutional Animal Care and Use Committee of Chinese PLA general hospital. Rabbit meniscal fibrochondrocytes isolation and expansion were performed as described in our previous work[1]. Briefly, after anesthesia with intramuscular injections of ketamine and xylazine, the menisci were obtained from rabbit knee joints. Surrounding tissue were removed prior to cutting the menisci into small slices, which were then digested with collagenase (Sigma-Aldrich Ltd., St. Louis, MO, USA) in Dulbecco's modified Eagle's medium (DMEM; Corning, Glendale, AZ, USA) for 40 min. Following tissue digestion, meniscal fibrochondrocytes isolation was centrifuged at 1500 rpm for 5 min followed by culturing in DMEM containing 10% (v/v) fetal bovine serum (FBS) and 1%(v/v) penicillin -streptomycin (GIBCO, Biosciences, Ireland) at a humidified atmosphere with 5% CO_2 at 37℃. Meniscal fibrochondrocytes expanded to passage 3 were used for following experiments.

1.4 FTIR analysis

Bruker Tensor 27 spectrometer (Ettlingen, Germany) was used to analyze the functional group differences in the PCL scaffolds before and after etching. Samples were cut into 1×1 cm^2 square pieces and used to perform the FTIR analysis in reflection mode. FTIR spectra were recorded in the spectral region of 4000-650 cm^{-1} with a resolution of 1 cm^{-1}.

1.5 Water contact angle

A drop-shaped analysis system (Nuona SL-200B, Shanghai, China) was used to analyze the surface contact angle differences in the PCL scaffolds before and after etching. Pictures of the drop were captured a few seconds after the drop was set onto the sample. The contact angles were calculated by analyzing

the shape of the drop, and five parallel replicates were used to analyze each group.

1.6 SEM

The PCL scaffolds before and after etching were fixed on an aluminum stage and sputter-coated with gold before analysis. The micro architecture was observed by SEM, operating at 5k eV and 10 mA.

2. Results

2.1 Identification of the MECM

The prepared MECM was compared with native meniscus by histological staining and biochemical assays (Figures S1A, B). Hoechst 33258 and H&E staining revealed the absence of DNA and nuclei in the MECM. Further DNA quantification showed that the DNA content was less than 20 pg/mg, indicating that the DNA in the obtained MECM was effectively removed. Positive TB and safranin O staining in the MECM suggested that GAG components were well preserved in the MECM, which was further confirmed by the quantitative GAG assay. Abundant collagen fibers, such as red or yellow fibers, in the MECM and native meniscus were observed by picrosirius red staining. No significant difference in collagen content was found between the MECM and the native meniscus, indicating that collagen was not remarkably reduced during the decellularization process.

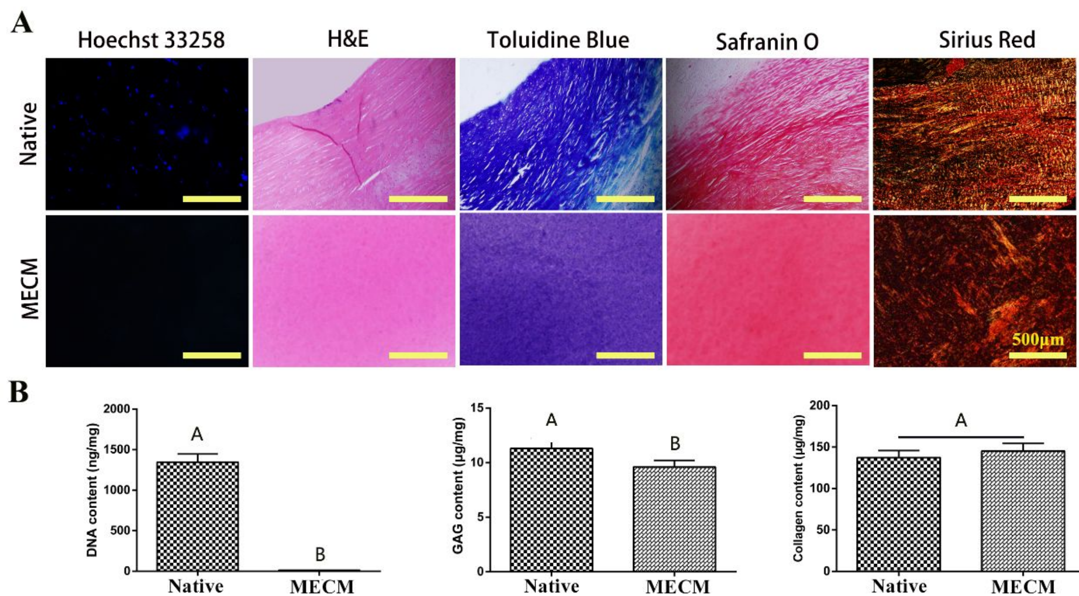

Figure S1. Histological staining (A) and biochemical assays (B) of MECM and native meniscus

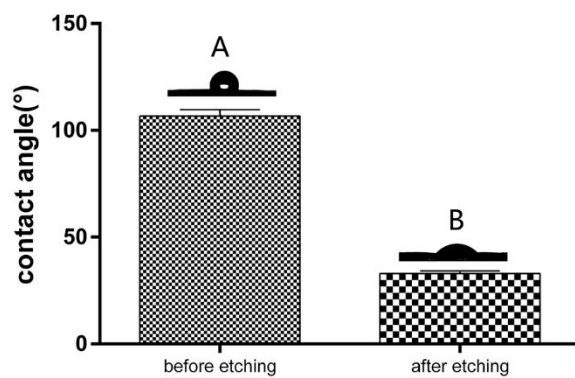

Figure S2. Water contact angles of PCL before and after etching

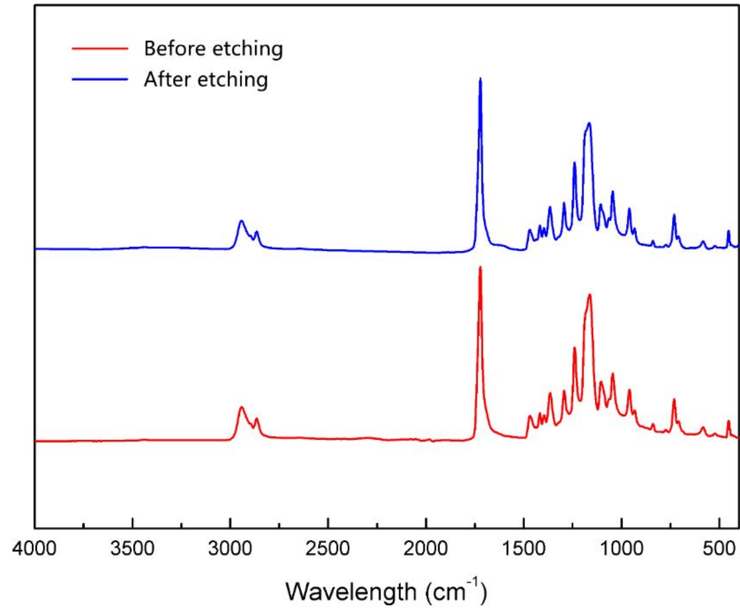

Figure S3. FTIR spectra of PCL before and after etching

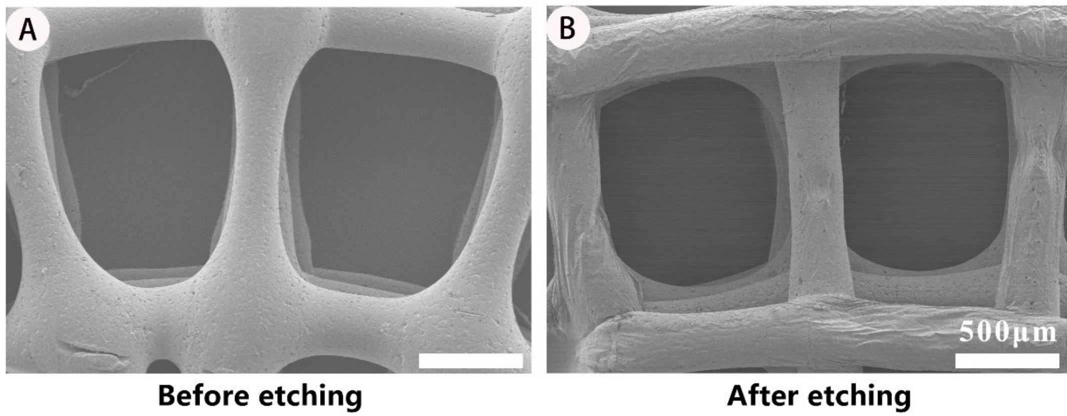

Figure S4. SEM images of PCL (A) before and (B) after etching

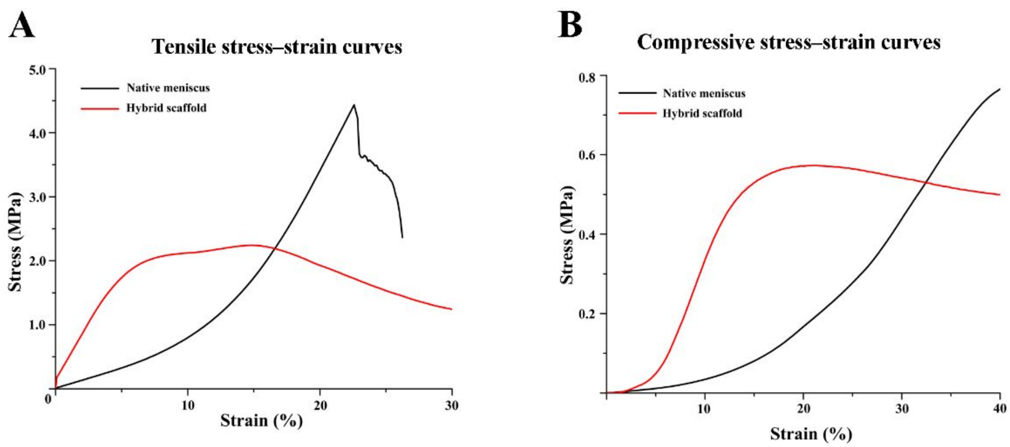

Figure S5. Tensile (A) and compressive (B) stress–strain curves of the native meniscus and the hybrid scaffold

Table S1. Primer sequences of target genes used for RT-PCR

Target	Forward primer 5' → 3'	Reverse primer 5' → 3'	Category
GAPDH	5' CAAGAAGGTGGTGAAGCAGG 3'	5' CACTGTTGAAGTCGCAG 3'	Reference gene
Aggrecan	5' GCAGTTTGTCAA 3'	5' TGTCCATCCGACCAGCGAAA 3'	Meniscus-related matrix gene
Collagen Ia2	5'GCCACCTGCCAGTCTTTACA 3'	5' CCATCATCACCATCTCTGCCT 3'	Meniscus-related matrix gene
Collagen IIa1	5' CACGCTCAAGTCCCTCAACA 3'	5' TCTATCCAGTAGTCACCGCTCT	Meniscus-related matrix gene
SOX9	5' GCGGAAGTCGGTGAAGAAT 3'	5' AAGATGGCGTTGGGCAT 3'	Transcription factor gene

Reference

(1) Yuan, Z.; Liu, S.; Hao, C.; Guo, W.; Gao, S.; Wang, M.; Chen, M.; Sun, Z.; Xu, Y.; Wang, Y.; Peng, J.; Yuan, M.; Guo, Q. Y. AMECM/DCB scaffold prompts successful total meniscus reconstruction in a rabbit total meniscectomy model. *Biomaterials* **2016,** *111*, 13-26.