Electronic Supplementary Material (ESI) for Polymer Chemistry

Supporting information

Facilely Prepared Inexpensive and Biocompatible Self-healing Hydrogel: A new injectable cell therapy carrier

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Experimental section

1. Materials and characterizations

Glycol Chitosan (Wako Pure Chemical Industry, Ltd.), cell culture media and fluorometric reagent were purchased from Invitrogen and used directly. DF-PEG was synthesized as our previous report[1]. Other reagents were purchased from Sinopharm Chemical Reagent and used without further purification.

Rheology analyses are performed on a TA-AR G2 rheometer with parallel plate geometry (20 mm in diameter) at 25 ^{o}C. Confocal microscopy images are performed on a Zeiss710-3channel confocal microscope.

2. Methods

2.1 Preparation of the hydrogel

As a typical method, a 3% (w/w) glycol chitosan solution was prepared by dissolving certain amounts of glycol chitosan in deionized water, and adjusts pH value to 7.0 by acetic acid. A 10% (w/w) DF-PEG solution was obtained by dissolving 1.0 g of the polymer in 9.0 g of deionized water. As a typical hydrogel preparation, DF-PEG$_{4000}$ aqueous solution was added to chitosan solution at 20°C (1.5 wt% glycol chitosan + 2.5 wt% DF-PEG$_{4000}$). The gelation occurred within ~60 s of vortex.

The gels for all other analyses were prepared using the same procedures. All % concentrations of solutions were presented based on mass (w/w).

2.2 Rheology analyses

The rheology analyses of the hydrogel were performed as our previous reports. A series of hydrogels were prepared with different wt% DF-PEG$_{4000}$ to test their mechanical properties. As a typical operation, glycol chitosan solution (0.23 g, 3% in acetic acid aqueous solution) was spread on a parallel plate (diameter: 20 mm). Then, DF-PEG aqueous solution (0.08 g, 10%) and deionized water (0.20 g) was evenly added dropwise onto the chitosan solution surface. The storage moduli G’ and loss moduli G’’ were measured as a function of time (Fig. 1A). For the modulus values

versus frequency analyses, the samples were prepared using the same method followed by the data collection (Fig. 1B).

2.3 Self-healing analyses

1) The dynamic hydrogel (stained with trypan blue) was induced to gel formation in a barrel of a syringe. And as a comparison, the gelatin (stained with rhodamine B) was induced to gel formation in another barrel of a syringe. After the gelation, gels were extruded through a 21-gauge needle directly into a water-containing bottle. The photos were taken after 30min (Fig. 2A). Additional experiment was carried out by punching a hole in the middle of both the self-healable hydrogel and the gelatin hydrogel. Photographs at different time intervals were taken to record the appearance of the united gel (Fig. S1).

2) Rheology analyses were carried out to monitor qualitatively the self-healing process. In brief, the profile of G' values and G" values to different amplitude were subsequently tested. Amplitude oscillatory forces were changed from γ = 200 to 1% under the same frequency (1.0 Hz) to test the recovery of mechanical properties of the hydrogel (Fig. 2B), and the process was repeated twice. Additional rheology analysis was also carried out to monitor the self-healing process. In brief, a gel was prepared as above described (1.5 wt% GCS and 2.2 wt% DF-PEG) and tested for the storage moduli G' (~2000 Pa, Fig. 2C). The gel was subsequently cut into 9 pieces on the plate, and the G' values versus time of the broken gel were recorded (Fig. 2D). After 15 min, the storage moduli G' of the self-healed hydrogel were recorded and compared with the original hydrogel.

2.4 Cytotoxicity evaluation of the DF-PEG

Cytotoxicity evaluation of DF-PEG4000 on Hela cells was determined by MTT assay. Cells were seeded in 96-well microplates at a density of 5×10^4 cells mL^{-1} in 160 μL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 0.5, 1.0, 3.0, 6.0, 9.0 mg mL–1 of DF-PEG_{4000} for 24 h. Then DF-PEG_{4000} was removed and cells were washed with PBS for three times. 100 μg of MTT dye dissolved in 200 μL of DMEM cell culture media was added to each well

and incubated for 4 h at 37 °C. The media was removed and 150 µL DMSO was added each wall. Plates were then analyzed with a microplate reader (VictorIII, Perkin-Elmer). Measurements of dye absorbance were carried out at 490 nm. The values were proportional to the number of live cells. The percent reduction of MTT dye was compared to controls (cells not exposed to DF-PEG_{4000}), which represented 100% MTT dye reduction. Five replicate wells were used for each control and test concentrations per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as mean ± standard deviation (SD). PEG_{4000} was also assayed using the same method (Fig. S2).

2.5 Cell Culture and Confocal Microscopy.

2.5.1 Cell Culture

Hela cells were cultured in RPMI1640 supplemented with 10% FBS, 5% penicillin and incubated at 37°C, 5% CO_2. The medium was changed every two days. The cells were harvested with PBS containing 0.025 (w/v) % trypsin and 0.01% EDTA, centrifuged and resuspended in the RPMI-1640 medium.

2.5.2 3D cell encapsulation

Hela cells were expanded to passage 4 in RPMI-1640 medium. Hela cells were resuspended in RP1640 media and mixed with glycol chitosan dissolved in the same media. The Hela/glycol chitosan solution was pipetted into a petri-dish, and DF-PEG_{4000} in RPMI-1640 media was then pipetted into the same dish and gently mixed to induce gel formation. All encapsulation studies were performed with 5×10^6 cells mL^{-1} in 1.5 wt% glycol chitosan + 1.0 wt% DF-PEG_{4000} gels. The gels were incubated at 37°C, 5% CO_2, and imaged either 24 h or 72 h later. To assess viability, constructs were rinsed with PBS, stained with FDA and PI, and visualized using confocal microscopy. Constructs were excited at 488 nm and 543 nm wavelengths to visualize live and dead cells, respectively, and z-stacks were taken through the depth of the gels to validate an even distribution of cells throughout. Viability is reported as the percentage of FDA stained cells compared to total cells (Fig. 3).

2.5.3 Injection Experiment

Hela cells were expanded to passage 4 in RPMI-1640 medium. Hela cells were resuspended in RPMI-1640 media and mixed with glycol chitosan dissolved in the same media. The Hela/glycol chitosan solution was pipetted into a barrel of a syringe, and DF-PEG_{4000} in RPMI-1640 media was then pipetted into the same barrel and gently mixed to induce gel formation. All encapsulation studies were performed with 5×10^6 cells mL-1 in 1.5 wt% glycol chitosan + 1.0 wt% DF-PEG_{4000} gels. Gels were extruded through a 21-gauge needle directly into a petri-dish, covered with excess RPMI-1640 media. After injection, the gels were incubated at 37°C, 5% CO_2, and imaged either 2 h or 24 h later. To assess viability, constructs were rinsed with PBS, stained with FDA and PI, and visualized using confocal microscopy. Constructs were excited at 488 nm and 543 nm wavelengths to visualize live and dead cells, respectively, and z-stacks were taken through the depth of the gels to validate an even distribution of cells throughout. Viability is reported as the percentage of FDA stained cells compared to total cells (Fig. 4).

Supporting data

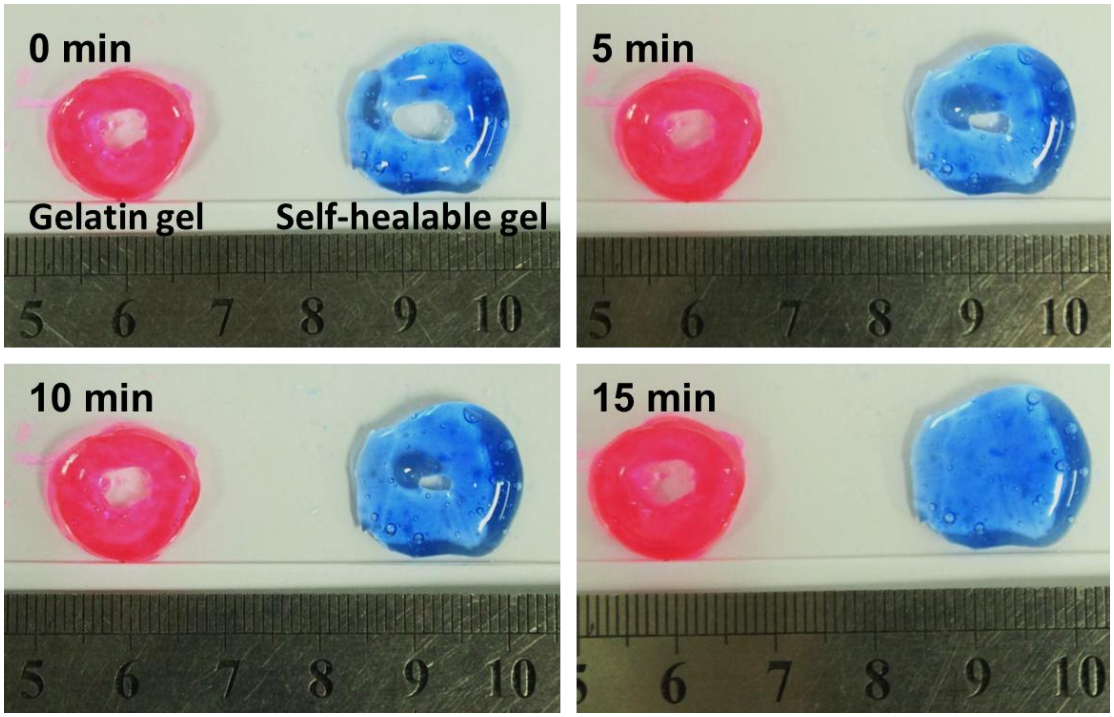

Figure S1. Appearance of self-healable hydrogels versus time (5% gelatin gel as control).

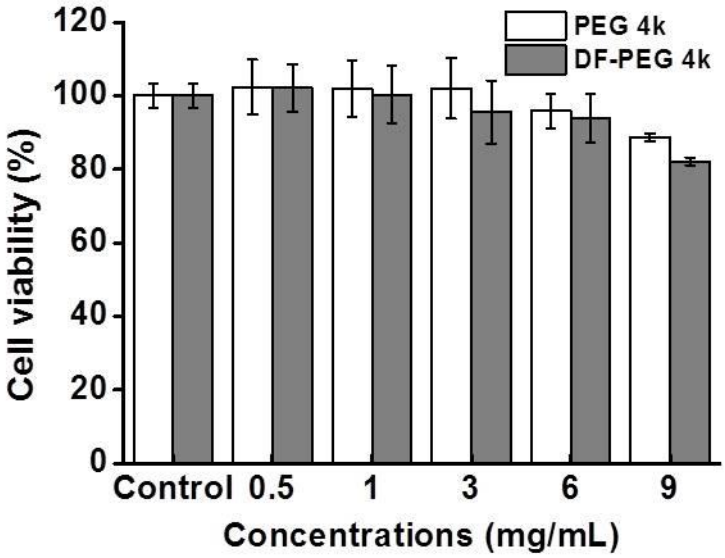

Figure S2. Cytotoxicity evaluation of the synthetic DF-PEG_{4000}, PEG_{4000} was used as the control.

(1) Y. Zhang, L. Tao, S. Li and Y. Wei *Biomacromolecules* 2011, **12**, 2894.