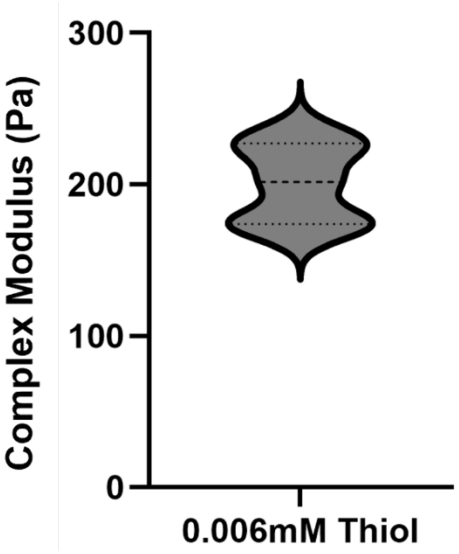
Supplementary Information (SI) for Journal of Materials Chemistry B.

SUPPLEMENTARY SECTION



Supplementary Figure 1: Before every encapsulation, rheology was conducted on 9mm hydrogels made with the same chemistry as those used for encapsulation to confirm the mechanical properties were maintained. The complex modulus was 201 +/- 11 Pa between all the studies (N=5).

Target	Definition
TUNEL Assay	This assay employs a method to detect fragmented DNA in apoptotic cells that have undergone 3D cell culture. It incorporates modified dUTP (which is fluorescently tagged) at the 3'-OH end of fragmented DNA, which then can be detected with fluorescence microscopy.
LIVE/DEAD™ Fixable Green Kit	The dye reacts with free amines in both the interior of cells with compromised membranes and on the surface of cells
EdU	EdU (5-ethynyl-2'-deoxyuridine), is a thymidine analog which is incorporated into newly synthesized DNA by cells within a sample over a controlled period
Ki-67	Cellular marker for proliferation because it is present in the cell nucleus during interphase and on the chromosome surface during mitosis
SOX2	A transcription factor that is important for maintaining pluripotency and had been associated with ribosomal RNA synthesis. Also has a role in maintaining neural stem cell characteristics
βIII-tubulin	A neuronal-specific protein that plays a vital role in the structure and function of neurons. It is involved in neuronal development and can be used as an immature neuronal marker
NeuN	Belongs to a family of tissue-specific splicing regulators and is exclusively present in post-mitotic neurons and absent from neural progenitors, oligodendrocytes and astrocytes
GFAP	Glial fibrillary acidic protein is an intermediate filament protein that can be used as a marker for astrocytes
Oligodendrocyte Clone NS-1 a.k.a. RIP	Recognizes oligodendrocytes and their myelin sheaths. Stains both early and mature oligodendrocytes

Supplementary Table 1: Immunostaining Targets

Target	Primary Antibody	Dilution Factor (Primary)

Ki-67	Novus (NB500-170)	1:100
SOX2	ThermoFisher Scientific (48-1400)	1:100
βIII-tubulin	Millipore Sigma (AB9354)	1:1000
NeuN	Millipore Sigma (MAB377)	1:100
GFAP	AVES (GFAP)	1:500
Oligodendrocyte Clone NS-1 a.k.a. RIP	Millipore Sigma (MAB1580)	1:1000
Target	**Secondary Antibody**	**Dilution Factor (Secondary)**
Mouse	Life Technologies Donkey anti-mouse 488 (A-21202)	1:500
Rabbit	ThermoFisher Donkey anti-rabbit 555 (A-31572)	1:200
Chicken	Sigma Donkey anti-chicken 647 (SAB4600127)	1:200
Hoescht	ThermoFisher Scientific (66249)	1:5000

Supplementary Table 2: Primary and Secondary Antibody Dilutions

	Sphere Size	**Global Density**	**Interaction**	**Figure**
TUNEL	F = 15.32 p = 0.0005	F = 10.59 *p = 0.0028*	F = 98.57 p < 0.0001	2b
Ki-67	F = 0.9 ns	F = 4.2 p < 0.05	F = 4.4 p < 0.05	3c
SOX2	F = 0.3 ns	F = 4.3 p < 0.05	F = 0.6 ns	4c
βIII-tubulin	F = 13.8 p < 0.0005	F = 26.3 p < 0.0001	F = 18.6 p < 0.0001	5e
NeuN	F < 0.001 ns	F = 0.2 ns	F = 10.1 p < 0.005	5f
GFAP	F = 1.6 ns	F = 30.0 p < 0.0001	F = 3.5 p < 0.05	6b
RIP	F = 6.4 p < 0.05	F = 4.2 p < 0.05	F = 24.6 p < 0.0001	7b

Supplementary Table 3: ANOVA values (F and *p*) for sphere size (column factor), global density (row factor) and interaction factor for all image quantification