Supporting Information

Microencapsulation improves chondrogenesis *in vitro* and cartilaginous matrix stability *in vivo* compared to bulk encapsulation

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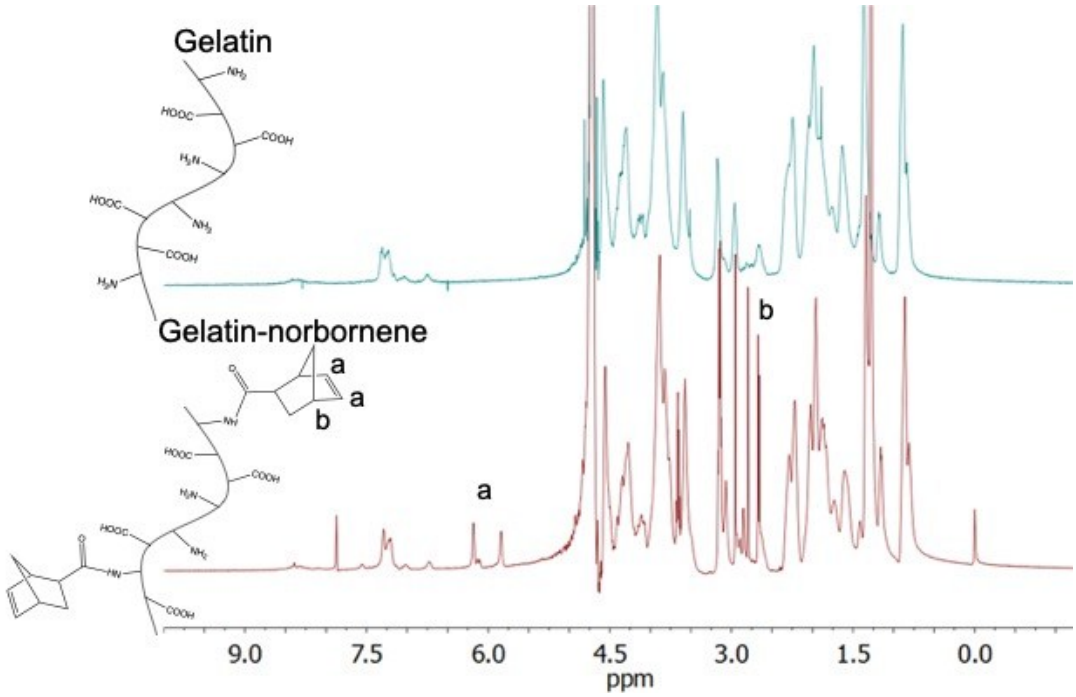
Gelatin-norbornene synthesis

5-norbornene-2-carboxylic acid (1.00 g, 7.20 mmol) (Sigma) was first dissolved in 15 mL dichloromethane (DCM) (Merck Millipore). Then, *N*-hydroxysuccinimide (1.12 g, 9.72 mmol) (Sigma) was dissolved in DCM (5 mL) and mixed into the previous norbornene solution with gentle swirling. To this mixture, 1.79 g of *N*-(3-(dimethylamino)propyl)-*N'*-ethylcarbodiimide hydrochloride (9.36 mmol) was added, the final mixture was stirred and the reaction was allowed to continue over 15 h. The mixture was washed with 40 mL deionised water, dried with anhydrous magnesium sulphate ($MgSO_4$) and concentrated *in vacuo*. The resultant product was used directly in the next step with no further purification.

Next, type A gelatin (1.00 g) (Porcine skin, Sigma) was dissolved in 20 mL *N,N*-dimethylformamide (DMF)/water (1:1 v/v), stirred at 37 °C and recovered to room temperature when fully dissolved. Then, the above intermediate product (59 mg, 0.025 mmol) was dissolved in 5 mL DMF and further added into the gelatin solution with additional 50 µL *N,N*-diisopropylethylamine (DIPEA) (Sigma) and stirred at ambient temperature for 10 h. The resultant product was transferred into a dialysis tube with 3.5 kDa cut-off and dialysed against deionised water in excess of water, changed over 5 days. The resulting purified solution was lyophilised to yield the final cotton-like GelNB product. Norbornene

functionalisation was confirmed via ^{1}H NMR. The percentage of substitution was characterised by fluoroaldehyde assay (ThermoFisher) by examining the content of amine groups before and post the funcrtionlisation process following the protocol provided by manufacturer. The norbornene substitution of the gelatin was measured to be 48% with the L-lysine as the reference.

A



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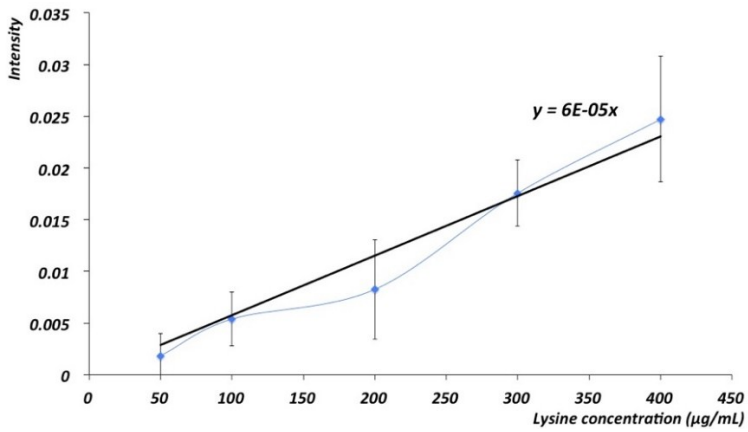

Figure S1. (A) ^{1}H NMR of raw gelatin and Gelatin-norbornene (GelNB), the sample contains trace of D_2O, (B) The standard curve of fluorescence intensity versus amine concentration based on different L-lysine concentrations

PEGdiSH synthesis

4 g of PEG (2 mM, Mw = 2kDa) and 600 mg of triethylamine (6 mM) were dissolved in DCM (20 mL) and cooled on ice. Then, 458 mg of methanesulfonyl chloride (4 mM) were added dropwise with stirring over 30 min. The mixture was stirred for 4 h, and a white powder was precipitated in 100 mL diethyl ether.

The resultant product with additional potassium thioacetate (912 mg, 8 mM) was dissolved in 10 mL DMF. The solution was stirred and allowed to continue overnight at room temperature before being concentrated *in vacuo*. The mixture was dissolved in DCM (20 mL) and washed with 10 mL of brine and 10 mL of water, then dried over anhydrous $MgSO_4$. The dried solution was then evaporated *in vacuo*. The concentrated product was subsequently redissolved in HCl (5 N, 20 mL) and heated to reflux at 120 °C under Dean-Stark conditions overnight. The resultant product was extracted in DCM (20 mL x 3), dried over anhydrous $MgSO_4$ and concentrated to 5 mL. The final product was precipitated into diethyl ether as a white powder. ^{1}H NMR confirmed the thiol functionalisation of PEG. Ellman's test was conducted to determine the amount of free thiol from the synthesised PEG-diSH. an aqueous solution of PEG-SH (1.5 mL, 0.05 mM, Tris buffered pH 7.4) in a quartz cuvette equipped with a stirring bar was first corrected as background on the Cary60. To this solution was added a solution of 5,5'-dithiobis-(2-nitrobenzoic acid) (0.2 mM in Tris buffered pH 7.4) The solution was allowed to react with stirring in the dark for 10 min and the absorbance at 412 nm was

recorded. L-cysteine solutions (0.025-0.2 mM) were used as standards for calculation of the amount of thiol release. Each measurement was done in triplicate.

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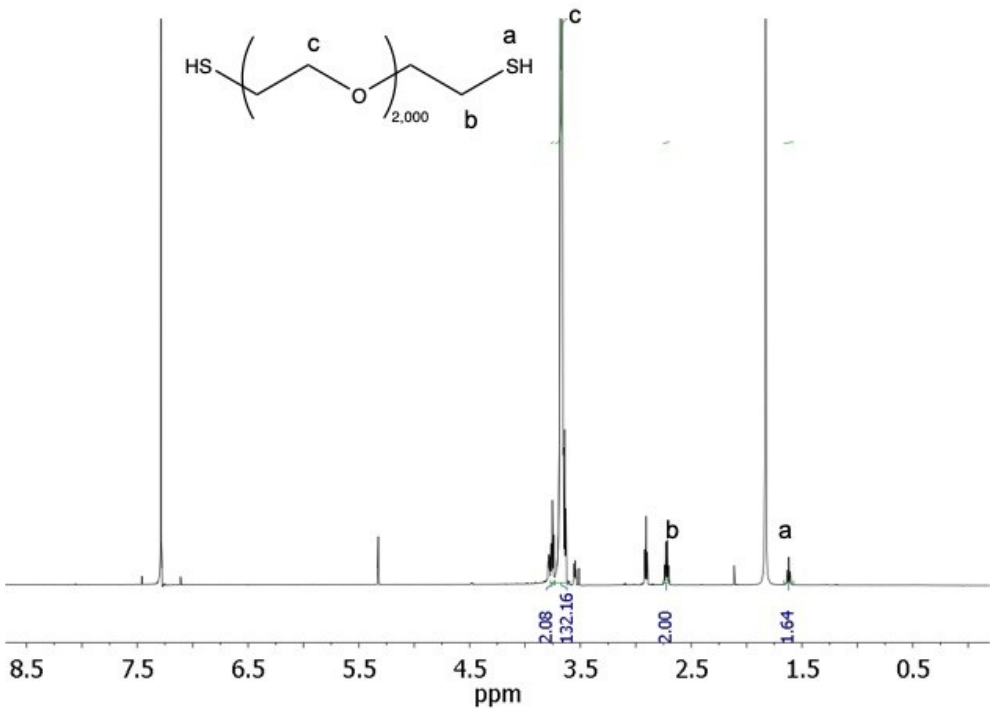

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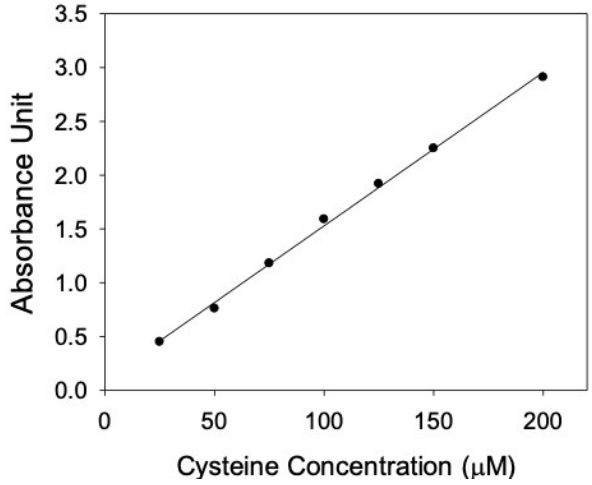

Figure S2. (A) ^{1}H NMR of PEG-diSH, the sample contains trace of acetone and dichloromethane, (B) Cysteine calibration curve for Ellman's test.

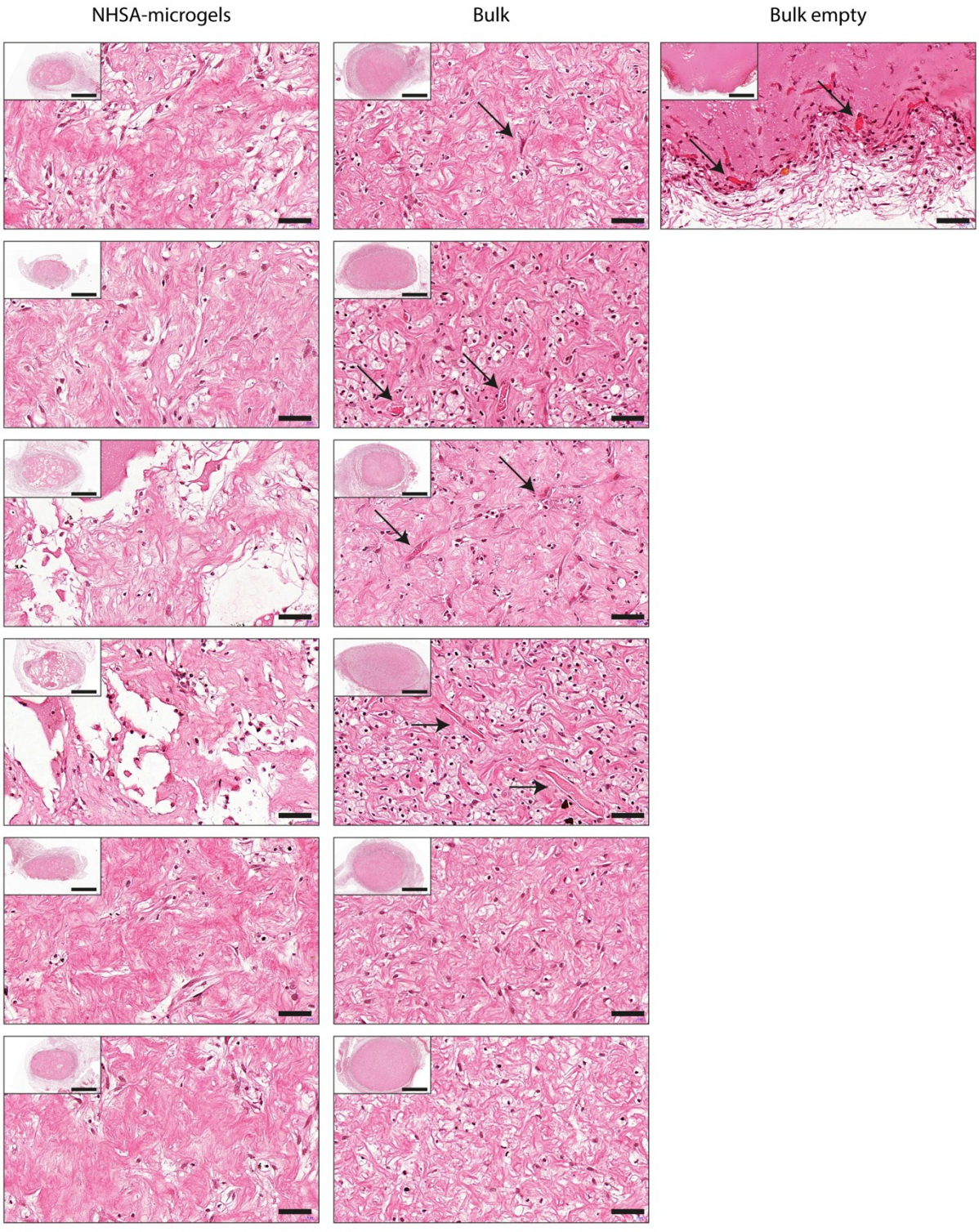

Figure S3. H&E staining for hCC-laden bulk hydrogels, NHSA-microgels and bulk empty gel (without cells) after in vivo chondrogenesis at day 49.

Table S1. Primers used for RT-qPCR

mRNA [Human]	Primers		Ref.
Sox-9	Forward	*5'-TCT GGA GAC TTC TGA ACG AGA GC-3'*	NM_000346.3
	Reverse	*5'-TGT AAT CCG GGT GGT CCT TC-3'*	
Aggrecan	Forward	*5'-GAA TGG GAA CCA GCC TAT ACC-3'*	NM_01135_3
	Reverse	*5'-TCT GTA CTT TCC TCT GTT GCT G-3'*	
Col2A1	Forward	*5'-GGA ATT CGG TGT GGA CAT AGG-3'*	NM_001844
	Reverse	*5'-ACT TGG GTC CTT TGG GTT TG-3'*	
Col1A1	Forward	*5'-CAG CCG CTT CAC CTA CAG C-3'*	NM_000088
	Reverse	*5'-TTT TGT ATT CAA TCA CTG TCG CC-3'*	
RPL13a [1]	Forward	*5'-AAG TAC CAG GCA GTG ACA G-3'*	NM_012423
	Reverse	*5'-CCT GTT TCC GTA GCC TCA TG-3'*	
GAPDH	Forward	*5'-AGT CAG CCG CAT CTT CTT TT-3'*	NM_002046
	Reverse	*5'-CCA ATA CGA CCA AAT CCG TTG-3'*	

1. D. Studer, S. Lischer, W. Jochum, M. Ehrbar, M. Zenobi-Wong and K. Maniura-Weber, *Tissue Engineering Part C: Methods*, 2012, **18**, 761-771.

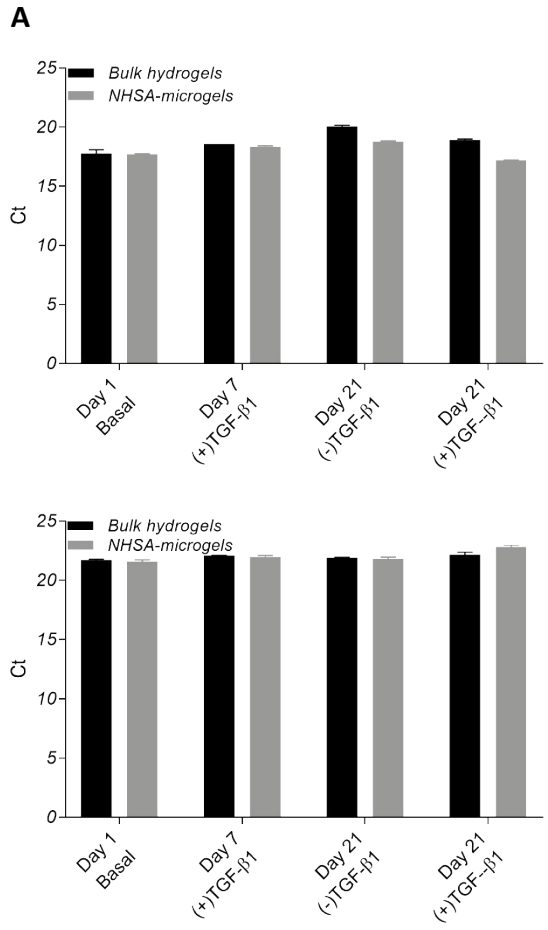

Figure S4. Housekeeping genes quantification Ct results. (A) GAPDH; (B) RPL13a. RPL13a values were more stable across all the experimental conditions compared to GAPDH data. RPL13a was determined as the reference gene for other target genes.