SUPPLEMENTARY METHODS

Chondrocyte isolation and culture. Primary bovine calf condylar chondrocytes were isolated as previously described\(^1\). Briefly, full-thickness articular cartilage slices were harvested from the femoral condyles of 6 bovine calves, 1–3 weeks old, and chondrocytes were isolated by sequential digestion for 1 hr with 0.2% (wt/vol) pronase (Sigma) and 16 hr with 0.016% (wt/vol) collagenase P (Roche Diagnostics). Chondrocytes were maintained in 3D alginate culture until use to stabilize cell phenotype\(^2\). Chondrocytes were resuspended in 1.2% (wt/vol) alginate (Keltone LV, Kelco) in 150 mM NaCl at 4 million cells mL\(^{-1}\), and then expressed from a 22-gage needle into 102 mM CaCl\(_2\) to polymerize small alginate beads containing encapsulated chondrocytes. The beads were cultured in DMEM/F12 with 10% fetal bovine serum (FBS) and additives (100 U mL\(^{-1}\) penicillin, 100 µg mL\(^{-1}\) streptomycin, 0.25 µg mL\(^{-1}\) Fungizone, 0.1 mM MEM non-essential amino acids, 0.4 mM L-proline, 2 mM L-glutamine) for 2–3 weeks with medium changes every 2–3 days (1 mL per million cells per day). Chondrocytes released from alginate using 55 mM sodium citrate in 150 mM NaCl retain a cell-associated matrix (CM) rich in collagen and proteoglycans\(^3\). For most experiments, the CM was stripped immediately prior to use to allow direct cell-cell interaction, by incubation with 2 mg mL\(^{-1}\) collagenase B (Roche) in DMEM with 10% FBS and additives for 4 hr at 37 °C under constant gentle stirring\(^4\). In one study (Discussion), cells with or without the CM were compared in patterned DCP hydrogels.

Matrix biosynthesis assay. The deposition of sulfated glycosaminoglycans (sGAG) by chondrocytes embedded within DCP hydrogels was characterized spatially and quantitatively via selective binding of Alcian Blue, a cationic dye. Hydrogels were washed twice with saline, fixed in 10% neutral-buffered formalin for 10 minutes, and incubated with a solution of 0.5% (wt/vol) Alcian Blue (8GX, Sigma) in 0.1N HCl in
water, pH 1.0, for 3.5–4 hr on a shaker. Hydrogels were then rinsed at least 5 times in water to remove unbound dye and imaged by brightfield microscopy or stored at 4 °C in saline. To quantify bulk sGAG deposition, stained hydrogels were trimmed to separate quadrants of distinct cell organization, and further into approximately 10–20 mm² sections. Section area was quantified via microscopy to normalize sGAG data to hydrogel volume and to cell number via measured volumetric seeding density. The Alcian Blue dye was disassociated from each hydrogel section in 100 µl Extraction Buffer (4 M guanidinium chloride, 50 mM Trizma-HCl, 0.1% (wt/vol) CHAPS (Sigma), pH 7.4) for 12–16 hr at room temperature on a shaker, transferred to a 96-well plate, and analyzed spectrophotometrically at 605 nm. Standard curves were formed using control hydrogel disks containing known concentrations of sGAG extracted from bovine calf articular cartilage using the procedure of Roughley et al.⁵ and quantified as previously described⁶. Absorbance values correlated linearly with proteoglycan concentration from 0–4.0 mg mL⁻¹ (R² = 0.998), a range that spanned all DCP hydrogel sections.

**DCP within thermogelling hydrogels.** To demonstrate method compatibility with alternative hydrogels, cells were patterned within agarose hydrogels (Fig. 2h,i). Agarose (Type IX-A: Ultra-low Gelling Temperature, Sigma) was dissolved in the electropatterning buffer by boiling and allowed to cool before mixing with an equal volume of the cell suspension. The prepolymer cell suspension, containing 1% (wt/vol) agarose, was introduced into the patterning chamber following sterilization and flushing with buffer and cell-free agarose. The chamber was maintained at 25–37 ºC during cell patterning, and then immediately cooled in ice water for 5 minutes to initiate agarose gelation. The cell-patterned agarose hydrogels were removed from the chamber and remained gelled in 37 ºC culture.
REFERENCES


