Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary information

ECM-based Bioadhesive Hydrogel for Sutureless Repair of Deep Anterior Corneal Defects

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This file contained

S1: GelMA synthesis and characterization.

S2: Corneal stromal stem cell isolation.

Movie S3: Ex vivo examination of the strong adhesion of the dCor/Gel-PEG hydrogel onto corneal defects.

S1. GelMA synthesis and characterization

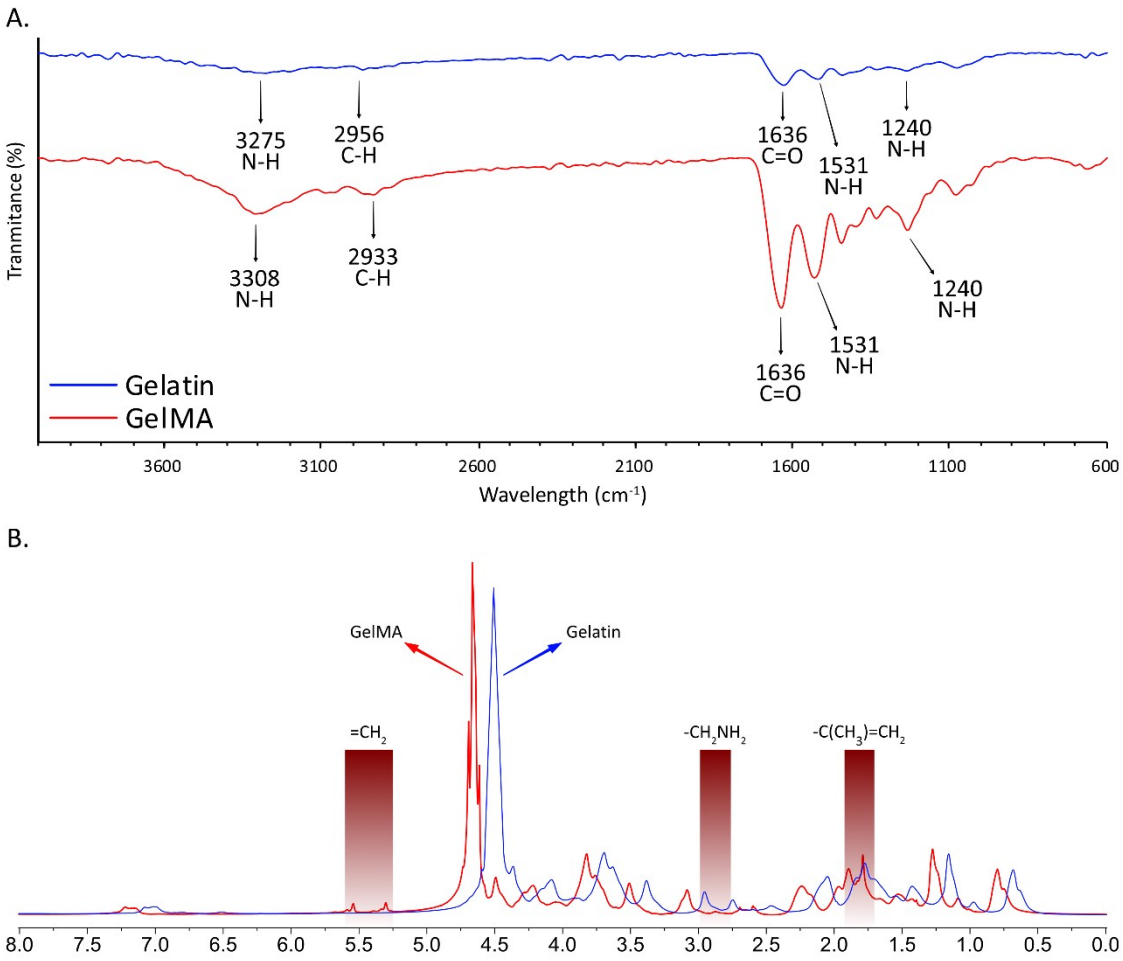
Porcine skin-derived gelatin type A (Sigma-Aldrich) was used to synthesize the GelMA. In brief, 5 mg gelatin was dissolved in 50 ml PBS at RT and slowly heated to 50°C in an oil bath via vigorous magnetic stirring for 1 h. As the gelatin dissolved completely to a clear yellow solution, the methacrylation process started with the dropwise addition of methacrylic anhydride (MA) to a final concentration of 6% (v/v), and stirring continued for 3h. PBS preheated at 40°C, in a volume of 150 ml was used to dilute the GelMA solution. The diluted GelMA solution was dialyzed against 500 ml deionized water for 7 days using a dialysis membrane with a 12-kDa MWCO at RT, and the water was changed daily. After 7 days of dialysis, GelMA was dehumidified by freeze-drier for 96 h and stored at -20°C.

1H-NMR spectroscopy was used to quantify the degree of functionalization (DOF) of the synthesized GelMA dissolved in deuterium oxide (5 mg in 600µL). ^{1}H-NMR spectra were recorded for raw gelatin and GelMA using a 400 MHz spectrometer. The data of the intended peaks were analyzed using the MestReNova software after performing baseline and phase correction. The phenylalanine signal at 7.2–7.5 ppm was considered as the internal reference to normalize the amine signals at 3.13–3.22 ppm of lysine. (1, 2). During the methacrylation of gelatin, MA reacts with primary amines on lysine residues; therefore, the degree of functionalization can be evaluated via the normalization of free amino groups of gelatin. The degree of functionalization (DOF) is calculated using the following equation:

$$DOF\% = \frac{lysine\ integration\ signal\ of\ GelMA}{lysine\ integration\ signal\ of\ gelatin} \times 100$$

DOF, which is defined as the percentage of functionalized amine groups in gelatin (lysine, hydroxylysine) with methacrylic anhydride (MA), was used to determine the successful methacrylation of gelatin for GelMA synthesis. The ^{1}H-NMR spectra of the unmodified gelatin and methacrylated gelatin are presented in Figure S1. Appearing new peaks at δ= 5.35 ppm and δ=5.68 ppm are related to acrylic protons in methacryloyl of lysine and hydroxyl lysine. The increasing peak around 1.8 ppm is representing methyl proton at methacryloyl grafts, while the decreasing signal around 3.13–3.2 ppm is representing free lysine(1, 2). The DOF was 75% due to decreasing free lysine signals at 3.13–3.2 ppm in GelMA after 3h reaction of gelatin with 6% (v/v) MA.

ATR-FTIR was performed to investigate the functional groups and chemical composition of Gelatin and GelMA. All spectra of gelatin and GelMA were obtained in the range 400–4000 cm^{-1}. The ATR-FTIR spectra of Gelatin and GelMA are shown in Fig. S1. The peak located at approximately 1240 cm^{-1} is related to Amide III, which is presented for C-H bending and stretching, Amide II is also present in the peak at 1531 cm^{-1} for C-N-H bonding, and the peak of Amide I is located at approximately 1636 cm^{-1} for C=O stretching. Amides A and B are represented by peaks around 3308 cm^{-1} and 2933 cm^{-1} for O-H/N-H stretching and vibration of C-H bonds, respectively. The successful synthesis of GelMA was confirmed by ATR-FTIR.

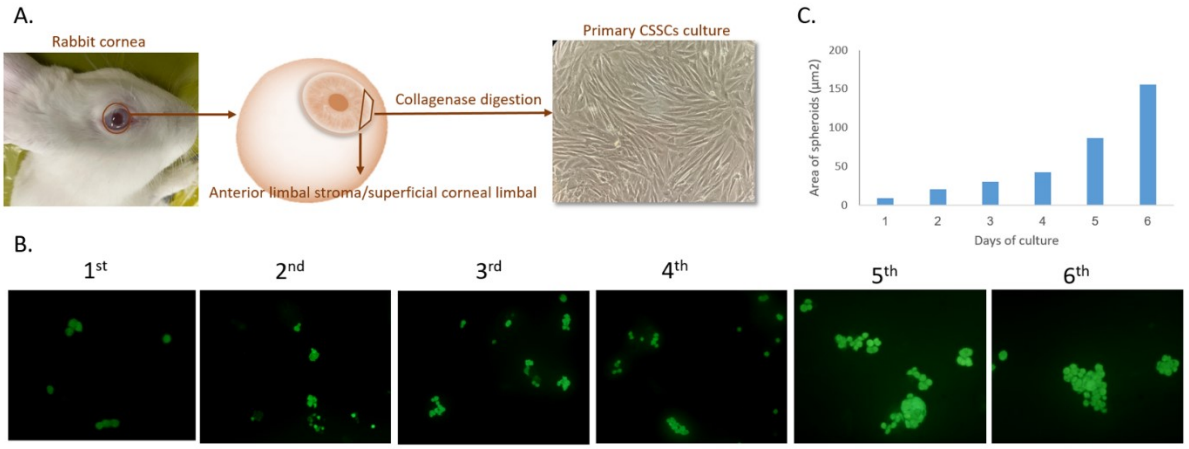


S1. ATR-FTIR spectroscopy (A), and ^{1}H-NMR- spectroscopy (B) of Gelatin and GelMA.

S2. Corneal stromal stem cell isolation and characterization

Corneal stromal stem cells (CSSCs) were isolated from the anterior limbal stroma (superficial corneal limbal) of the cornea following modified methods reported previously (3, 4). After disinfecting the orbital origin of the sacrificed rabbits, the corneal disc was cut with a 4 mm border of sclera using scissors, washed in PBS supplemented with 4X pen/strep antibiotics, placed into the culture medium without FBS, and incubated in an incubator until the process started. The following processes were performed in a laminar flow hood. The corneas were transferred to a petri dish (10×12 cm), and the epithelial and endothelial layers of the cornea were removed by scrubbing and rinsing. The anterior limbal stroma (0.5-1 mm wide, 0.1 mm deep) was dissected and chopped into small pieces (approximately 2×2 mm) by a blade while immersed in 1 ml serum-free culture medium containing 1 mg/ml collagenase I. The chopped anterior limbal stroma was incubated overnight in serum-free DMEM/F12 medium containing 1 mg/ml collagenase I (each anterior limbal stroma from one cornea was digested in 1 ml collagenase solution) at 37°C and 5% Co2. Digested anterior limbal stroma was transferred into a 15 ml falcon through a cell strainer (70 µm) and centrifuged at 12000 rpm for 10 min. The supernatant was discarded, and the cell pellet was carefully pipetted into 1 ml pre-warmed culture medium. A 25 cm^2 flask filled with pre-warmed culture medium (DMEM-F12, FBS 10%, 1% penicillin/streptomycin, and amphotericin B) and isolated cells were seeded into the flask and placed in an incubator. The medium was changed every 48 h, and at a confluency of 60 – 80% (S2. A).

A spheroid forming assay was performed to evaluate the spheroid formation ability of CSSCs as a specific characteristic of CSSCs stemness (5, 6). CSSCs (first passage) at a density of 2000 cells were seeded in each well of an ultra-low attachment 6 well plate in DMEM-F12 medium containing 5% FBS and antibiotics. Spheroid formation by CSSCs was captured by an inverted fluorescence microscope on different days of culture by fluorescein diacetate (FDA) staining. The spheroid area was measured using ImageJ software (S2. C). Cell aggregation and spheroid formation of CSSCs at their first passage were evaluated within 6 days, which supported the stemness of isolated CSSCs as a specific characterization for these cells (S2. B). First-passage CSSCs were used for biocompatibility and differentiation evaluation.



S2. Schematic of CSSCs isolation from anterior limbal stromal region of rabbit cornea. CSSCs isolation (A) and Spheroid formation assay (B) for stemness ability of isolated CSSCs within 6-day observation of CSSCs culture with DMEM-F12 -%5 FBS.

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