

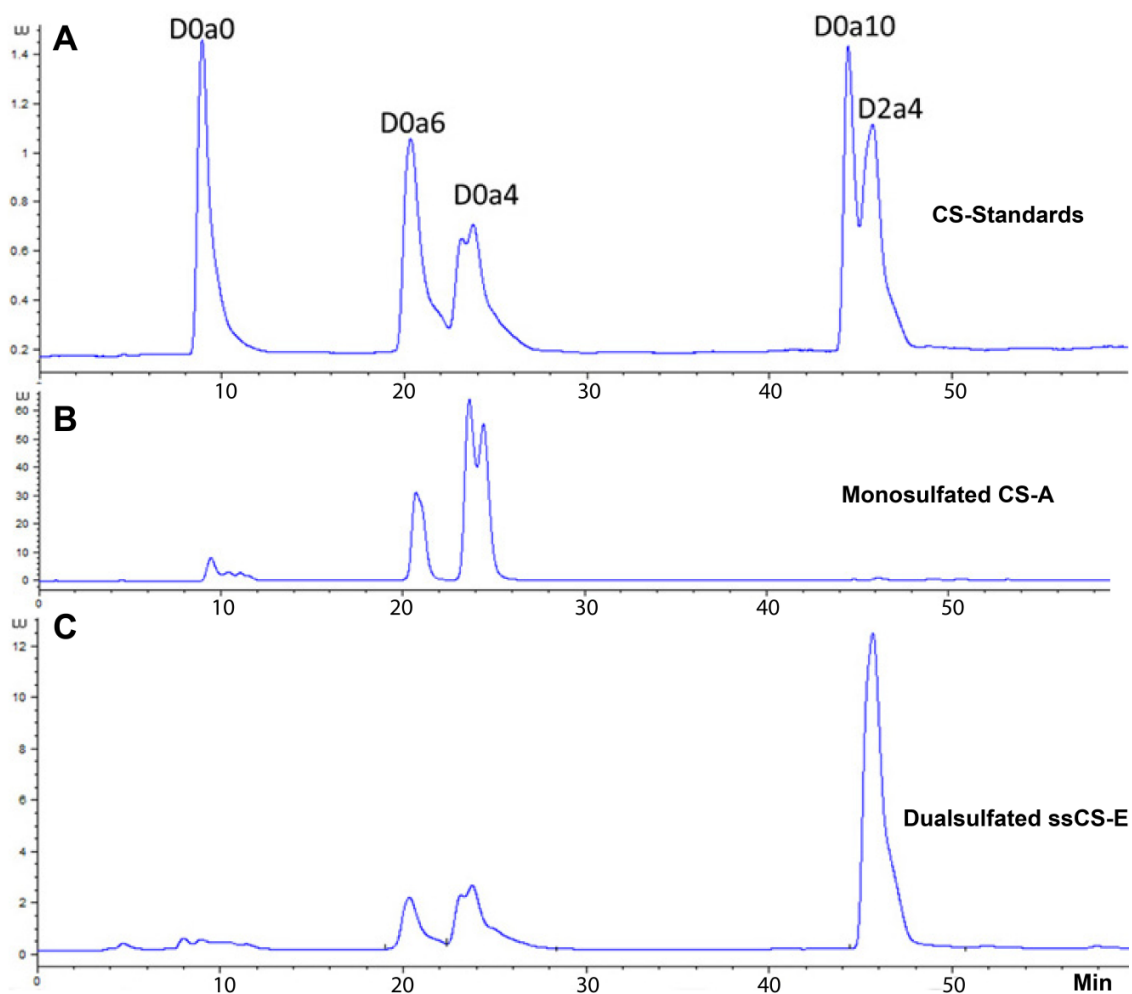
### Supplementary Table 1.

Estimated amounts ( $\mu\text{g}$ ) and corresponding percentage (w/w) of chondroitin sulfate as determined by SAX-HPLC. All values represent the amount estimated for total reaction volume. Total analysis volume of sample = 100  $\mu\text{L}$ , or 20  $\mu\text{g}$  of starting material. 'ND' = Not Detected; 0\* indicates a calculated value that is less than 1 percent.

	CS A/C		ssCS-E	
	Mass	%	Mass	%
<b>CS</b>				
D0a0	0.319	2	0.038	1
D0a6	2.35	13	0.504	17
D0a4	15.9	85	0.557	19
D2a0	ND		0.028	1
D2a6	0.020	0*	0.023	8
D0a10	0.092	0*	1.54	52
D2a4	0.051	0*	0.045	2
D2a12	ND		0.562	19
Total CS	3.00	100	2.944	100

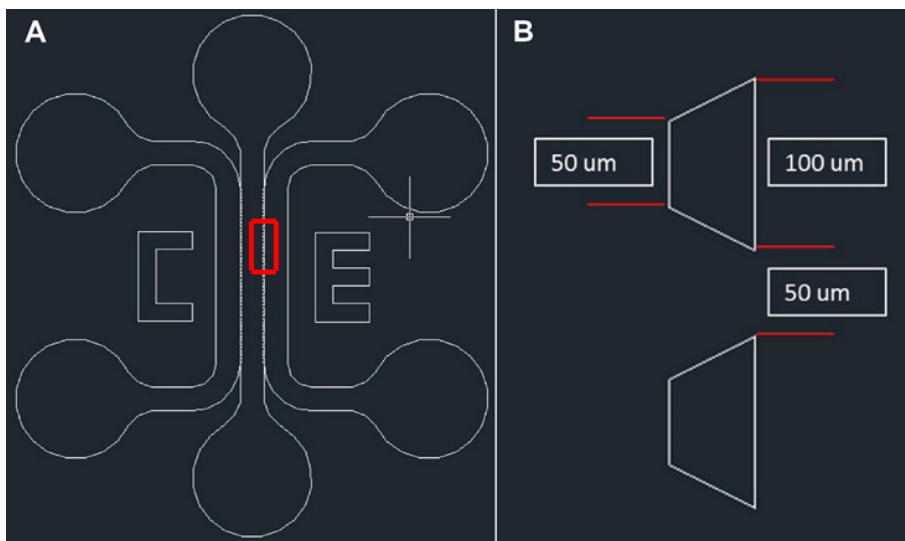
**Supplementary Fig. 1.**

Strong Anion Exchange (SAX) HPLC of: (A) CS- standards (B) monosulfated CS-A (D0a4), consisting of trace amounts of monosulfated CS-C (D0a0) and (C) Regioselective sulfation of CS-A yielding dual sulfated semisynthetic CS-E (Doa10, 52%), which along with minor increases in 2O sulfation also consists of 17% CS-C and 19% CS-A.



### Supplementary Fig. 2.

AutoCAD-generated schematic of the silicon wafer mold design used in fabricating the microfluidics devices for *in vitro* experiments. (A) The three main channels are 1000  $\mu\text{m}$  wide with wells at each end measuring 5 mm in diameter. Insert shows (B) trapezoidal barriers that line the inner channel, with dimensions chosen to allow for the selective cell migration between channels without allowing hydrogel contents to mix within the middle channel.



**Supplementary Figure 3.**

Representative brightfield images of U87MG-EGFP cells within AG, HA, CS-A, and COMP hydrogel matrices displaying differential cell morphology. Images were acquired 48h post-encapsulation. Scale bar = 50  $\mu\text{m}$ .

