Supplementary Information

Ultrafast Spreading Effect Induced Rapid Cells Trapping into Porous Scaffold with Superhydrophilic Surface

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Experimental Section

Preparation of chitosan scaffolds: 200mg chitosan was dissolved in 100ml 1% (v/v) acetic acid solution, which concentration was controlled to be 20 mg/ml. Three doping rates between chitosan and cross-linker genipin of 100:1, 30:1 and 10:1 were prepared by adding different amount of genipin into suitable volumetric chitosan solutions. After cross-linking, the hydrogels were freeze-dried for 12h to obtain the porous chitosan scaffold. Finally the scaffolds were cut to square in size of 8mm×8mm×3mm (length × width × height). The porosity was measured by a pycnometer method. In brief, ethanol was added in a pycnometer fully, which weight was recorded as W_1. Chitosan scaffold that weight was recorded as Ws was immersed in the ethanol and then degassed in a vacuum container for 1h. The level of ethanol in the pycnometer would become lower during this process because ethanol was filled in the internal pores of scaffold occupied formerly by air. After being taken out from the vacuum container the pycnometer was

refilled with ethanol, the weight was recorded as W_2. Then the sample was taken out from the pycnometer, the rest of ethanol and pycnometer was weighted and recorded as W_3. Finally the porosity (ε) was calculated by the formula 1:

$$\varepsilon = (W_2 - W_3 - Ws)/(W_1 - W_3) \quad (1)$$

To achieve ultrafast spreading property on the prepared porous chitosan scaffold surface, the prepared scaffolds were exposed under UV light by UV/Ozone ProCleaner™ (UVO; Bioforce Nanoscience Inc., USA) for 0h, 1h and 2h. The surface wettability was measured on the OCA20 Contact Angle system (DataPhysics Instruments GmbH, Germany). The surface tension of the culture medium was measured by pendant-drop method by the OCA20 Contact Angle system. The relative viscosity of culture medium was measured by Ubbelohde viscometer. In brief, the relative viscosity is calculated by the formula 2:

$$\eta_r = \eta/\eta_0 = \rho t/\rho_0 t_0 \quad (2)$$

The ρ and ρ_0 are on behalf of the density of cell culture medium and water, ρ_0 is 0.998g/ml and ρ is 1.062g/ml. The t and t_0 represented the efflux time of cell culture medium and water, which was measured by an Ubbelohde viscometer. The average efflux time of water was 5728.33ms; the efflux time of culture medium was 6020.33ms. Therefore the relative viscosity of culture medium was 1.1183.

Cell culture experiment: Murine osteoblastic MC3T3-E1 cells were purchased from Cell bank of Academia Sinica (Shanghai, China). MC3T3-E1 cells were cultured at 37 ℃ in a 5% CO_2 atmosphere in α-MEM medium contained 10% FBS (Gibco, UK), 100U/ml penicillin and

100μg/ml streptomycin. The chitosan scaffolds were transferred to 24 well plates after treated by ultraviolet irradiation. 20μl cell suspension with a concentration of 1.33×10^6 cells/ml was seeded onto the chitosan scaffold in a drop-wise manner. After each time point, following tests were done: MTS, SEM and fluorescence staining. MC3T3 cells were stained by DIPI. All tests were performed in triplicate.

MTS assay: MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl) -2H-tetrazolium) (promega, USA) test was performed to determine the attachment ability of MC3T3-E1 cells on the prepared chitosan scaffolds with or without ultrafast spreading and permeating properties. After each time point (10min, 30min, 60min, 1day, 3days, 7days), chitosan scaffolds were transferred to a new 24-well plate and washed twice with sterilized PBS. 300μl culture medium without FBS and phenol red was mixed with MTS in a 5:1 ratio, added to each well, which could totally cover the constructs, and incubated for 3h at 37°C in a 5% CO_2 incubator. After the incubation period, 100μl of medium mixture in each well were transferred to 96-well plate in triplicate and absorbance was measured at 490nm using the automated Dynex plate Reader (Synergy HT, BioTek). Statistical analysis: All of the data were evaluated using One-Way ANOVA. All of the data were expressed as the mean and standard deviation. The data was considered to be significantly different at p values <0.05 or < 0.01.

Table S1. Porosities of the prepared chitosan CG100, CG30 and CG10 scaffolds.

Sample	CG100	CG30	CG10
Porosity (%)	73.6	80.2	96.1

Table S2. Water and culture medium CAs on the smooth chitosan film.

Sample	CG100	CG30	CG10
Water CA	101.0 ± 1.4^{o}	98.8 ± 1.5^{o}	107.7 ± 2.4^{o}
Culture medium CA	100.4 ± 1.6^{o}	94.2 ± 6.0^{o}	99.5 ± 3.2^{o}

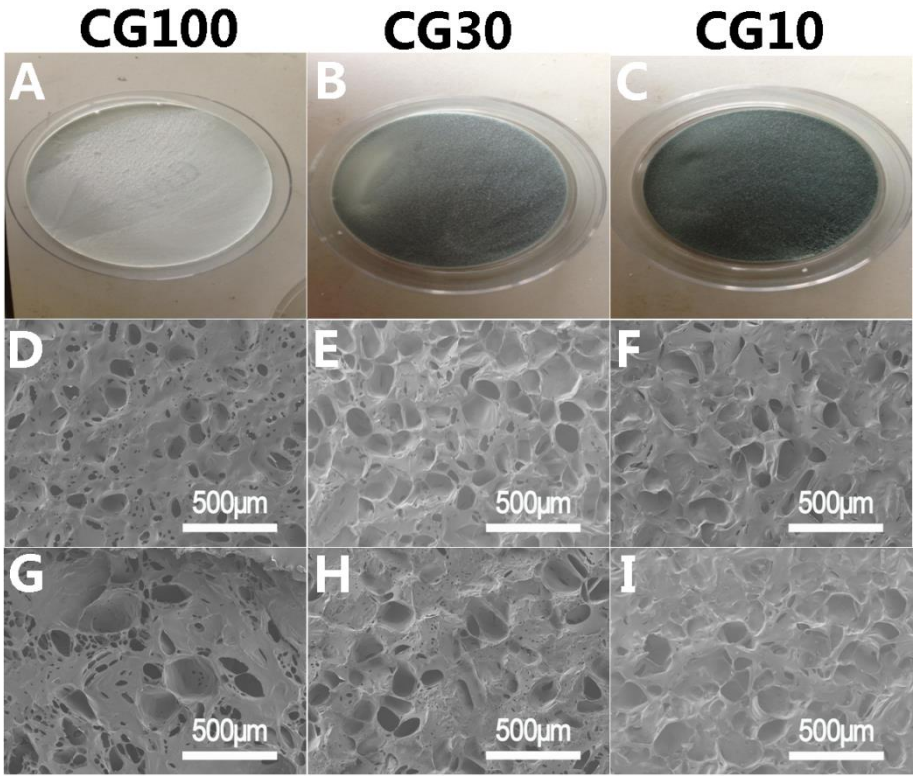

Figure S1 A), B) and C) Optical photos of prepared chitosan scaffolds CG100, CG30 and CG10; D), E) and F) SEM images of prepared untreated CG100, CG30 and CG10 chitosan scaffolds, correspondingly; G), H) and I) SEM images of CG100, CG30 and CG10 chitosan scaffolds treated by UV light for 2h correspondingly.

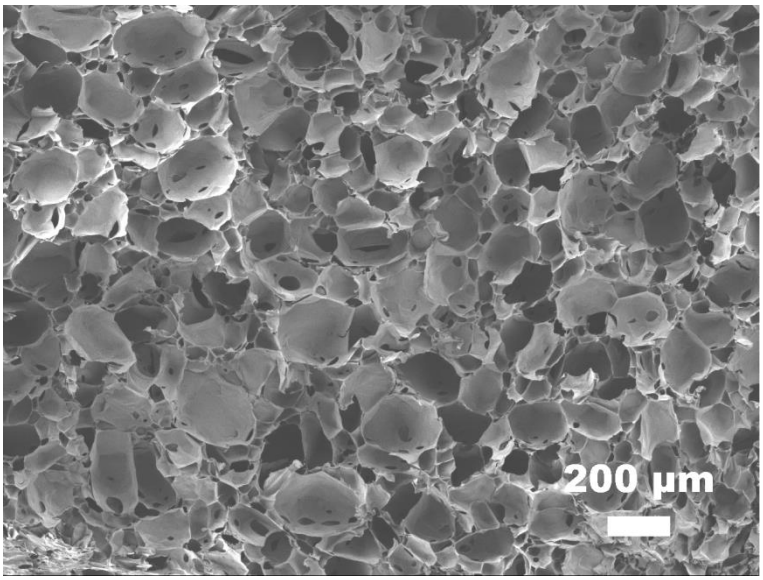

Figure S2 the SEM image of CG10 chitosan scaffold in profiled view.

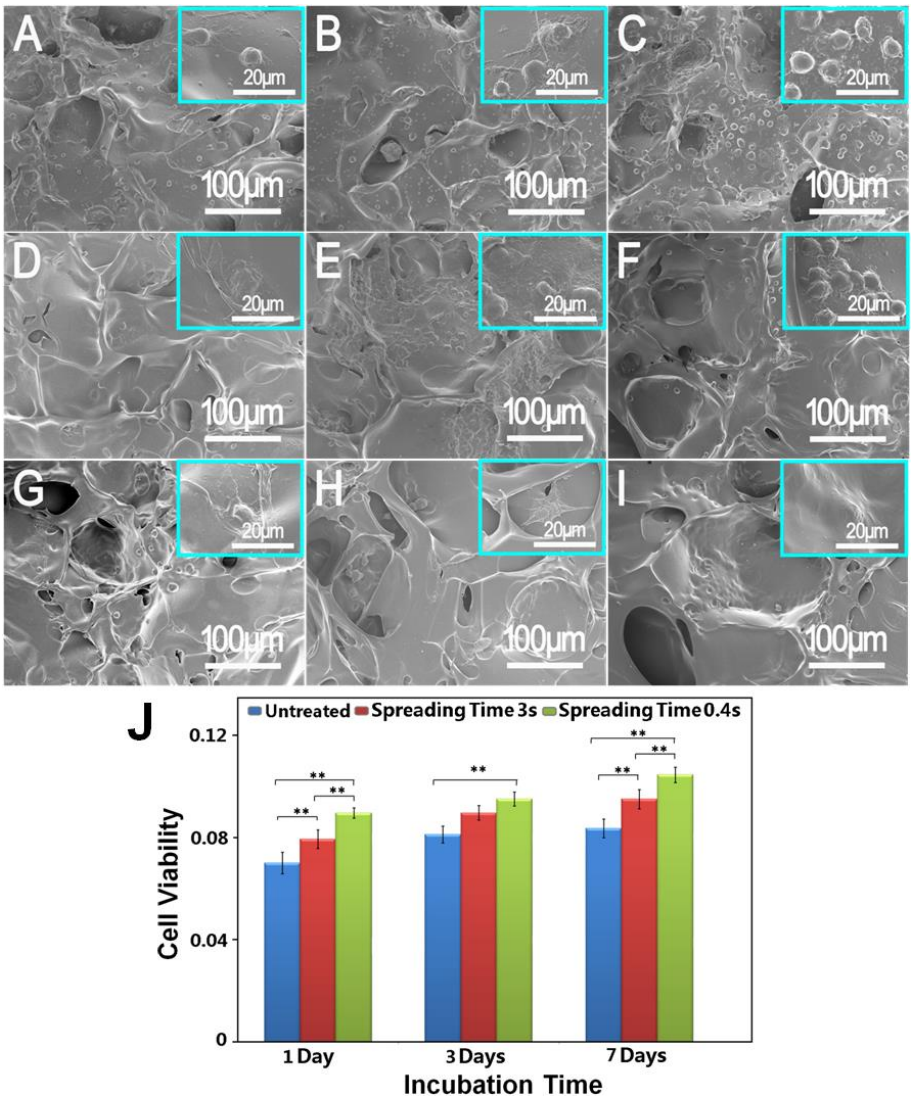

Figure S3 A), B) and C) SEM images of the untreated CG10 surface that were cultured by MC3T3 cells for 1day, 3days and 7days, correspondingly; D), E) and F) SEM images of the CG10 surface with 3s spreading time that were cultured by MC3T3 cells for 1day, 3days and 7days, correspondingly; G), H) and I) SEM images of the CG10 surfaces with 0.4s spreading time that were cultured by MC3T3 cells for 1day, 3days and 7days, correspondingly; J) Cell viability on CG10 surface with different spreading time in culture for 1day, 3days and 7days. Statistically significant difference compared to another group *($P<0.05$), **($P<0.01$).