Electronic Supplementary Material (ESI) for Biomaterials Science.

SUPPORTING INFORMATION

Chitosan-catechol: A writable bioink under serum culture media

Daiheon Lee,[a] Joseph P. Park,[a] Mi Young Koh,[b] Pureum Kim,[c] Jun Hee Lee,[c] Mikyung Shin,[a]* and Haeshin Lee[a]*

[a] Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, South Korea

[b] InnoTherapy Inc. 97 Uisadang-daero, Yeongdeungpo-gu, Seoul 07327, South Korea.

[c] Bio-Mechatronics Team, Division of Nano-Machinery, Korea Institute of Machinery and Materials(KIMM), Daejeon 34103, South Korea

* Co-corresponding authors equally contributed to this work.

Materials and Methods

Rheological properties of V-Chi-C solutions with various vanadyl concentrations

Chi-C was dissolved in DPBS solution (pH 7.4) at a concentration of 20 mg mL^{-1}. The vanadyl ion solution was prepared by dissolving vanadium oxide sulfate hydrate in DDW (0.2, 0.6, 1, 2, 20, 100, 200 and 2000 mM). To prepare V-Chi-C solutions, the vanadyl ion solution (50 µL; the final concentration = 0.01, 0.03, 0.05, 0.1, 1, 5, 10 and 100 mM) was added to Chi-C solutions (1 mL, 20 mg mL^{-1}). After 5 min, to evaluate rheological characteristics of V-Chi-C (20 mg mL^{-1}), a rotating rheometer (Bohlin Advanced Rheometer, Malvern Instruments, U.K.) was used. The solid-like behaviour (elastic modulus, G') and liquid-like behaviour (viscous modulus, G'') were recorded by a frequency sweep mode over the range from 0.1 to 10 Hz using a parallel plate with a diameter of 20 mm under a constant stress (100 Pa). To compare the G' moduli as a function of vanadyl ion concentration (0.01, 0.03, 0.05, 0.1, 1, 5, 10 and 100 mM), the G' measured at 1 Hz was used.

Cell cytotoxicity test in vanadyl ion-containing media

The L929 cells (100 µL, 1 x 10^5 cells per mL) were seeded in a 96-well plate (SPL Life Sciences, Korea). The cells were pre-cultured for 24 hrs in DMEM (FBS 10%) under 5% CO_2 at 37 ^oC. The vanadyl ion containing DMEM was prepared by dissolving vanadium oxide sulfate hydrate in DMEM (10% FBS) at a concentration of 0 to 2 mM. To evaluate the cell viability, the media of each well was replaced to vanadyl ion containing DMEM and incubated for 24 hrs. The viability (%) was determined by Cell Counting Kit-8 (CCK-8) assay kit (Dojindo Laboratories, Japan).

Long-term cell viability test

For encapsulating cells to the V-Chi-C scaffolds, L929 cells were cultured in DMEM (FBS 10%) under 5% CO_2 at 37 ^oC. The L929 cells (100 µL, 1 x 10^7 cells per mL) dispersed in DPBS were

mixed with V-Chi-C solution (1 mL, 20 mg mL^{-1}). The cell dispersed V-Chi-C solutions (300 µL) were injected into DMEM (3 mL, 10% FBS) using a 23 G needle (BD PrecisionGlide™). The media was freshly replaced every 2 days and incubated for 5 days. After incubation, the scaffolds were stained using LIVE⁄DEAD® Viability⁄Cytotoxicity Kit (Invitrogen, Carlsbad, CA, U.S.A.), containing calcein AM and ethidium homodimer (EthD-1). The scaffold was treated with 2 µM of calcein AM and 4 µM of EthD-1 for 30 min. The fluorescent images were observed using a laser scanning confocal microscope (Eclipse Ti, Nikon Instruments, Japan). The cell viability was calculated by measuring the raw integrated density values (sum of pixel values) of green dots for live and red ones for dead cells using Image-J software. The 6 layers were randomly chosen within the printed scaffold, and were used for analysing raw integrated density values.

Results

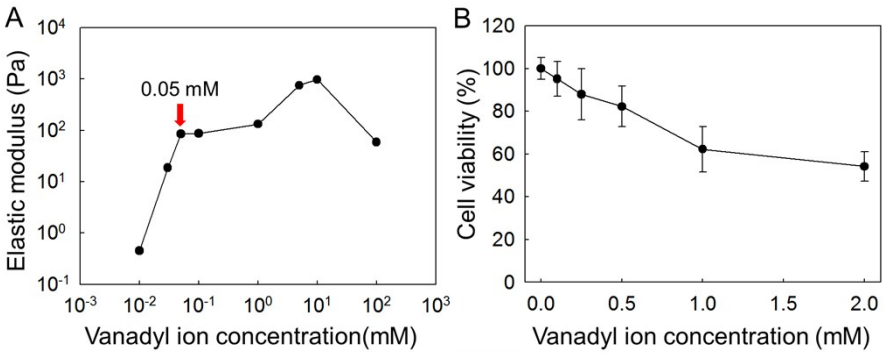

Fig. S1 A. Elastic modulus of V-Chi-C as a function of vanadyl ion concentration. B. Evaluation of L929 cell viability cultured in vanadyl ion containing DMEM for 24 hrs.

Table S1 The raw integrated density values measured from the six layers of the printed V-Chi-C scaffold.

Layer number	Green dots	Red dots	Total dots	Green dots /Total %
1	968,545	175,986	1,144,531	84.6
2	737,285	78,020	815,305	90.4
3	447,549	26,023	473,572	94.5
4	645,824	123,543	769,367	83.9
5	326,625	11,760	338,385	96.5
6	1,533,013	130,875	1,663,888	92.1

Average	90.4

St. Dev.	4.7

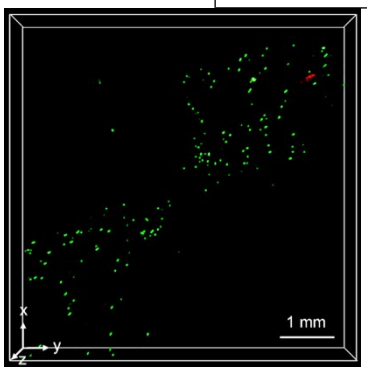

Fig. S2 Z-stacked confocal image of the L929 cell encapsulated in V-Chi-C bioink after 5 days incubation. Green dots for live and red ones for dead cells.

Table S2 The raw integrated density values detected in six layers of the structure injected with V-Chi-C bioink after 5 days incubation.

Layer number	Green dots	Red dots	Total dots	Green dots /Total %
1	34,000	1,020	35,020	97.1
2	37,745	2,774	40,519	93.2
3	66,558	13,022	79,580	83.6
4	92,092	29,639	121,731	75.7
5	56,296	11,312	67,608	83.3
6	25,330	425	25,755	98.3

Average	88.5
St. Dev.	8.3