

## **Supporting information**

### **Cross-reactive alginate derivatives for the production of dual ionic-covalent hydrogel microspheres presenting tunable properties for cell microencapsulation**

Luca Szabó,<sup>1</sup> Carmen Gonelle-Gispert,<sup>2</sup> Elisa Montanari,<sup>2</sup> François Noverraz,<sup>1</sup> Aurélien Bornet,<sup>3</sup> Léo H. Bühler,  
Sandrine Gerber-Lemaire<sup>1\*</sup>

<sup>1</sup>Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne, Group for Functionalized Biomaterials, EPFL SB ISIC SCI-SB-SG, Station 6, CH-1015 Lausanne, Switzerland

<sup>2</sup>University Hospital of Geneva, Surgical Research Unit, CMU-1, CH-1211 Geneva, Switzerland

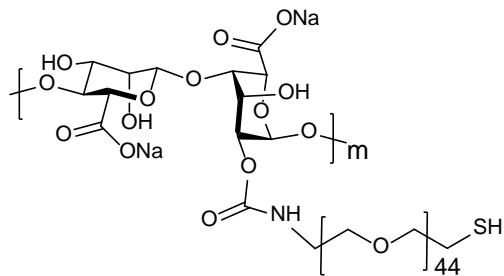
<sup>3</sup>Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne, NMR service, Batochime, CH-1015 Lausanne, Switzerland

\*E-mail: [sandrine.gerber@epfl.ch](mailto:sandrine.gerber@epfl.ch)

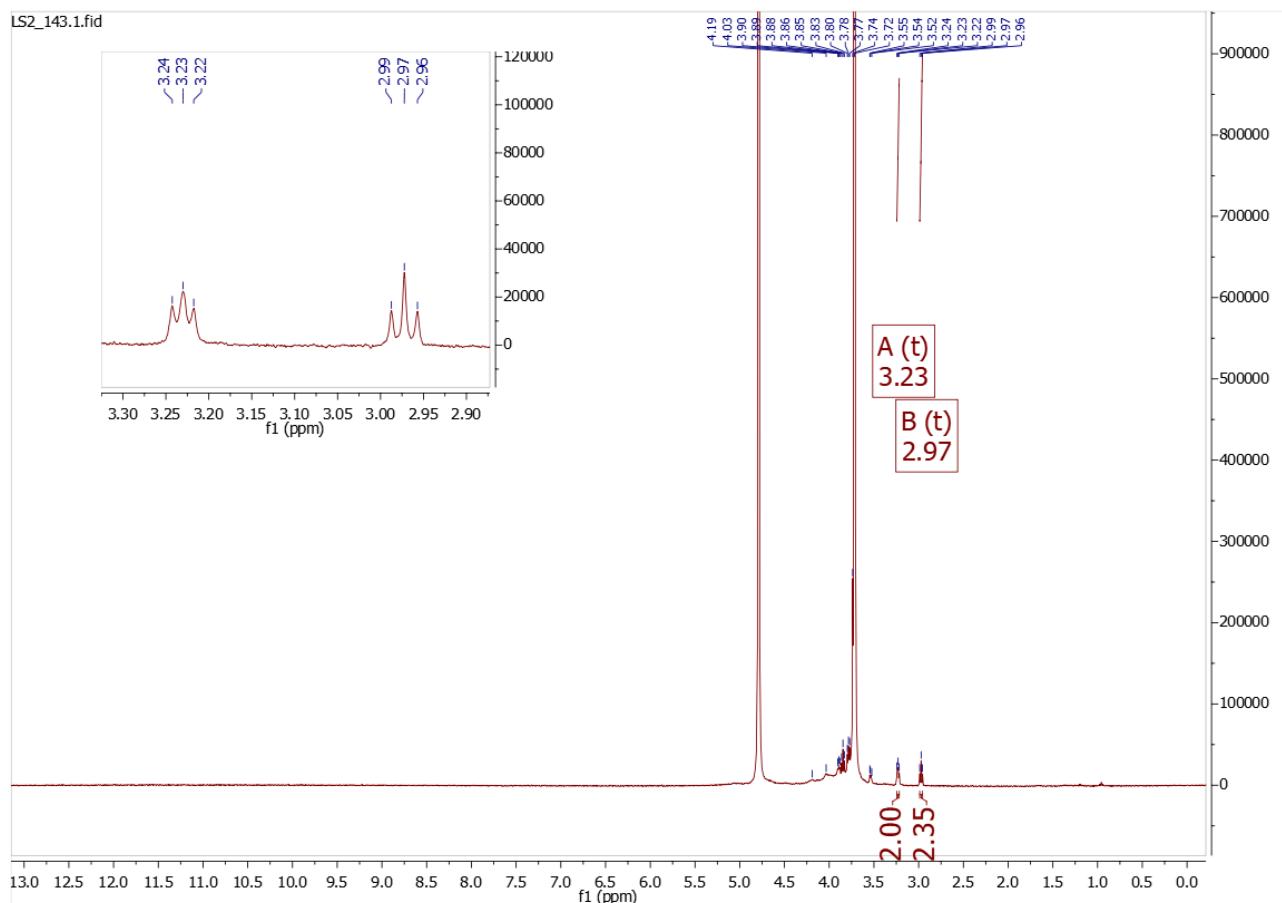
## Table of contents

Preparation of <b>Alg-SH</b>	S-3
Preparation of <b>Alg-ACR</b>	S-4
Preparation of <b>Alg-MAL</b>	S-5
Representative DOSY NMR spectrum of <b>Alg-ACR</b>	S-6
Superimposition of deconvoluted spectra and raw <sup>1</sup> H NMR data	S-7
Exposition of Alg-SH:Alg-ACR and Alg-SH:Alg-MAL MS to monovalent non-gelling ions	S-8
Encapsulation of Huh7 cells in Alg-SH:Alg-ACR and Alg-SH:Alg-MAL MS	S-9
Transplantation of Alg-SH:Alg-ACR and Alg-SH:Alg-MAL MS in immune competent mice	S-10

### Preparation of Alg-SH

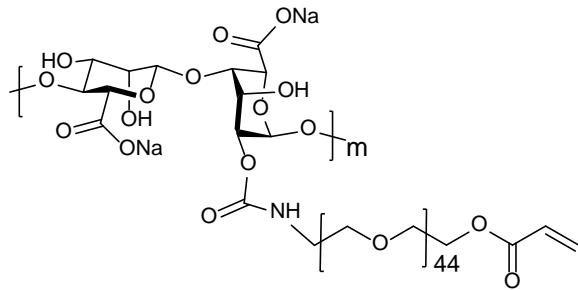


<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ 4.19 – 3.52 (m, CH-Alg + CH<sub>2</sub>-CH<sub>2</sub>-O), 3.23 (t, J = 5.0 Hz, 2H, CH<sub>2</sub>-SH), 2.97 (t, J = 6.1 Hz 2H, CH<sub>2</sub>-NH-C(O)-O).

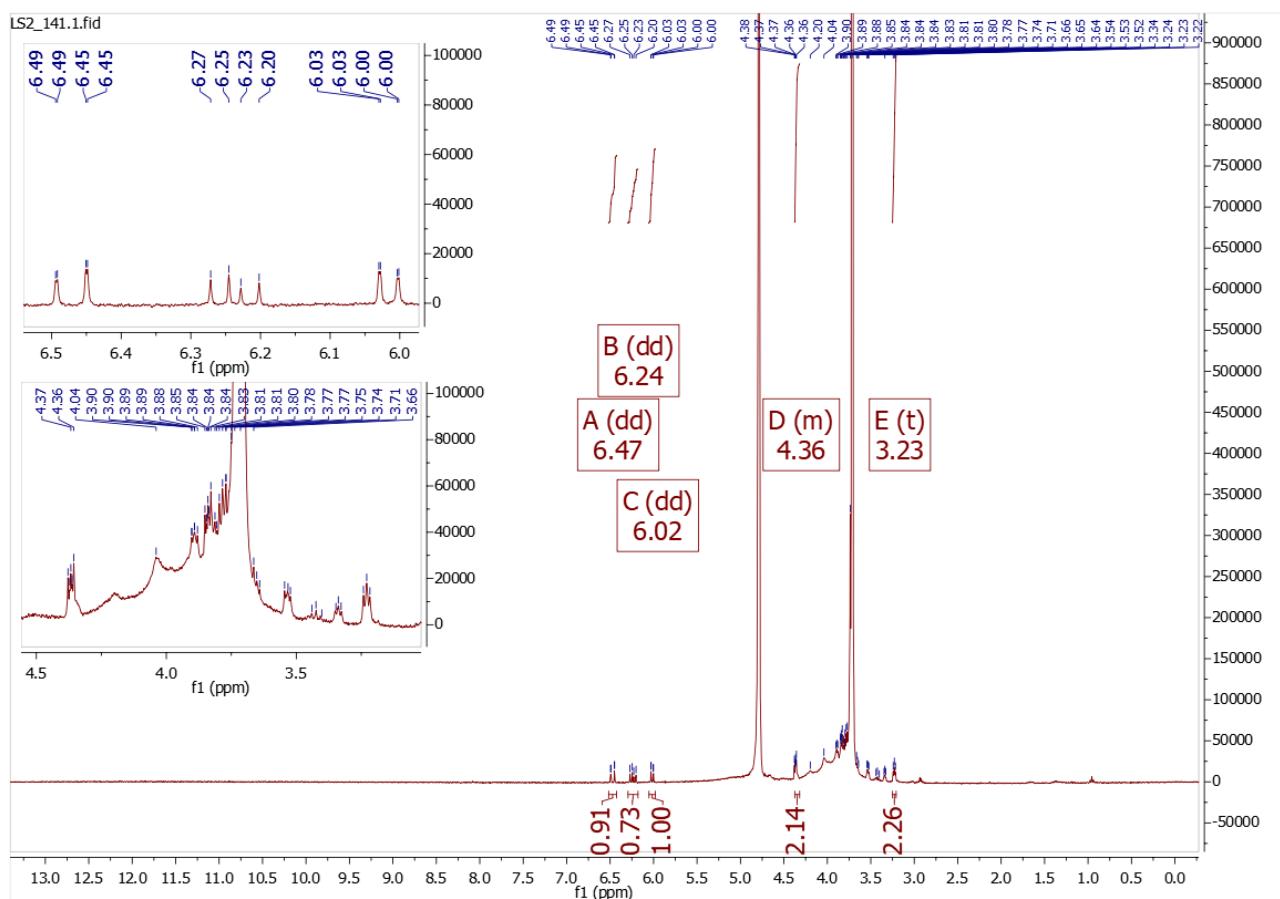


**Figure S1.** <sup>1</sup>H NMR spectrum of Alg-SH

**Preparation of Alg-ACR**

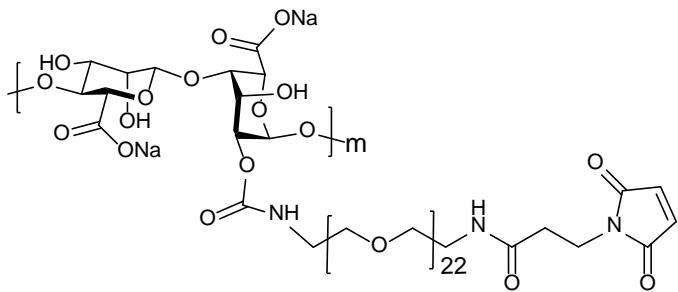


$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  6.47 (dd,  $J = 17.3, 1.0$  Hz 1H,  $\text{CH}_2=\text{CH}-\text{O}$ ), 6.24 (dd,  $J = 17.3, 10.5$  Hz 1H,  $\text{CH}_2=\text{CH}-\text{O}$ ), 6.02 (dd,  $J = 10.7, 0.9$  Hz, 1H,  $\text{CH}_2=\text{CH}-\text{O}$ ), 4.37 – 4.36 (m, 2H,  $\text{CH}_2-\text{O}-\text{C}(\text{O})$ ), 4.20–3.33 (m,  $\text{CH}$ -Alg +  $\text{CH}_2-\text{CH}_2-\text{O}$ ), 3.23 (t,  $J = 4.9$  Hz, 2H,  $\text{CH}_2-\text{NH}$ ).

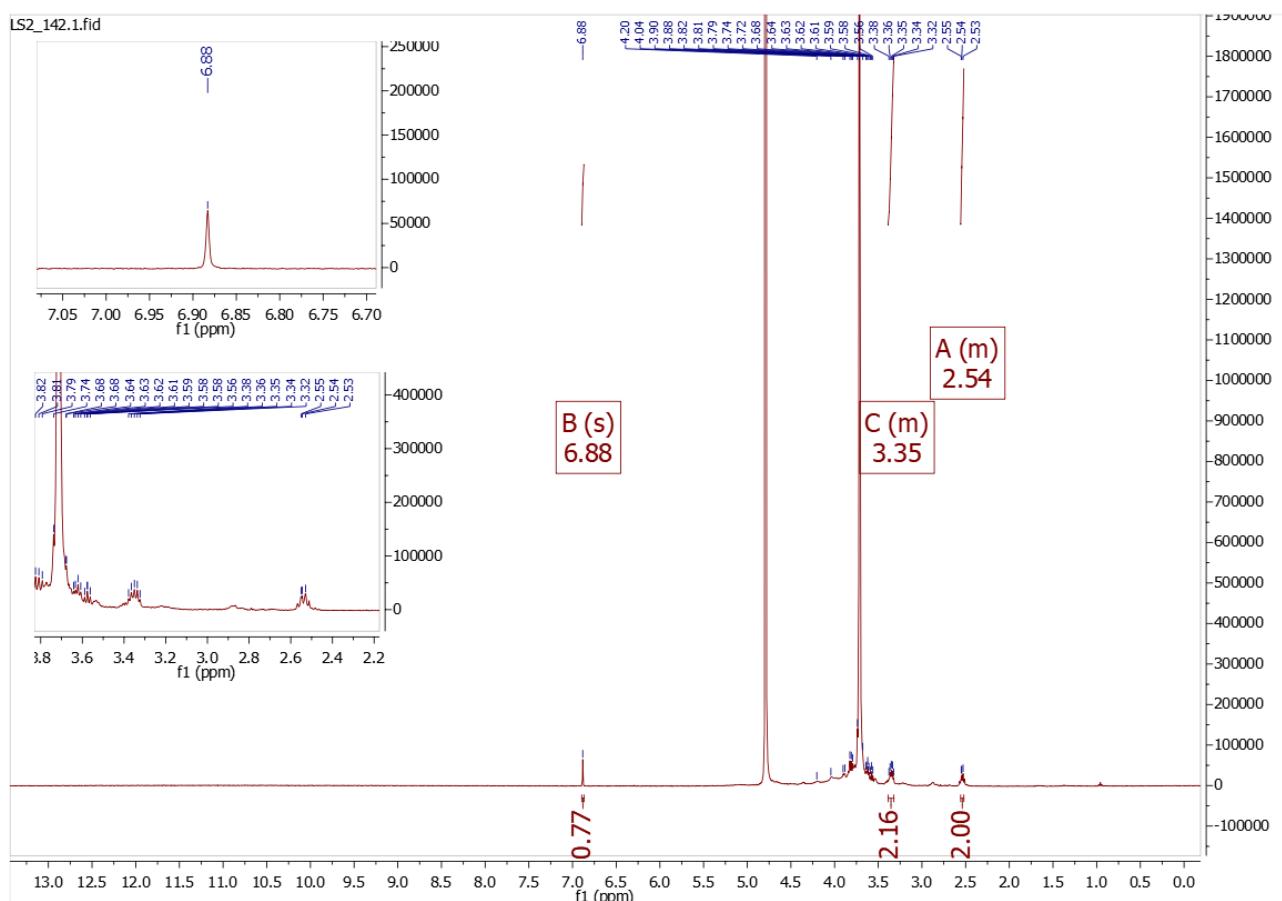


**Figure S2.**  $^1\text{H}$  NMR spectrum of Alg-ACR

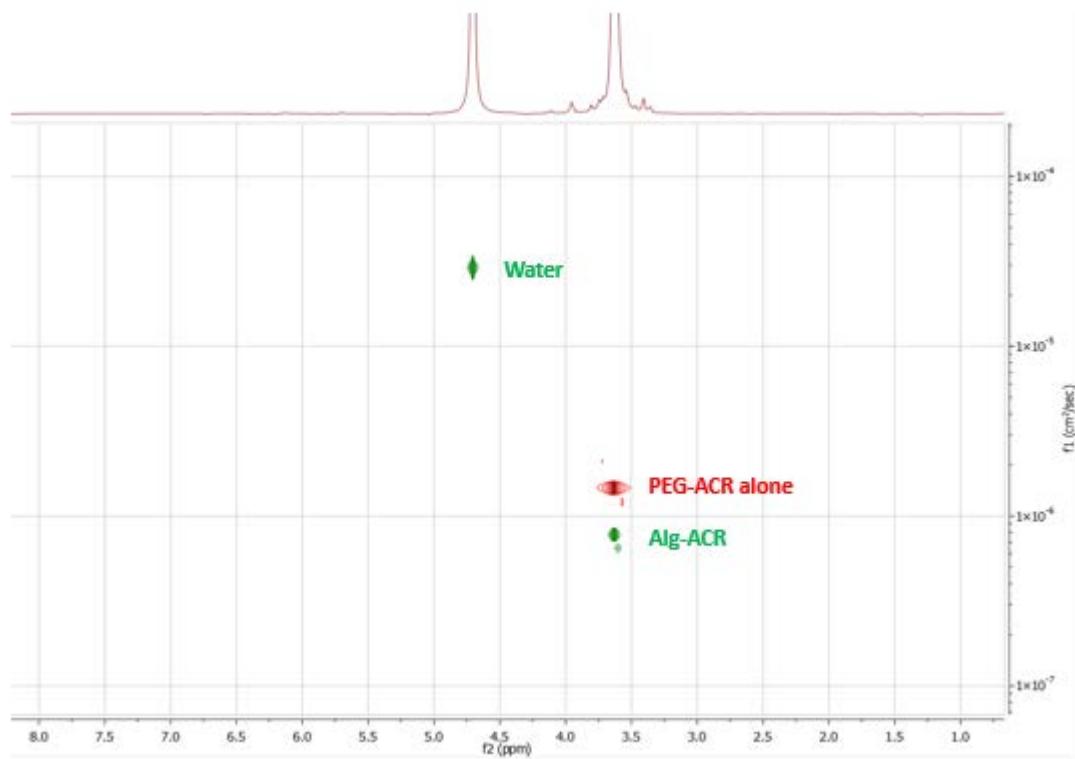
### Preparation of Alg-MAL.



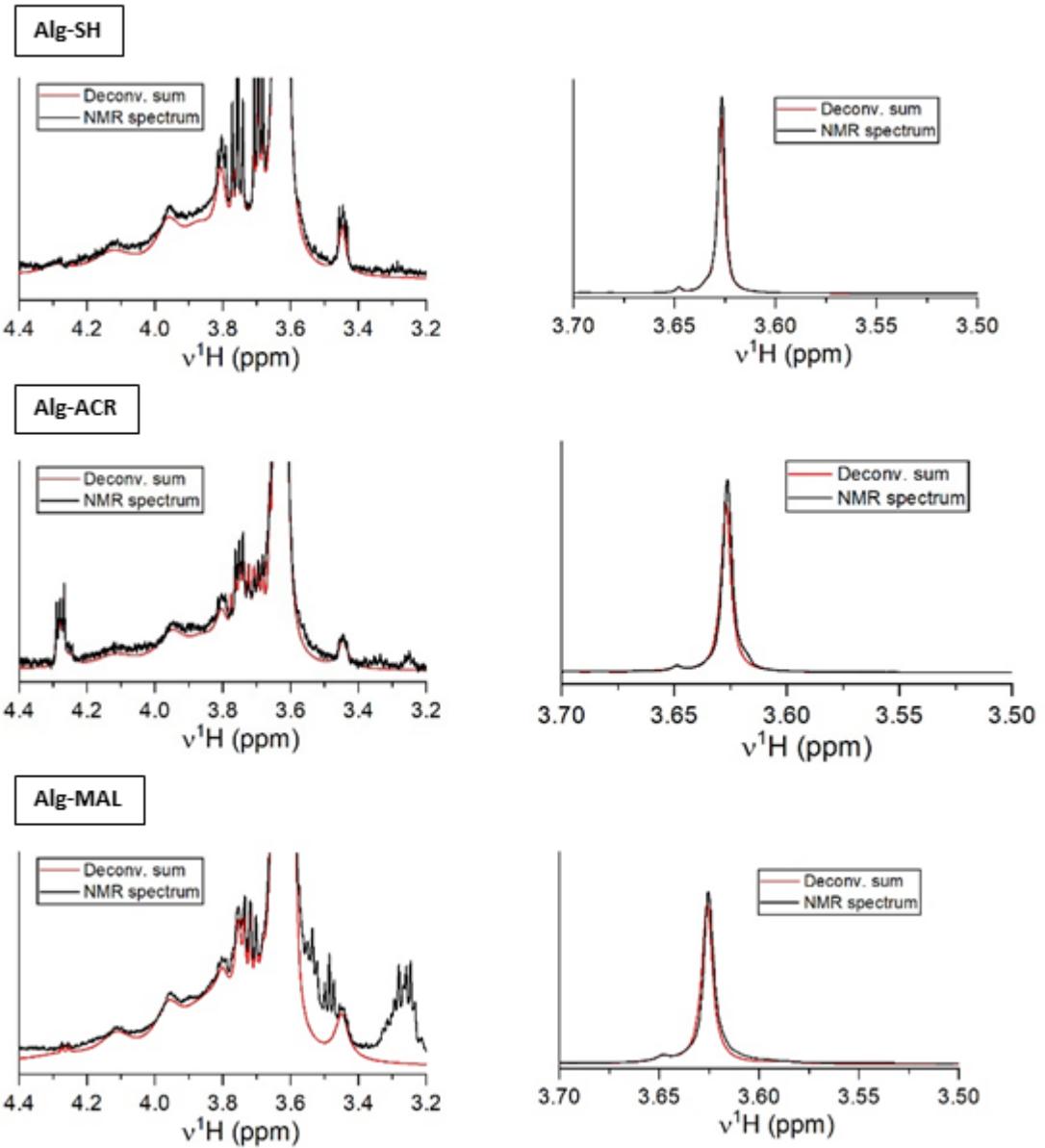
<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ 6.88 (s, 2H, 2x =CH), 4.20 – 3.56 (m, CH-Alg + CH<sub>2</sub>-CH<sub>2</sub>-O), 3.39 – 3.32 (m, 2H, CH<sub>2</sub>-NH), 2.56 – 2.52 (m, 2H, CH<sub>2</sub>-C(O)-NH).



**Figure S3.**  $^1\text{H}$  NMR spectrum of Alg-MAL



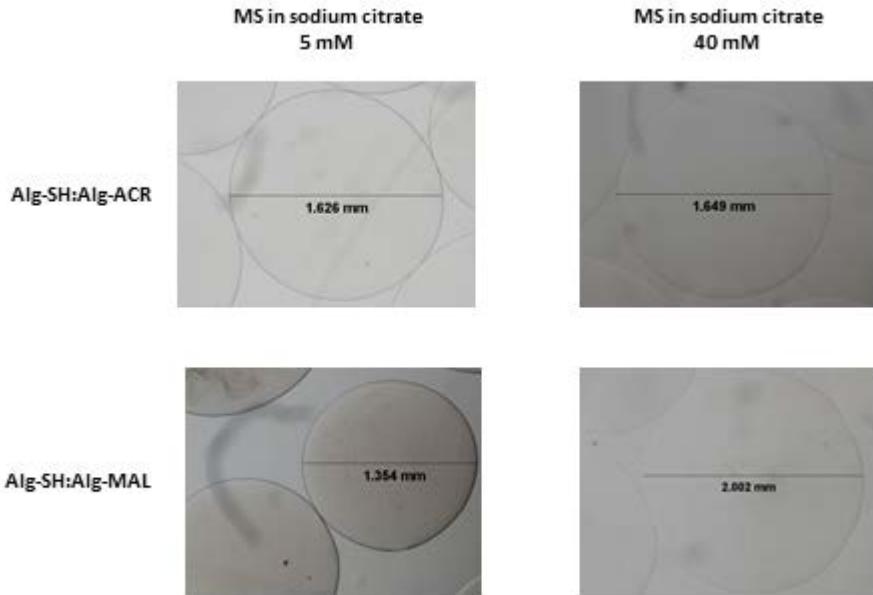
**Figure S4.** Representative DOSY NMR spectrum of Alg-ACR



**Figure S5.** Superimposition of deconvoluted spectra and raw  $^1\text{H}$  NMR data. Left row, zoom at alginate scale; right row, zoom at PEG scale

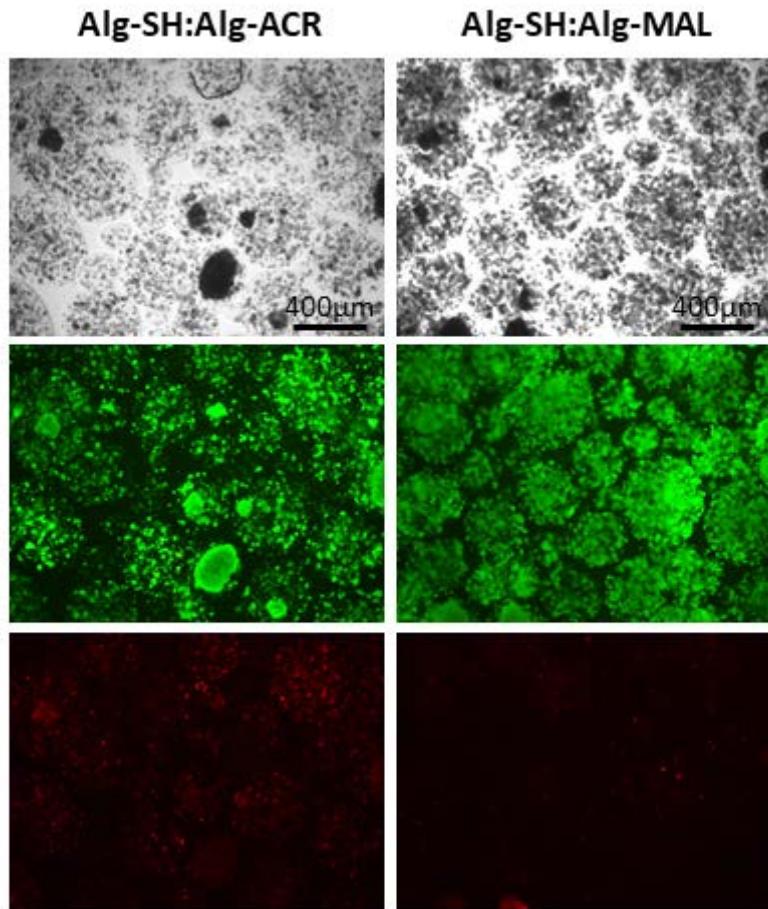
### **Exposition of Alg-SH:Alg-ACR and Alg-SH:Alg-MAL MS to monovalent non-gelling ions**

Ca-alg, Alg-SH:Alg-ACR and Alg-SH:Alg-MAL MS were recovered by filtration (70 µm), dried, and immersed in 2 mL of 5, 10, 20, 30 and 40 mM Na-citrate solutions. After 24 h, the sample were analyzed by microscopy (Olympus AX70 microscope).



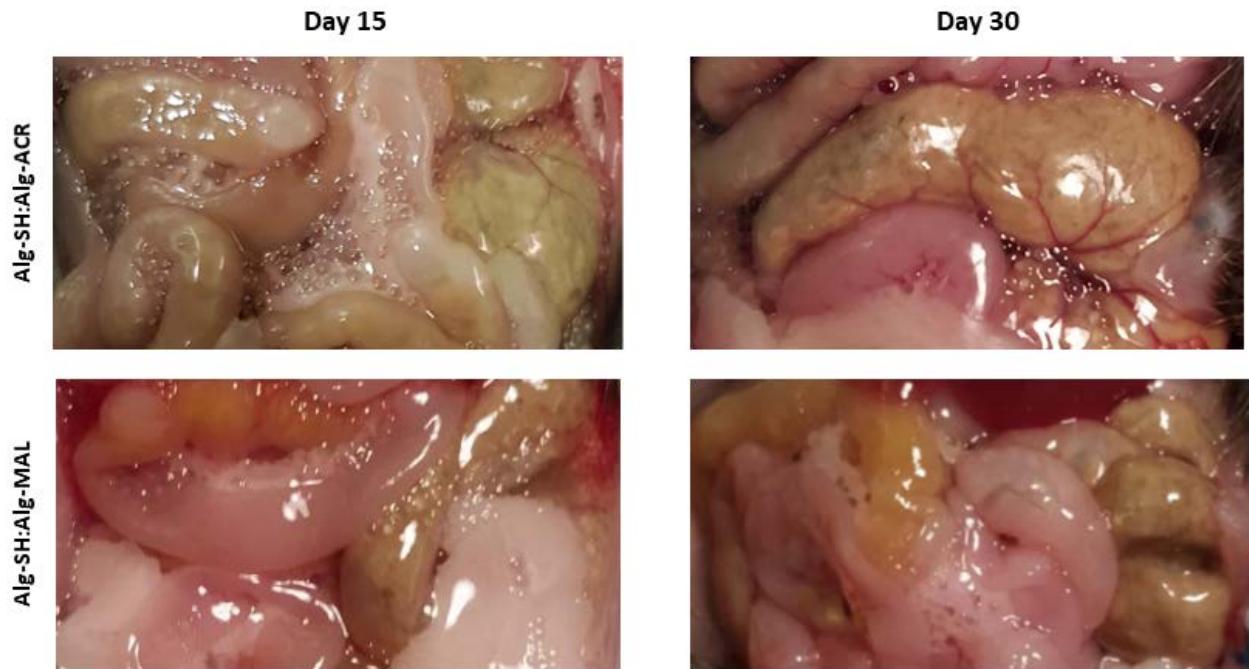
**Figure S6.** Representative images of Alg-SH:Alg-ACR and Alg-SH:Alg-MAL MS after 24 hour exposure to sodium citrate solutions (5 and 40 mM)

**Encapsulation of Huh7 cells in Alg-SH:Alg-ACR and Alg-SH:Alg-MAL MS**



**Figure S7.** Representative images of Huh7 cells microencapsulated in Alg-SH:Alg-ACR and Alg-SH:Alg-MAL MS at day 4 after encapsulation. Upper panel: light microscopy. Middle panel: staining of live cells with fluorescein diacetate (FDA). Lower panel: staining of dead cells with propidium iodide (PI).

**Transplantation of Alg-SH:Alg-ACR and Alg-SH:Alg-MAL MS in immune competent mice**



**Figure S8.** Macroscopic inspection at 15 and 30 days after intraperitoneal transplantation of Alg-SH:Alg-ACR and Alg-SH:Alg-MAL MS. Representative images or experiments executed in 4 replicates.