

Supporting Information

Fast contact of solid-liquid interface created high strength multi-layered cellulose hydrogels with controllable size

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Cell viability assay

The samples were cut into powder and sterilized by autoclaving, and then used to prepare the extract. According to ISO 10993-5, a cell line of L929 was re-suspended in the culture medium and plated (200 μ L/well) into 96-well micrometer plates at 1×10^4 cells/well, which were incubated at 37 °C in a 5% CO₂ atmosphere for 24 h.¹ The medium was then replaced by 50 μ L/well sterilized extract, using the culture medium itself as a control. After incubating for 24, 48 and 72 h, the cells were treated with 20 μ L/well of MTT (5 mg/mL in PBS filtered for sterilization), to reach a final concentration of 0.5 mg MTT per mL, and incubated for a further 4 h at 37 °C in a 5% CO₂ atmosphere. At this stage, the MTT was removed and 150 μ L/well of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The plates were placed in an incubator at 37 °C to shake for 10 min. The absorbance values were read in triplicate against a reagent blank at a test wavelength of 490 nm (Tecan GENios, Tecan Austria GmbH, Salzburg, Austria).² Cell viability was calculated using the

following equation:

$$\text{Cell viability (\%)} = A_{\text{test}}/A_{\text{control}} \times 100\%$$

where A_{test} and A_{control} were the absorbance values of the test and control groups, respectively.

(1) C. Liu, Y. Chen, X. Wang, J. Huang, P. R. Chang, D. P. Anderson, Improvement in physical properties and cytocompatibility of zein by incorporation of pea protein isolate. *Journal of materials science* **2010**, *45*, 6775-6785.

(2) M. Serrano, R. Pagani, M. Vallet-Regí, J. Pena, A. Ramila, I. Izquierdo, M. Portoles, In vitro biocompatibility assessment of poly (ϵ -caprolactone) films using L929 mouse fibroblasts. *Biomaterials* **2004**, *25*, 5603-5611.

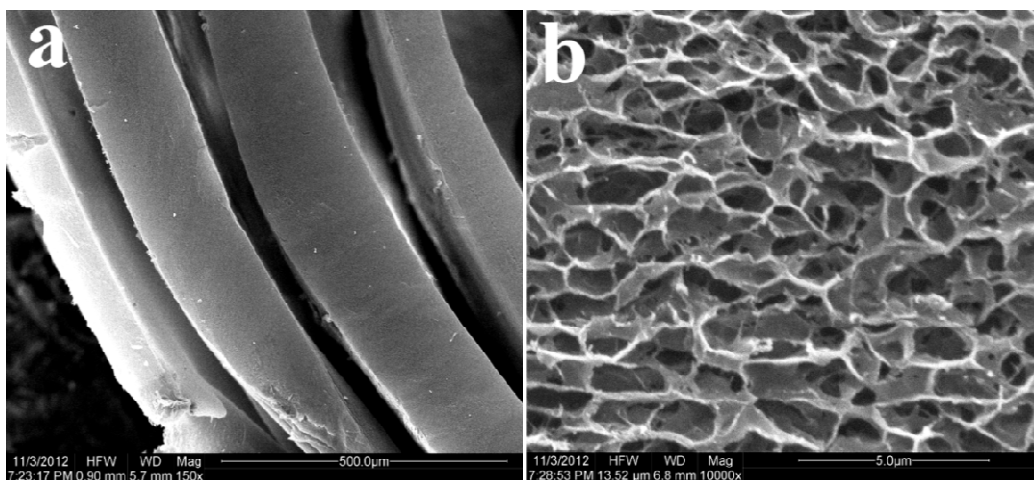


Figure S1. SEM images of the freeze-dried multi-layered cellulose gels of R3-10 frozen by liquid nitrogen.

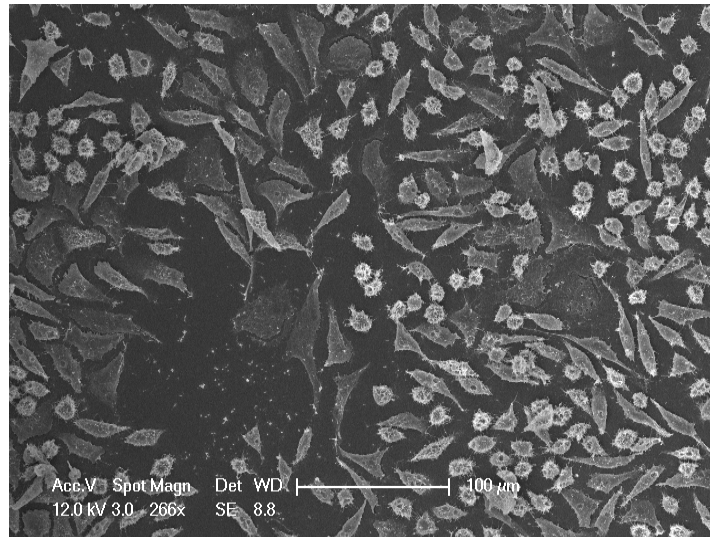


Figure S2. Morphology of the L929 cells cultured for 2 days on the control.

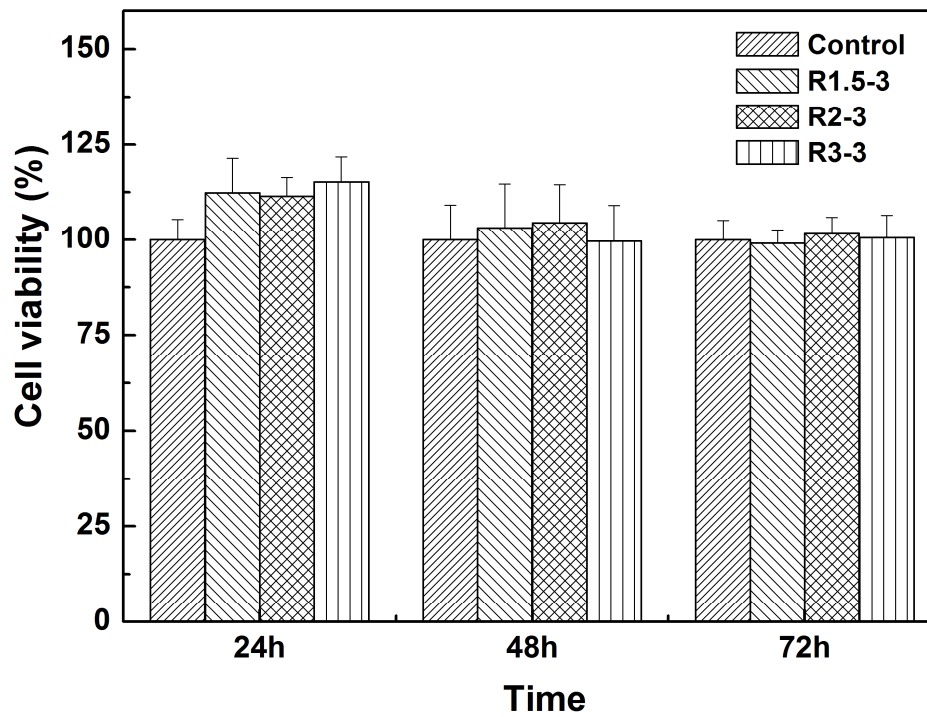


Figure S3. The results of the cytotoxicity tests of the R1.5-3, R2-3 and R3-3 gels.