upplemental Information

Study on the properties of biomass film with different compositions

1. Sample preparation

Mainly prepared by solution casting method can absorb membrane. Chitosan was dissolved in 2%v/v acetic acid at room temperature to prepare 1.5%w/v chitosan solution, and gelatin was dissolved in water at 80 ℃ to prepare 1.5%w/v gelatin solution. The mixture was mixed according to the proportions and 30%wt glycerol was added to mix well. The composite solution was made by stirring at 40 ℃ for 60 minutes, and the bubbles were completely removed by ultrasonic treatment for 30 minutes for use. The chitosan and gelatin solutions were divided into the following proportions: 1:0, 9:1, 8:2, 7:3, 6:4, and 1:1, The composite solutions of different ratios were cast in a smooth mold and then dried at 65℃ for 24 hours to release the mold. The smooth surface films are denoted as SCM1-0, SCM9-1, SCM8-2, SCM7-3, SCM6-4, and SCM1-1 (smooth construction membrane), respectively. A dry and ventilated place was stored for more than 48 hours to allow the acetic acid to fully evaporate before subsequent testing.

2. Characterization of biomass absorbable membrane with different compositions

2.1 Physical characterization of biomass absorbable membrane with different compositions

2.1.1 Morphology characterization

To study the surface topology, the surface morphology of the film was characterized. With a conductive adhesive film on the sample stage, using an ion sputtering apparatus (HITACHI E – 1010, HITACH, Japan) with gold sputtering coating surface, then under 15.00 kV acceleration voltage surface observed microstructure by scanning electron microscope (SEM, SUPRA 35, LEO, Germany).

At the same time use an EDS spectrometer measuring surface element distribution.

2.1.2 Functional group and phase analysis

Using Fourier infrared spectrometer (FTIR, Cary 630, Agilent, Germany)) analysis of the sample composition and internal crosslinking, the scanning range is 4000 cm^{-1}-400 cm^{-1}, with a resolution of 2 cm^{-1}.

The phase and crystallization properties of each sample were tested and characterized by X-ray diffraction (XRD, D8 Advance, Bruker, Germany), scanning in the Angle range of 5-80° (2θ). Wherein SCM0-1 is a pure gelatin film made of 1.5%w/v gelatin solution according to the preparation process described in 2.2.

2.1.3 Degradation property

The films were cut into 90 mm diameter discs, immersed in phosphate buffer (PBS) at 37 ℃, and shaken in a 37 ℃ constant temperature gas bath oscillator. In 7 days and 14 days, dry surface moisture absorption, after completely dry weighs its degradation rate, expressed in the weightlessness rate measured three times average in each sample. The weightlessness rate formula is expressed as:

$$Degradation = {(m_0 - m_n)}/{m_0} * 100\%$$

Where:

M_0 - the quality of the samples before soaking; M_n - the quality of the sample soaked in n days

2.1.4 Hydrophilia

For smooth films with six components, the hydrophilicity of the different films was tested using a contact Angle measuring instrument. Fasten the films to a flat surface, using a contact Angle measurement instrument to test samples of static water contact Angle, averaging three times each sample test.

2.1.5 Mechanical property

The tensile properties of the different samples were tested using an electronic tensile testing machine (AG-X, Shimadzu, Japan), and the samples were cut into 25×75mm strips with 3 samples in each group. The thickness of three samples was measured using a spiral micrometer before testing, and each sample was measured three times and averaged. The strain was stretched at 1mm/min until the fracture experiment was stopped. The Young's modulus, tensile strength, and elongation at the break of each sample were obtained by drawing tensile stress-strain curves.

2.2 The biocompatibility of the different compositions of biomass absorbable membrane

Each film will be cut into 16 mm diameter wafers after UV irradiation sterilization and fully placed in 24-Well Cell Culture Plates, the Mouse calvarial preosteoblast cell line MC3T3-E1 to 1.5 × 10^4 / hole density in the thin film surface. The cells were cultured in a constant temperature incubator (5%CO_2, 37 ℃) for 5 days, and the Cell Counting Kit-8 assay (CCK-8, Beyotime, China) was used to evaluate cell proliferation every day. In brief, cells were washed twice with PBS and then treated with 550 μL CCK-8 working solution (550 μL culture medium containing 10%CCK-8 working solution (v/v)). The cell plate at 37 ℃ under 2.5 hours after incubation, with each hole 150 μL of the solution to 96-well plates, the absorbance (OD) at 450 nm was read using a microplate reader (Spectra Max M5, Molecular Devices, USA).

3. Results

3.1 Properties of biomass films with different compositions

As shown in **Fig. S1**, 500 times the magnification of biomass flat membrane surface is smooth, without impurities and larger dissolved polymer particles, the chitosan and gelatin in the system are completely dissolved and dispersed

homogeneously and did not produce obvious physical separation. The biomass films of each component were prepared by the same process and had a dense smooth surface structure. Spectrum EDS electron microscopy is used to determine different composition distributions of the material surface elements, all membrane mainly consists of two elements of C, and O, atomic ratio is close to 6:4.

Fig. S2 shows all the films in 3100-3500 cm^{-1} show a broad peak, this is due to the stretching vibration of intermolecular hydrogen bond O-H (3200-3500 cm^{-1}) and N - H stretching vibration absorption (3100-350cm^{-1}), the composite films in 1645 cm^{-1} (amide I, C-O stretching vibration), 1540 cm^{-1} (amide II, N-H bending and C-N tensile) and 1155 cm^{-1} (amide III, C-N, N-H stretching vibration) has the characteristics of the belt, which attributed to the formation of hydrogen bonds between chitosan and gelatin and electrostatic interactions. In the FTIR of pure chitosan, there are 895 cm^{-1} β-glycosidic bond configuration absorption peak, 1155 cm^{-1} C-O absorption peak on the ring, and 1655 cm-^{1} C=O stretching vibration peak[1]. In the FTIR of pure gelatin film, the absorption peaks of the amide I band, amide II band, and amide III band were 1637 cm^{-1}, 1541 cm^{-1}, and 1240 cm^{-1}, respectively [2]. After gelatin was blended with the chitosan membrane, the absorption peak of the amide I band at 1637cm^{-1} shifted to a higher wave number, and the absorption band became wider, indicating that there was a strong hydrogen bond between chitosan and gelatin molecules.

Fig. S3 shows the parcel peak can be seen that all the material for the amorphous structure, no apparent crystalline phase, and no fine spectrum peak structure. By pure substance XRD diagram can be seen that chitosan in 2 theta is 10.2° and 20.6° respectively in the crystallization, at about 15° appear smaller without water of crystallization peak [3]. Gelatin shows amorphous diffraction peaks around 19.9° and 7.5° at 2θ, which are characteristic diffraction peaks of gelatin [4]. If there is no interaction between chitosan and gelatin molecules or the interaction is very weak, they will have their crystallization area in the composite membrane, the diffraction pattern is characterized by chitosan and gelatin diffraction pattern in proportion to the simple

superposition. 20.6° in composite membrane of chitosan characteristic peak gradually with the addition of gelatin to left shift, and not in 2 theta 7.5° and 10.2° and 15° occurrence characteristics of diffraction peak, these instructions between chitosan and gelatin strong interaction, disrupting the crystallization of chitosan and gelatin in their respective situations.

It can be seen from **Fig. S4** that the degradation of pure chitosan is slower than that of gelatin. With the increase in the amount of gelatin added, the degradation rate of biomass film is gradually accelerated. When the ratio of the two components is 1:1, the degradation rate of 7 days reaches about 60%. Further degradation was observed at 14 days compared to 7 days.

The hydrophilic and hydrophobic properties of the material surface are one of the important factors affecting cell adhesion and proliferation. According to relevant reports, both hydrophilic and too hydrophobic surfaces are not suitable for cell adhesion and proliferation [5]. Cell adhesion usually depends on protein adhesion in the cells initially, while hydrophobic surfaces are more conducive to protein adhesion, which can bring favorable effects on cell adhesion and proliferation. Material of hydrophilic and hydrophobic is to describe the nature of the interaction between fluid and solid surface, usually with Water Contact Angle measurement. If the droplets on the material surface form a smaller Contact Angle (less than 90 degrees Celsius), suggests that the material has good hydrophilicity, on the contrary, when the water contact Angle is greater than 90° it shows that the material surface is hydrophobic. **Fig. S5** illustrates the static water contact Angle plots of the six component biomass films. It can be seen from **Fig. S6** that the water contact Angle of the 6 smooth biomass films formed by chitosan/gelatin composite with different components is about 120°, which is a hydrophobic surface, consistent with the surface hydrophobicity of cell adhesion and proliferation, and can be used as a biological material to promote cell proliferation and differentiation.

Mechanical properties are one of the important indexes to evaluate whether the

biomass film can be used as a tendon-bone interface graft. To ensure that the material acting on the affected site can compensate for the insufficient mechanical properties of the original interface and encapsulate the tendon, it is necessary to have good mechanical properties of the membrane. It can be seen from **Fig. S7** and **Fig. S8** that chitosan/gelatin biomass films with different compositions belong to brittle materials, with elongation at break of about 5% and high tensile strength, all of which can reach more than 20 MPa, which can meet the requirements of tendon-bone interface.

As promotes tendon-to-bone interface healing tissue engineering material, the most basic requirements of biomass film are a non-toxic effect in vivo, good biocompatibility, and promotion of cell proliferation. The films of all components were therefore co-cultured with the Mouse calvarial preosteoblast cell line MC3T3-E1, through the Cell Counting Kit-8 measurement to evaluate cell proliferation. As shown in **Fig. S9**, all material surface inoculation cells, trained in five days, with the extension of incubation time, the absorbance on the surface of the material all showed a trend of increase, indicating that the material was not toxic to cells, had good biocompatibility, can accommodate cell growth and proliferation. There was no significant difference in the first 3 days of co-culture, but after 5 days of co-culture, the group with a chitosan/gelatin ratio of 9:1 had a higher absorbance and better ability to promote cell proliferation. On the fourth and fifth day, the absorbance of SCM9-1 material was significantly different from that of the other five groups of materials. As you can see, chitosan and gelatin both are good biocompatibility materials, proper proportion can improve the promoting effect, and is conducive to a large number of cell proliferation.

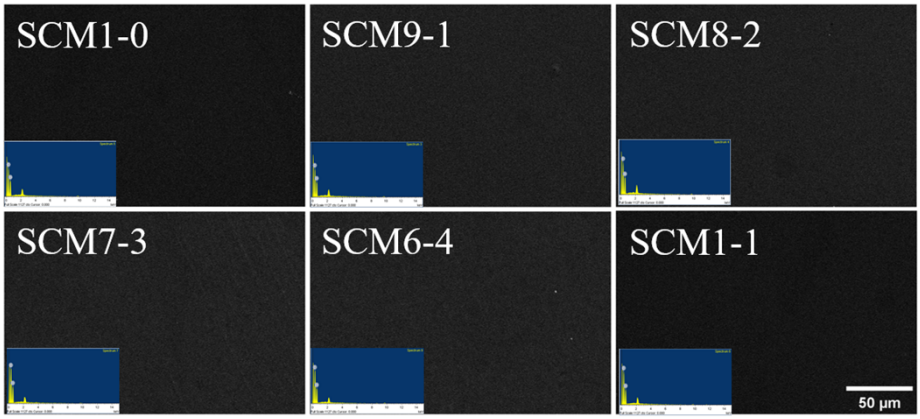

Fig. S1 SEM images and EDS energy spectra of biomass films with different compositions.

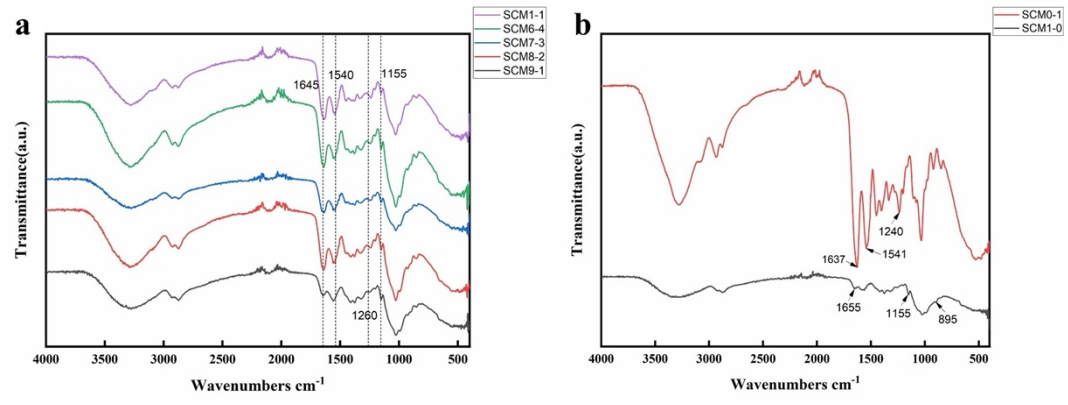

Fig. S2 FTIR spectra of biomass films with different compositions (a) and two kinds of pure substance (b).

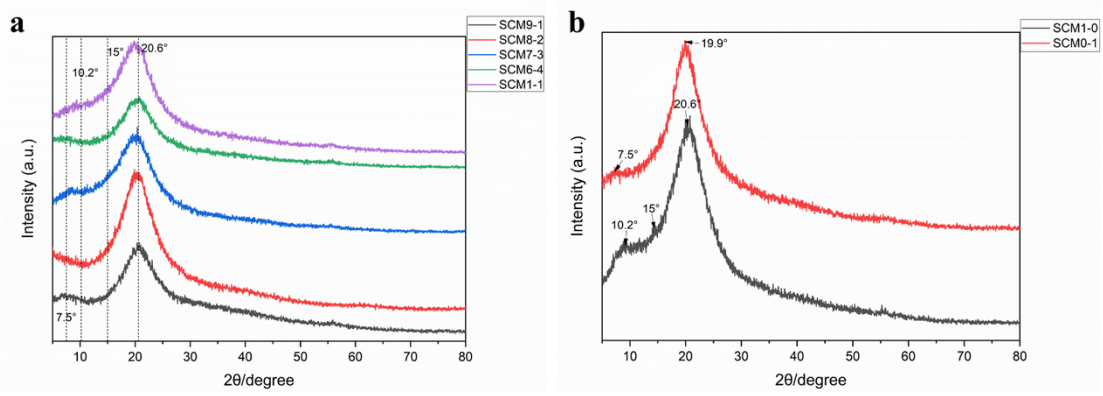

Fig. S3 XRD profiles of biomass films with different compositions (a) and two kinds of pure substance (b).

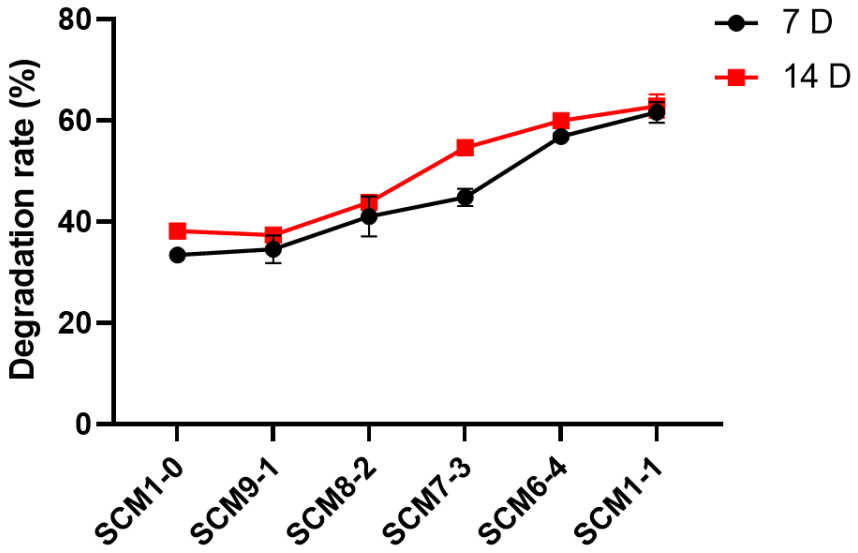

Fig.S4 Degradation curve in vitro.

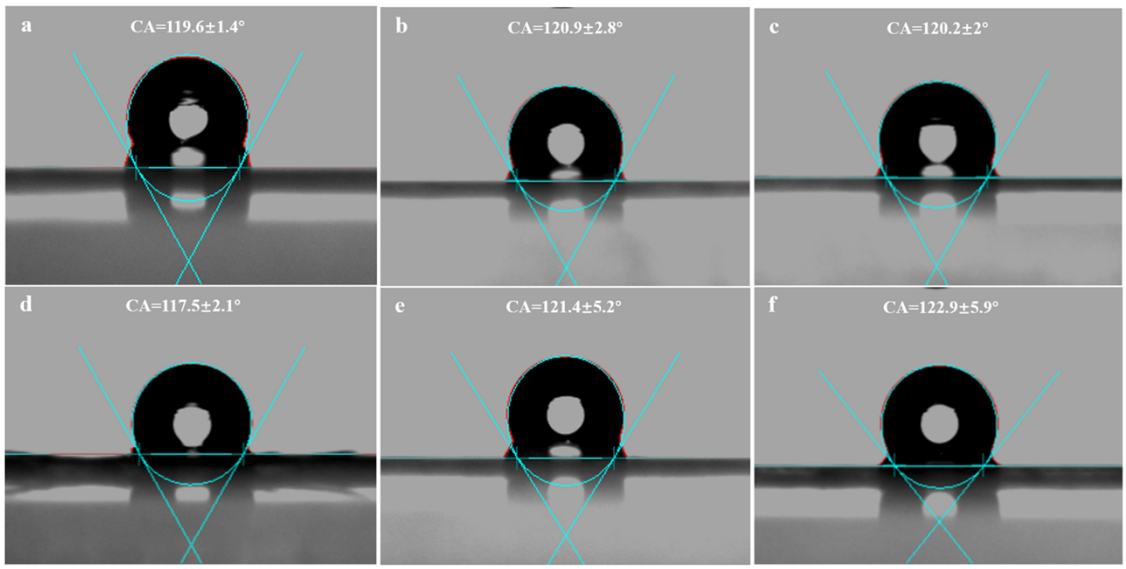

Fig. S5 Static water contact Angle plots of biomass films with different compositions.

(a)SCM0-1. (b)SCM9-1. (c)SCM8-2. (d)SCM7-3. (e)SCM6-4. (f)SCM1-1.

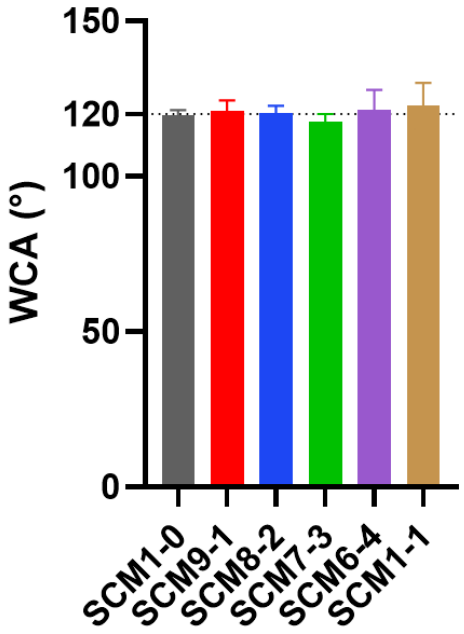

Fig. S6 Water contact angle of biomass films with different compositions.

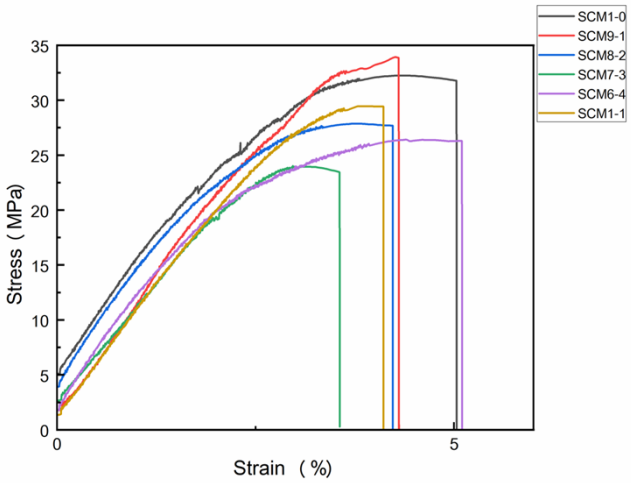

Fig. S7 Stress-strain curve.

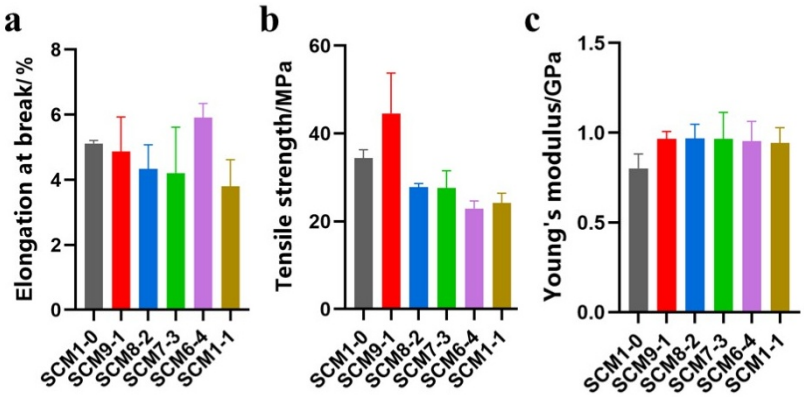

Fig. S8 The Elongation at the break(a), Tensile strength(b), and the Young's modulus(c) of biomass films with different compositions.

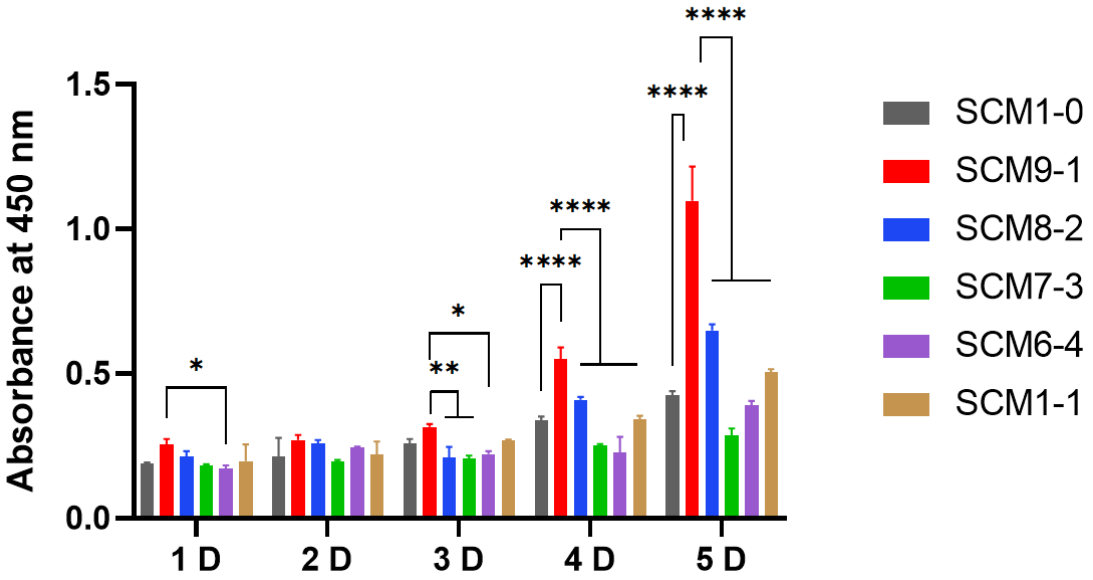

Fig. S9 CCK 8 cell proliferation of biomass films with different compositions. Data are presented as the mean±SD. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

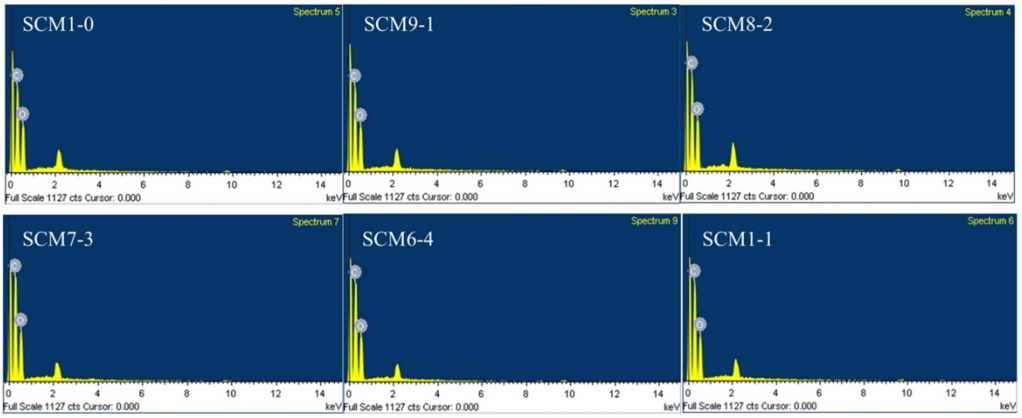

Fig. S10 EDS energy spectra of biomass films with different compositions.

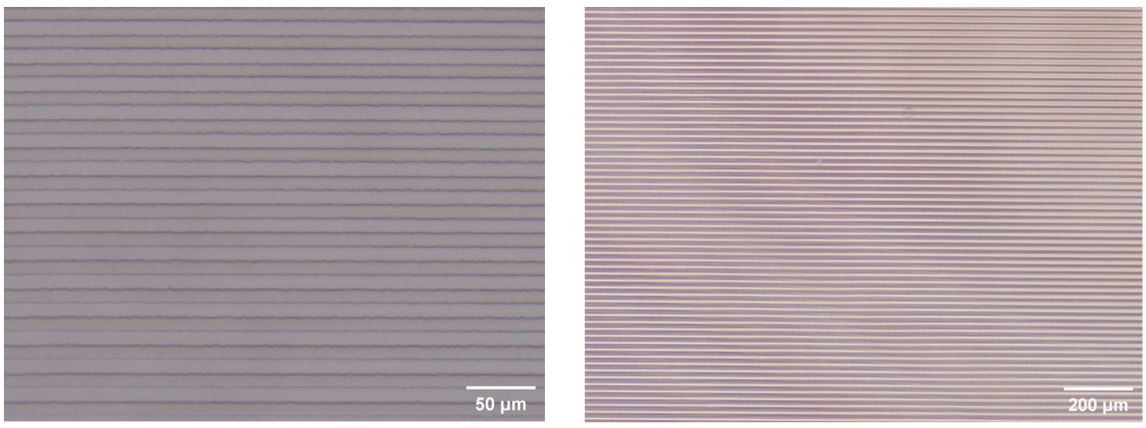

Fig. S11 Morphological light microscopy of PDMs replica prepared by soft lithography.

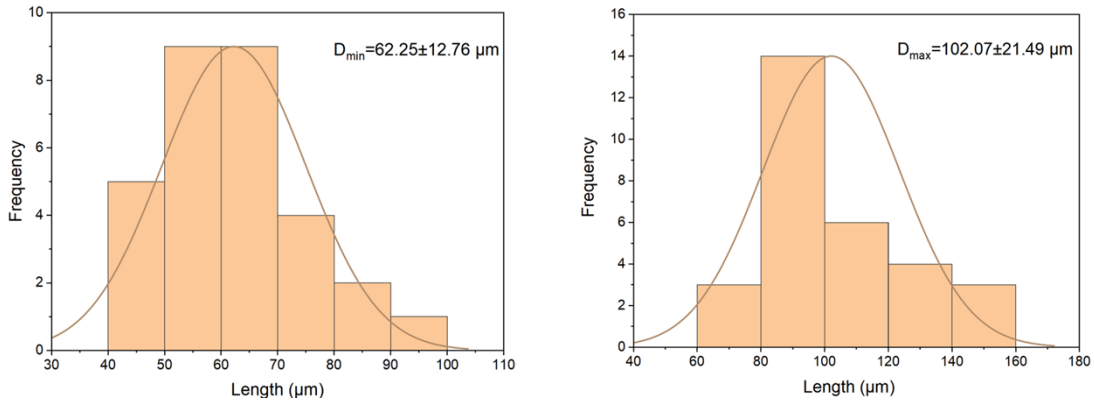

Fig. S12 The length of the long diameter and short diameter distribution of PCM.

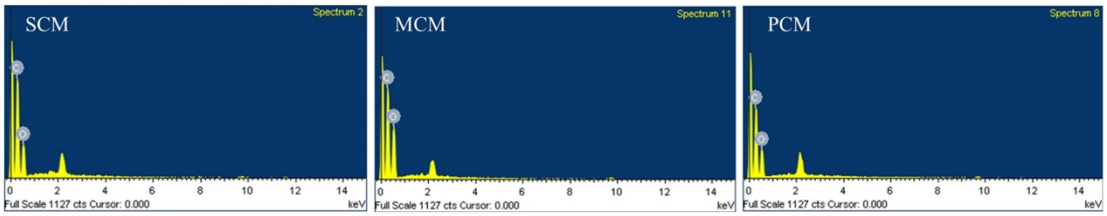

Fig. S13 EDS energy spectra of biomass films with different morphologies.

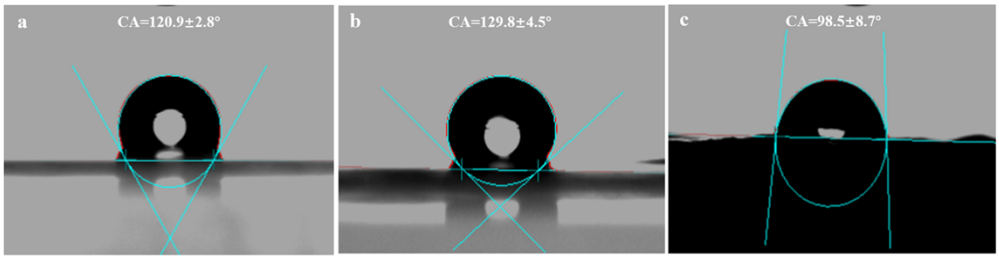

Fig. S14 Static water contact Angle plots of biomass films with different morphologies.

(a)SCM. (b)MCM. (c)PCM.

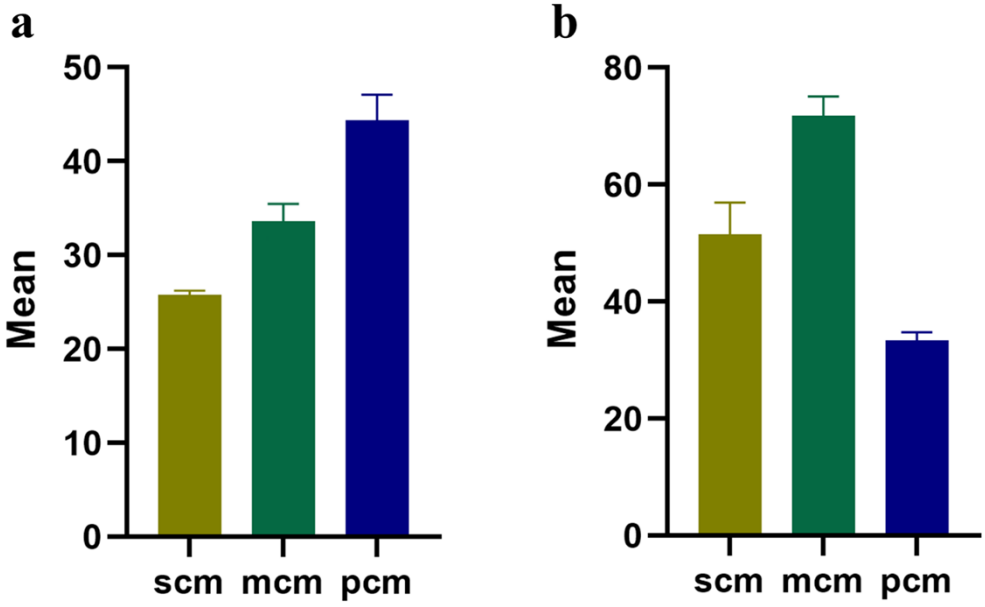

Fig. S15 Immunofluorescence intensity of specific protein secreted by cell coculture on the surface of the material. (a)The fluorescence intensity of RUNX2 secreted by MC3T3-E1 cells. (b)The fluorescence intensity of α-SMA secreted by NIH3T3 cells.

Supplemental References

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