

## **Pulmonary tissue-mimetic hydrogel niches for small cell lung cancer cell culture**

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(Supplementary data)

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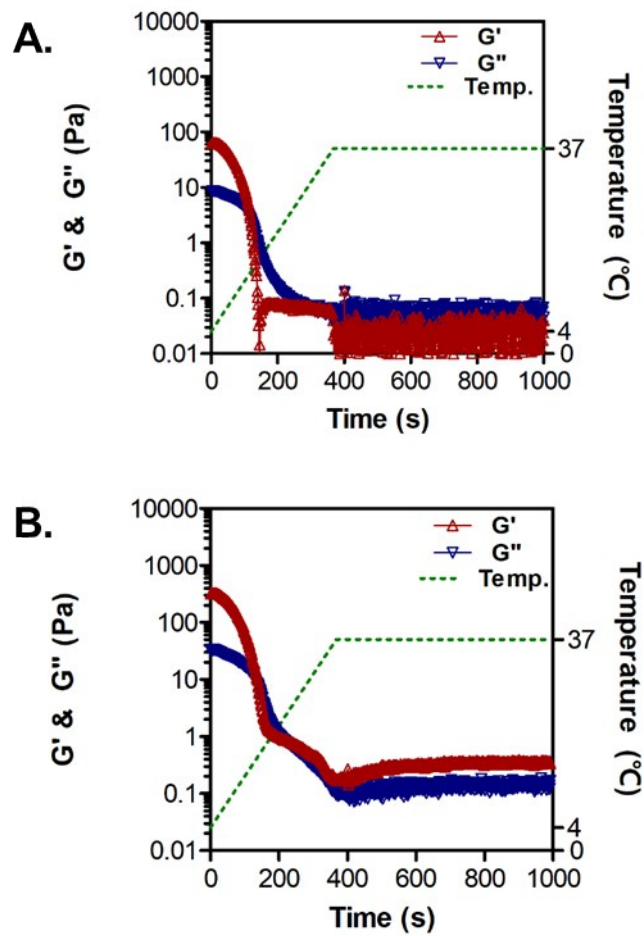
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Associate Professor

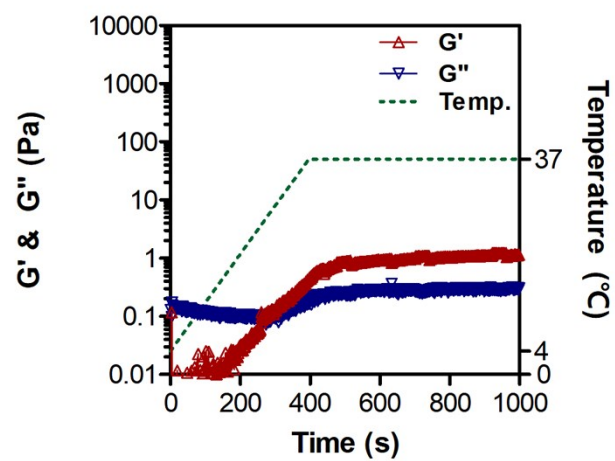
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**Table S1.** Sequence of CD44 forward and reverse primers

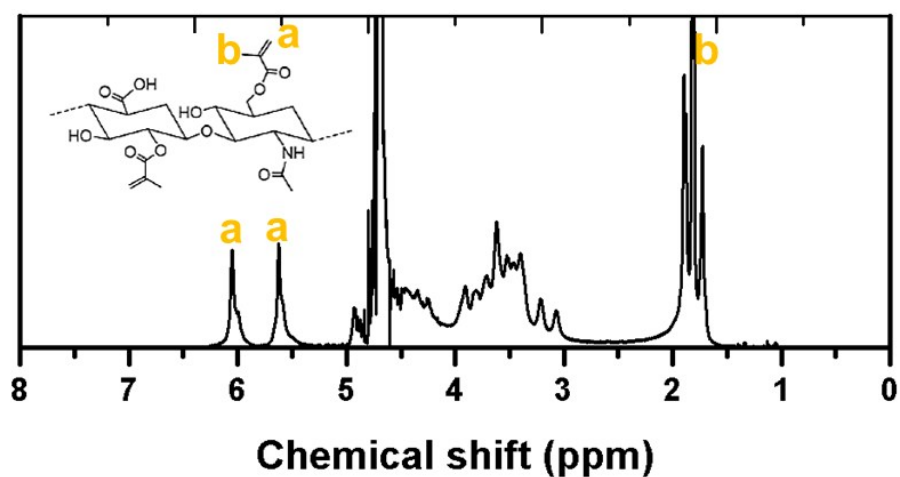
<b>Genes</b>	<b>Primer sequence (5' – 3')</b>	<b>Ref.</b>
<b>CD44</b>	F-TCC AAC ACC TCC CAG TAT GAC A R-GGC AGG TCT GTG ACT GAT GTA CA	[38]
<b>GAPDH</b>	F-GAG TCA ACG GAT TTG GTC GT R-TTG ATT TTG GAG GGA TCT CG	[39]



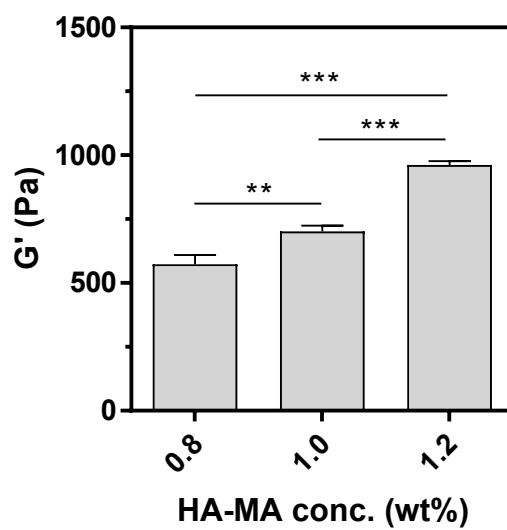
**Fig. S1.** Shear moduli ( $G'$  &  $G''$ ) of dECM solutions in a temperature sweep mode. (A) 10 wt% and (B) 15 wt%.



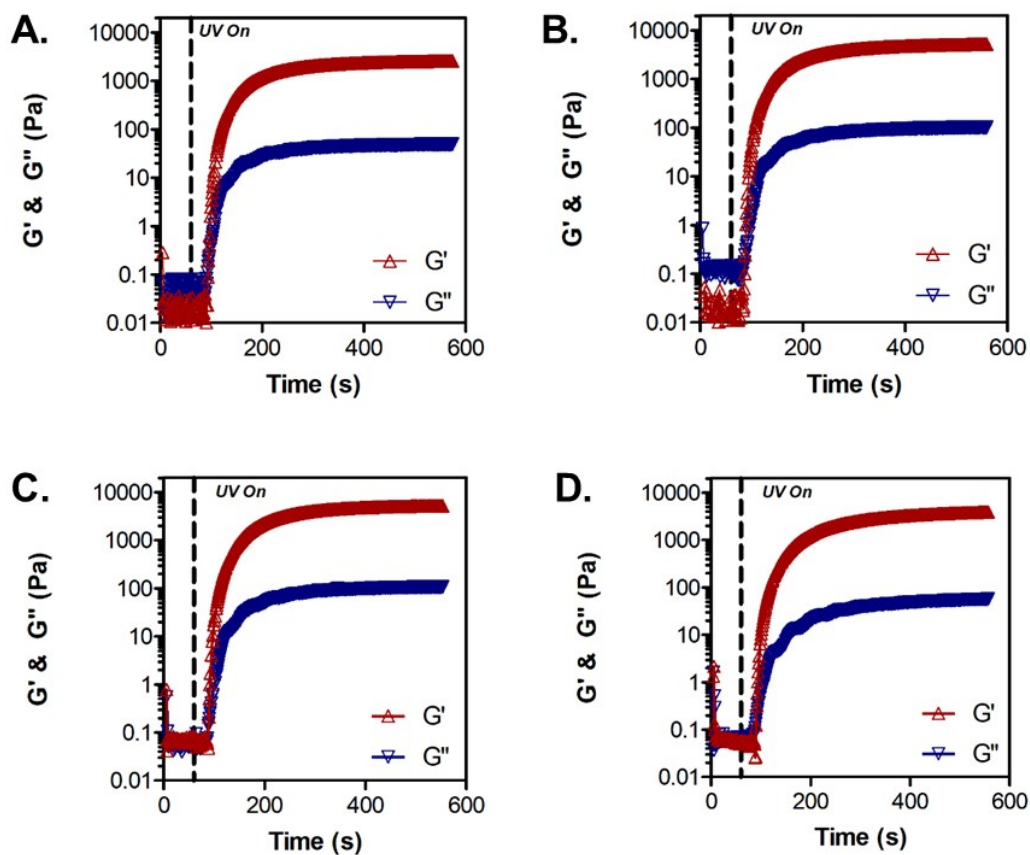
**Fig. S2.** Shear moduli ( $G'$  &  $G''$ ) of type I collagen solution (2 mg/mL) in a temperature sweep mode.



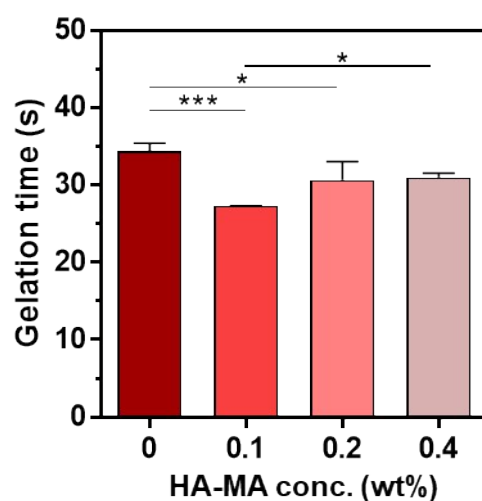
**Fig. S3.**  $^1\text{H}$ -NMR spectrum of HA-MA.



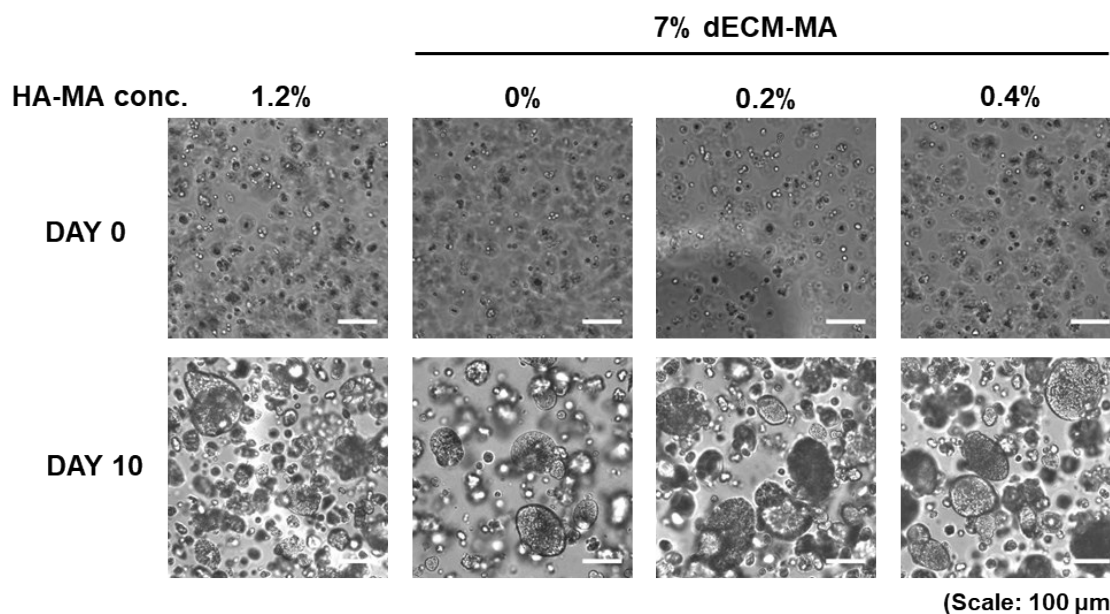
**Fig. S4.** Shear storage moduli of hyaluronic acid hydrogels formed at varying concentration of HA-MA in prepolymer solution ( $n = 3$ , mean  $\pm$  SD).



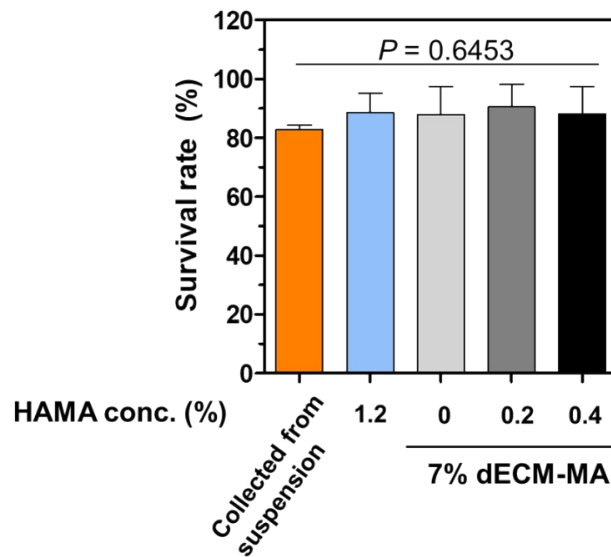
**Fig. S5.** In situ photorheometry results of pulmonary tissue mimetic hydrogels formed with various formulations. (A) 7 wt% dECM-MA with 1 mM LAP; (B) 7 wt% dECM-MA and 0.1 wt% HA-MA with 1 mM LAP; (C) 7 wt% dECM-MA and 0.2 wt% HA-MA with 0.8 mM LAP; (D) 7 wt% dECM-MA and 0.4 wt% HA-MA with 0.7 mM LAP. UV light was turned on at 60 s after the onset of rheometrical measurement.



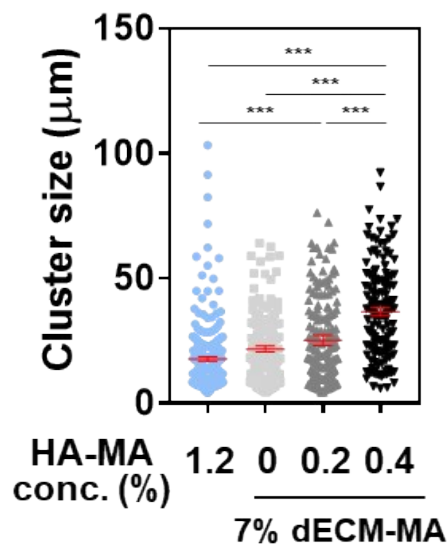
**Fig. S6.** Gelation times of pulmonary tissue mimetic hydrogels formed with various formulations (n = 3, mean  $\pm$  SD). Gelation time was defined as the time when the shear storage modulus ( $G'$ ) surpassed the shear loss modulus ( $G''$ ) from the in situ photorheometry results.



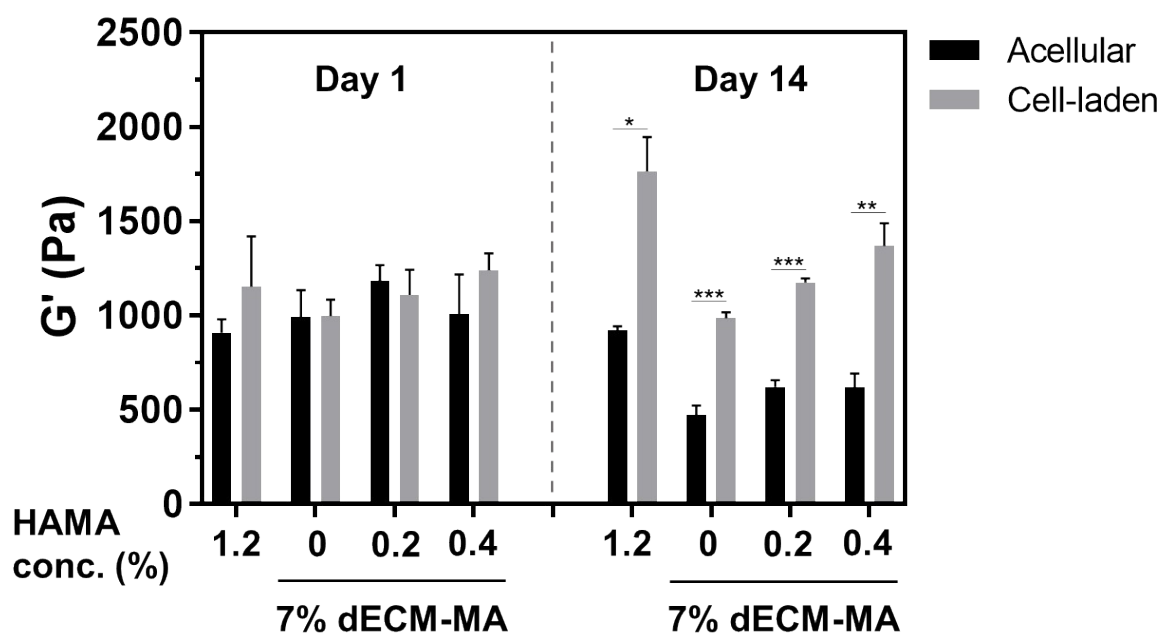
**Fig. S7.** Bright field images of NCI-H69 cells encapsulated in hydrogels at 1 h and 10-day post-encapsulation.



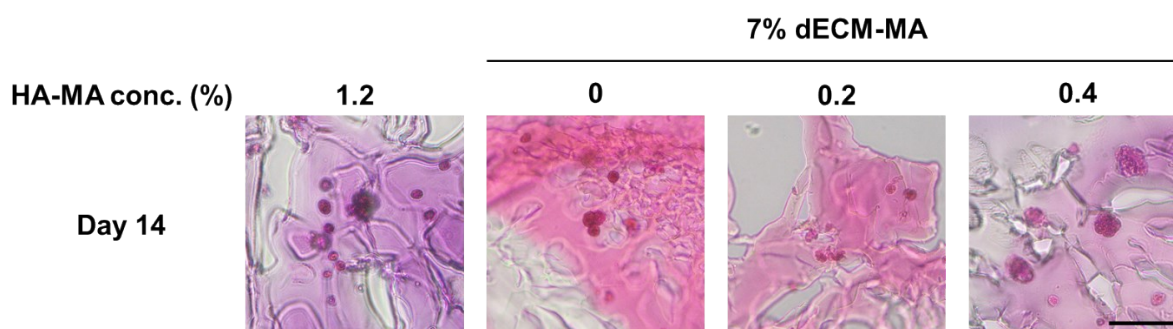
**Fig. S8.** Cell survival rates of NCI-H69 cells that were collected from suspension and encapsulated in pulmonary tissue mimetic hydrogels 1-h post-encapsulation. The survival rates were obtained by counting live/dead cells from randomly taken 8 images for each group (n = 8, mean  $\pm$  standard deviation). The P value indicates a statistical significance level determined by one-way analysis of variance.



**Fig. S9.** Cell cluster sizes of NCI-H69 cells in pulmonary tissue mimetic hydrogels on day 10 after encapsulation. The cell cluster size was determined as a diameter of the equivalent circle of cross-sectional area of each cell cluster (mean  $\pm$  standard error of mean).



**Fig. S10.** Shear elastic moduli of acellular and cell-laden pulmonary tissue-mimetic hydrogels on day 1 and 14 after gel formation. The acellular hydrogels were incubated in PBS and the cell-laden hydrogels were incubated in the culture medium ( $n = 3$ , mean  $\pm$  standard deviation).



**Fig. S11.** H&E stain images of NCI-H69 cells encapsulated in pulmonary tissue-mimetic hydrogels on day 14 post-encapsulation. The cell-laden hydrogels were fixed with 4% paraformaldehyde and cyrosectioned, followed by haematoxylin and eosin staining. The intense eosin stain was observed in boundary cell clusters (scale: 100  $\mu$ m).