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Supporting Materials

Self-organizing Gelatin-polycaprplactone Materials with Good Fluid Transmission Can Promote Full-thickness Skin Regeneration

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Experimental Section

Main Experimental Materials and Reagents

Materials in the experiments included collagenase type I (309 Umg-1, Worthington, USA), Fluorescein sodium (FS, $C_{20}H_{10}Na_2O_5$) was purchased from Sigma-Aldrich, USA. Experimental animals Wistar closed group rats were purchased from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd. (animal production license number: SCXK (Beijing) 2016-0011, No. 11400700214295). Chloral hydrate (Chloral hydrate, $CCl_3CH(OH)_2$) for anesthesia was purchased from Shenggong Bioengineering (Shanghai) and Stereo microscope (XTL1200C, Shanghai Wanheng Precision Optical Instrument Factory).

The polymer material polycaprolactone (PCL) used in the high-voltage electrospinning technology in this study was purchased from Sigma-Aldrich, USA, with a number average molecular mass of 70,000 to 90,000, and a purity of more than 99%. Dichloromethane (CH_2Cl_2) and N,N-Dimethylformamide (Dimethyl formamide Formyldimethylamine, DMF, C_3H_7NO) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. The purity was analytical grade. The magnetic stirrer is RCT basic type (safety control type) IKAMAG (IKA, Germany) and the high-voltage electrospinning equipment is self-built. The high-voltage power supply is purchased from Dongwen High Voltage Power Supply (Tianjin) Co., Ltd., the model is DW-P303-1ACF5, the output voltage is DC 0～30000V, and the output current is 1mA. High-voltage electrospinning flat wire take-up device, rotary wire take-up device, dish-shaped wire take-up device, micro syringe pump and various electrospinning needles were purchased from Shenzhen Tongli Micron Technology Co., Ltd. In addition, the high-voltage electrospinning moving shaft is self-built, with a positioning accuracy of 2μm, an effective stroke of 200mm, and a speed range of 0~500 mm/s. It can be

controlled by a handle or computer and the control box comes with two speed modes. The vacuum drying oven is DZF-6030B (Shanghai Yiheng Scientific Instrument Co., Ltd.).

In the in vitro simulation structure experiment, acrylamide solution (Acrylamide 40%, w/v) and methylene acrylamide (Bis-acrylamide 2%, w/v), Tetramethylethylenediamine (TEMED), persulfuric acid Ammonium (Ammonium persulfate 10% w/v, APS) and Glutaraldehyde in phosphate-buffered saline (0.5%, v/v) were purchased from Sigma-Aldrich, USA. Dichlorodimethylsilane (DCDMS) and 3-Aminopropyltriethoxysilane (APES) were purchased from Tixiai (Shanghai) Chemical Industry Development Co., Ltd. 1X Phosphate-buffered saline (PBS) was purchased from Beijing Soleibao Technology Co., Ltd. The laser confocal culture dish was purchased from Wuxi Nice Biotechnology Co., Ltd., with a diameter of 35 mm. Optical slides were purchased from Thermo Fisher Scientific, USA. The hydrogel was shaken and mixed using a Votex Genius vortex oscillator (IKA, Germany). To observe the organization structure and the physical structure of materials, use electron microscope (environmental scanning electronic microscope, SEM, Quanta 200) and transmission electron microscope (Hitachi, H-7500, Japan), Other solutions were prepared with deionized water.

In the sample preparation for scanning electron microscopy and transmission electron microscopy, glutaraldehyde in phosphate-buffered saline (0.5%, v/v) was purchased from Sigma-Aldrich, USA. Osmium tetroxide (Osmium tetroxide, OsO4, 1%, w/v) was purchased from PELCO, USA. Uranyl acetate (also known as Uranyl acetate, $C_4H_{10}O_8U$, 1-3%) Lead citrate ($C_6H_5O_7Pb$) was purchased from Structure Probe Company, USA. Epon-812 epoxy resin set was purchased from SPI-CHEM Company, USA. The copper mesh used in the transmission electron microscope samples is 100 meshes, purchased from China Zhongjing Science and Technology Company. The ultramicrotome is Leica EM UC7 ultramicrotome (Ultratome, Leica, Germany).

Preparation of polycaprolactone by high-voltage electrospinning technology

The micro injection pump is used for pushing the electrospinning liquid to carry out high-voltage electrostatic spinning at the pushing speed of 0.5ml/h, the voltage of 15-20kv, the type of a sampling needle of 7G-9G, the yarn taking-up distance of 15cm, the electrospinning temperature of 35-40℃ and the electrospinning humidity of 10-20%. When the humidity and the temperature in the electrospinning box reach the requirements of the corresponding ranges, the micro injection pump can generate continuous and stable liquid propulsion at the needle head, and the high-voltage power supply is stably pressurized to a specified voltage, the electrospinning can be collected. The wire take-up device is a flat-plate wire take-up device for spreading aluminum foil. The collection time is 10-20 minute. The collected electrospun films were placed in a vacuum oven for more than 5 hours to ensure complete volatilization of all volatile solvents.

Preparation and characterization of Gt-PCL micro-nano composite material

High-voltage electrospinning was prepared in a two-liquid phase system and the materials used were polycaprolactone (PCL, Mn=70000~90000), gelatin and collagen, and the solvent was trifluoroethanol (TFE, $C_2H_3F_3O$). The electrospinning solution was formulated with polycaprolactone, gelatin, and collagen in a 2: 1: 1 (w/w/w) ratio, and 2g of solute was added per 10ml of solvent. The solute and the solvent need to be continuously stirred for 4 hours under the action of the magnetic stirrer, and the heating temperature is 40℃, so that the solute is fully dissolved into a uniform liquid. The micro injection pump was used to promote the electrospinning liquid for high-voltage electrostatic spinning. The advance speed is 0.5ml/h, that voltage is 15-20kv, the type of the sample needle is 7G-9G, the yarn collection distance is 20cm, the

electrospinning temperature is 35-40℃, and the electrospinning humidity is 10-20%. When the humidity and the temperature in the electrospinning box reach the requirements of the corresponding ranges, the micro injection pump can generate continuous and stable liquid propulsion at the needle head, and the high-voltage power supply is stably pressurized to a specified voltage, the electrospinning can be collected. The wire take-up device is a flat-plate wire take-up device for spreading aluminum foil. The collection time is 10-20 minute. The collected electrospun films were placed in a vacuum oven for more than 5 hours to ensure complete volatilization of all volatile solvents. All fibrous films are stored in a drying oven.

Preparation of Gt-PCL polyacrylamide hydrogel Methods

The hydrogel preparation method proposed by Engler in 2010 was used for reference [1]. The composite Gt-PCL was removed from the aluminum foil, cut into 5mm*10mm rectangular pieces, and immersed completely overnight in distilled water. A polyacrylamide hydrogel prepolymer solution was prepared in a 10ml solution containing 2.5 mL of acrylamide solution (acrylamide 40%, w/v), 0.5 mL of methylene acrylamide (bis-acrylamide 2%, w/v), and 7ml of pure water. Fully oscillating and uniformly mixing the mixed liquid on a vortex oscillator for later use. As used, add 1% (using the total volume) of ammonium persulfate solution (10% w/v, APS) and 1‰ (using the total volume) of tetramethylethylenediamine (TEMED) and mix well with shaking. A total of 100μl of the above mixture was dropwise added to the bottom of the laser confocal dish, and a completely immersed electrospun film was taken out, placed on the droplets and completely spread. Then 100-150μl of mixed solution was dropwise added onto the film, and the washed optical slide and the edge of the groove at the bottom of the dish were fastened onto the liquid drop at an interval of 2 mm. After the liquid drop was completely polymerized, 1ml of pure water was added into the dish and the water was changed for three or more times to wash off the

unpolymerized monomer. The sample needs to be stored in water prior to the fluorescence experiments.

Sample preparation for transmission electron microscope

The composite material was cut into 2 * 2 * 2cm^3 small pieces and fixed in 2.5% glutaraldehyde for 3-5 hours at 4℃. The sample was completely immersed in phosphate buffer for 3 times and fixed in 1% osmium tetroxide for 3 hours before gradient dehydration. The sample was embedded in an embedding plate with Epon-812 resin for infiltration for 6 hours and the resin was polymerized by the gradient temperature rise method. After the samples were sliced by an ultra-thin microtome, they were stained by lead citrate-uranyl acetate double staining and observed under a transmission electron microscope.

Total mRNA isolation

The total RNA from each sample was isolated using a Trizol Reagent (Tiangen, Beijing) and assessed with Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) and Qubit Fluorometer (Invitrogen). The RNA integrity was checked by SMA 3000 spectrophotometry and 1.5% formaldehyde denaturant gel electrophoresis. Total RNA samples that meet the following requirements were used in subsequent experiments: RNA integrity number (RIN) > 7.0 and a 28S:18S ratio > 1.8[2].

Differentially expressed genes analysis

The random-variance model F-test was used to filter the differentially expressed genes for both the control and experiment groups. The differentially expressed genes

were identified according to the p-value threshold ($P < 0.05$) and $|\log 2\ FC| >= 1$. Log 2 FC is the differential expression multiple and takes the base 2 logarithm that log 2 FC>=1 is the up-regulated differential gene, and log 2 FC<=-1 is the down-regulated differential gene[3].

Analysis of differential gene expression levels on different material substrates

Gene differential expression analysis is an independent statistical hypothesis test for thousands of genes, and a differential test P-value is obtained, then the p-value is corrected by the FDR method. The lower the P-value or q-value, the more significant the difference in gene expression. We used Cuffquant and Cuffnorm software for expression analysis and used Cuffdiff software to analyze differentially expressed genes between samples[4].

KEGG pathway and Gene ontology (GO) analysis

The Gene Ontology (GO) database （http://geneontology.org/ ）is a database created by the Gene Ontology Consortium and GO Analysis is applied to determine the differently expressed genes belonging to the main function according to the Gene Ontology, which is the key functional classification of NCBI[5]. In general, Fisher's exact test and χ^2 test are applied to classify the GO category, and the false discovery rate (FDR) is calculated to correct the P-value. Similarly, pathway analysis was used to analyze the significant pathways of the differential genes according to the Kyoto encyclopedia of genes and genomes (KEGG) database（https://www.genome.jp/kegg/）[6]. Two-side Fisher's exact test and χ^2-test were applied to select the significant pathway category, and the FDR was used to correct the p-value.

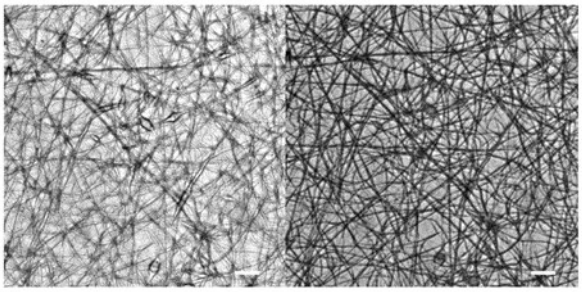

Fig.S1 Bright field images of single PCL fiber, scale bar: 50μm.

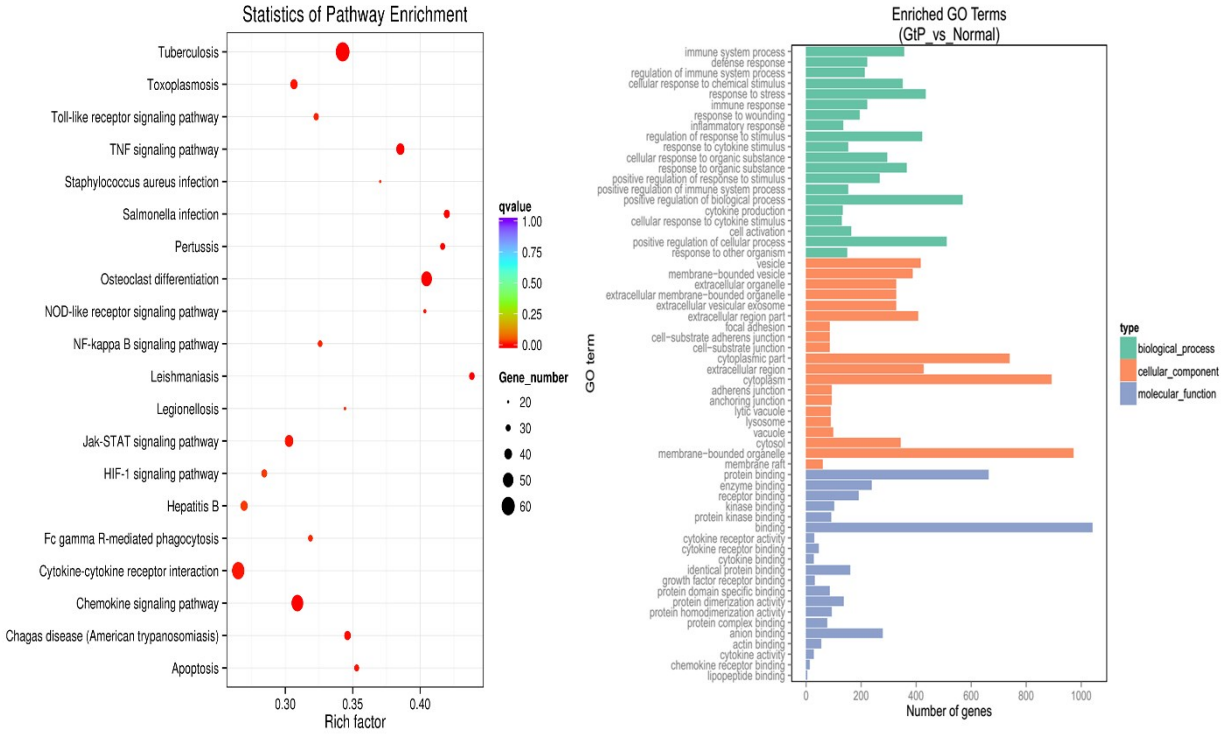

Fig.S2 KEGG Pathway and GO terms of down-regulated of genes, Gt-PCL vs Normal. The results showed that the GO gene functions were concentrated in the material group and the negative group, in which green represented the genes involved in biological processes, red represented the genes involved in cell component synthesis, and blue represented the genes involved in functional adjustment.

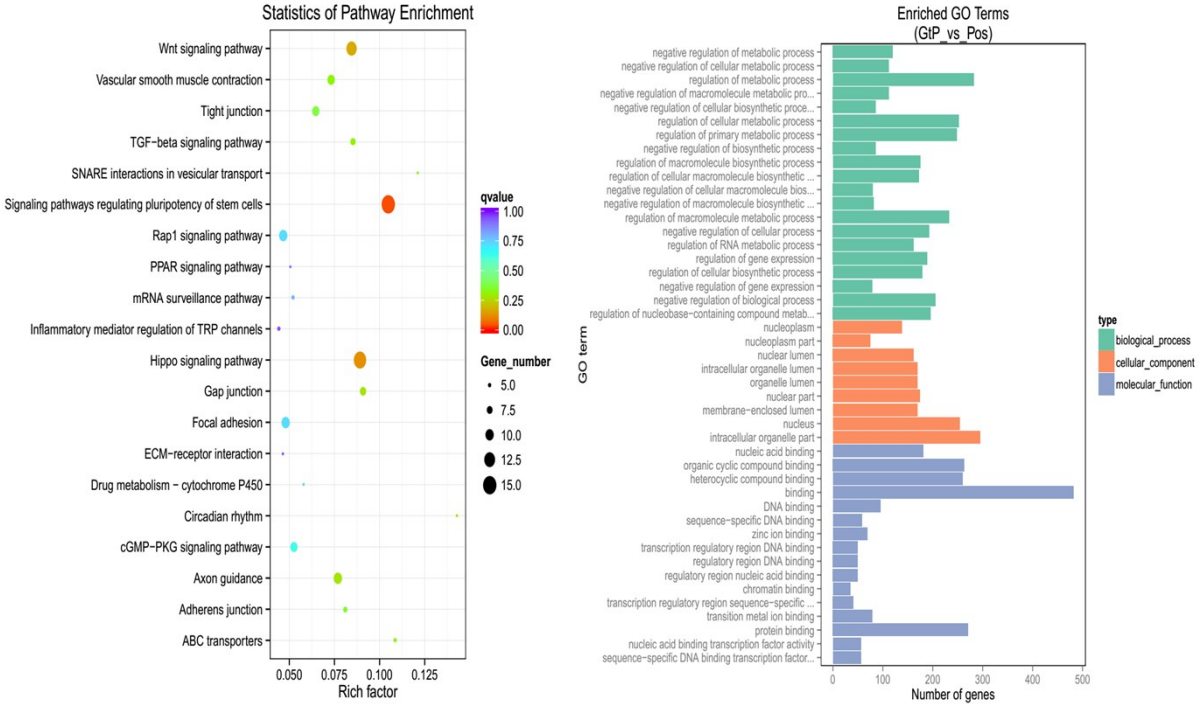

Fig.S3 KEGG Pathway and GO terms of up-regulated of genes, Gt-PCL vs Positive group.

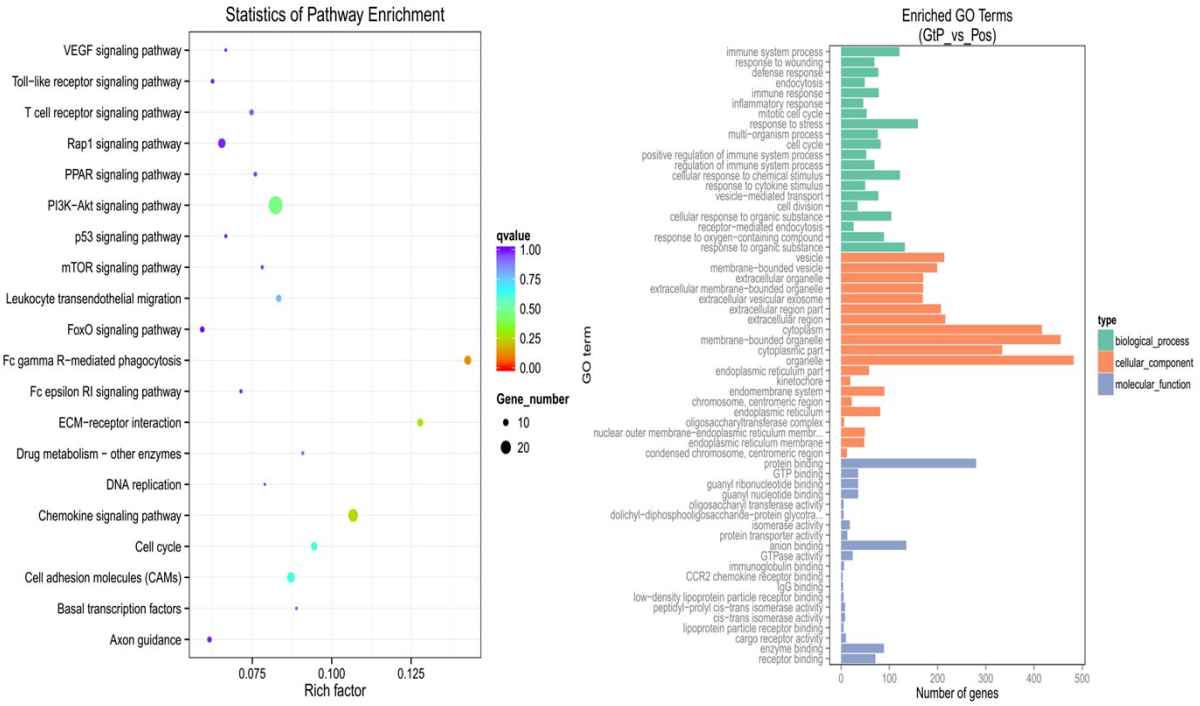

Fig.S4 KEGG Pathway and GO terms of down-regulated of genes, Gt-PCL vs Positive group.

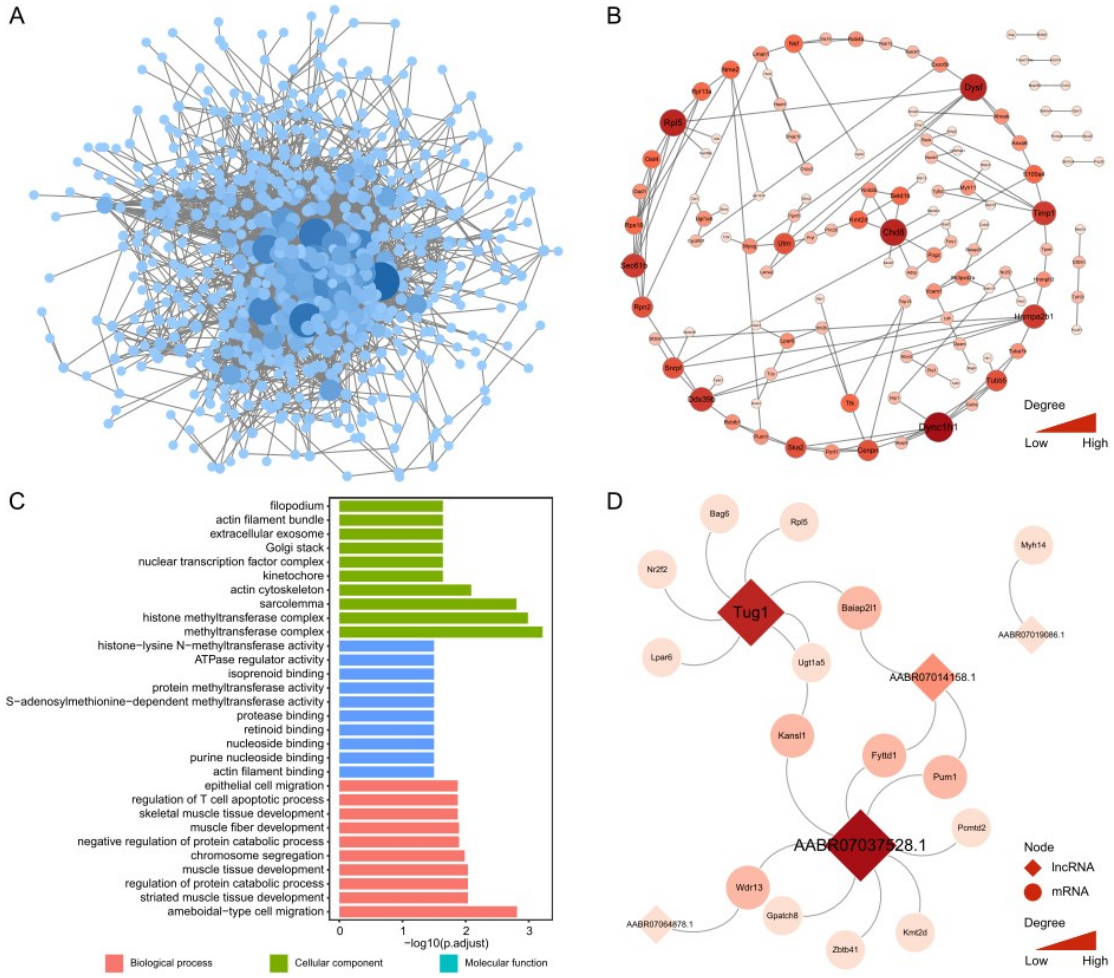

Fig.S5 Analysis and interaction network of three groups of differentially expressed mRNAs and lncRNAs.

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