Biomaterials science

Supplementary materials

A Chondroitin Sulfate based Injectable Hydrogel for Delivery of Stem

Cells in Cartilage Regeneration

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Materials and Methods

Materials

Chondroitin sulfate (A sodium salt from bovine trachea, chondroitin-4-sulfate), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI), dithiothreitol (DTT), deuterium oxide, sodium phosphate, Ethylenediaminetetraacetic acid (EDTA), L-Cysteine hydrochloride monohydrate and 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) were obtained from Merck, and 3,3'-Dithiobis(propionohydrazide) (DTP) was purchased from Alfa chemistry.

Synthesis and characterisation of CS-SH

Thiol functionalised CS was synthesised as reported previously¹: CS (1 g) and DTP (0. 95g) was dissolved thoroughly in deionised water (100 mL) and stirred at room temperature. The pH of the reaction solution was maintained at 4.75 for 2 h. EDCI (770 mg) was then added into the solution and the reaction was stopped by raising the pH of the mixture to 7.0. Before adjusting the pH to 8.5, 4.01 g of DTT was added. The mixture was then stirred at room temperature overnight. The reaction mixture was adjusted to pH 3.5 and transferred to dialysis tubing (Mw cut off 8 kDa). After dialysis against deionised water for three times, the CS-SH was lyophilised and stored in a -20 °C freezer under the protection of argon.

The substitution degree (SD) of CS-SH was characterised by proton nuclear magnetic resonance (¹H-NMR). ¹H-NMR analysis was carried out on a 400 MHz Varian NMR system spectrometer with MestReNova processing software (Mestre Laboratories, Spain). The chemical shifts were referenced to the deuterium oxide (D₂O, 4.80 ppm).

The thiol content of CS-SH was analysed using a revised Ellman's method and light absorption of thiolated polymer aqueous solution was measured with a wavelength of 412 nm *via* UV-vis spectrometer (Molecular Devices, Spectramax M3). All samples were measured in triplicates. Table S1. Gene name and primer ID applied for RT-qPCR analysis.

Reference ID	Gene Name
Rn99999916_s1	GAPDH
Rn01751070_m1	Sox9
Rn01637087_m1	Col2a1
Rn00573424_m1	Acan
Rn00580432_m1	ll1b
Rn01410330_m1	IL6



Fig. S1 The summary of storage modulus hydrogels fabricated with 0.5%, 1%, and 2% CS-SH and various HB-PEG concentrations of 2.5% and 5% after 30 mins at 37 $^{\circ}$ C.



Fig. S2 Percentage of live cells to total cell number at 7 days post encapsulation determined by LIVE/DEAD assay (Mean \pm SD, n = 3). Note: there is no significant difference between all groups (p < 0.05).

Reference

1 X. Z. Shu, Y. Liu, Y. Luo, M. C. Roberts and G. D. Prestwich, 2002, 1304–1311.