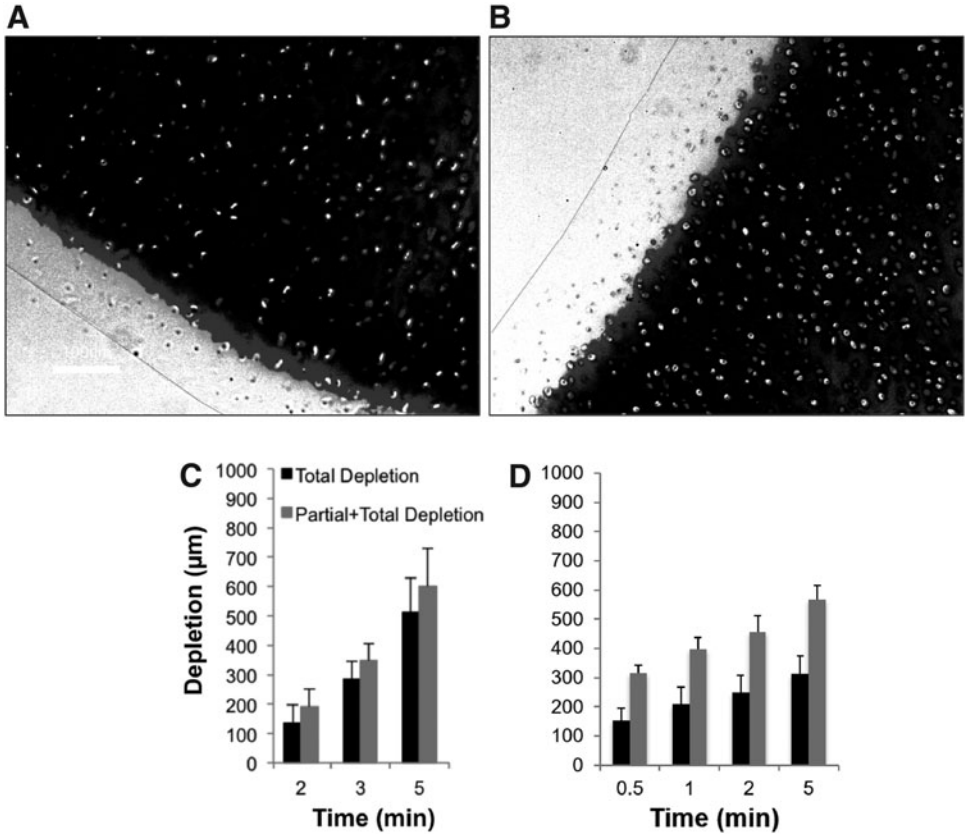
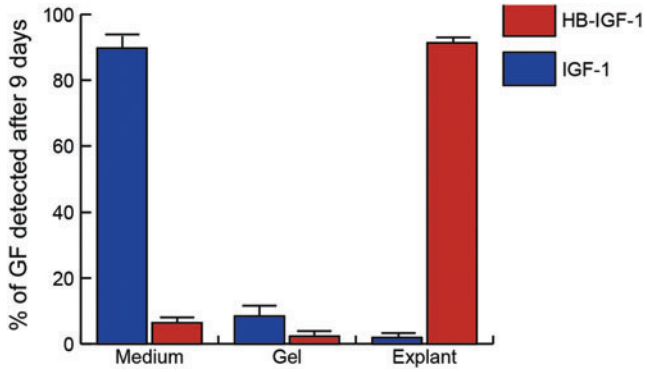
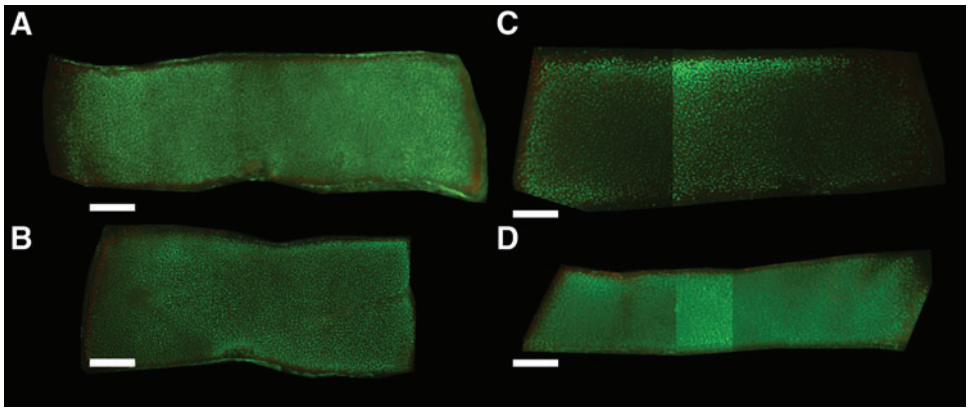
Supplementary Data



SUPPLEMENTARY FIG. S1. Quantification of sGAG depletion distance from toluidine blue staining of trypsin-treated cartilage annuli (gray-scale). Fifty microliters of the trypsin-PBS solution at 10 mg/mL (1%),[S1] 25 mg/mL (2.5%),[S2,S3] 50 μg/mL,[S4] or 500 μg/mL, was applied to the center of an annulus for 0.5–30 min at room temperature, aspirated, and followed by an equal duration application of 50 μL of FBS to neutralize the trypsin. Following enzyme pretreatment, the explant rings were immediately fixed in 10% neutral-buffered formalin for 24 h at 4°C, and subsequently dehydrated in ethanol and xylene before paraffin embedding. To determine the extent of GAG depletion attributable to the initial defect creation, annuli not subjected to the trypsin pretreatment were fixed and imaged as controls. The specimens were axially sliced, deparaffinized, and stained with toluidine blue to visualize the GAG content.[S5] Cross-sectional images of each annulus sectioned halfway through the height of the cylinder were obtained at 10× magnification. Using ImageJ (NIH), the contrast of each RGB image was enhanced based on a 50% cutoff threshold to clearly define the gradation of toluidine blue staining that corresponded to GAG content and subsequently converted to an 8-bit gray-scale image. This adjustment resulted in one to two zones of depletion: the innermost zone was defined as an area of ''substantially depleted sGAG,'' where sGAG was depleted to an extent that the toluidine blue dye binding was substantially reduced. The next zone was considered ''partially depleted,'' where the enzyme had time to only partially degrade the proteoglycan content before removal and/or neutralization. The distance of GAG depletion was quantified using a custom MATLAB (MathWorks, Inc., Natick, MA) script that assessed both total and partial and GAG depletion using the zones defined above and a manually marked inner edge of the defect, and averaged around the circumference of the specimen. **(A, B)** Representative gray-scale images of toluidine blue staining following exposure to 50 μg/mL of trypsin for **(A)** 2 min and **(B)** 5 min. **(C, D)** Quantification of sGAG depletion distance over time at **(C)** 50 μg/mL and **(D)** 500 μg/mL of trypsin. FBS, fetal bovine serum; PBS, phosphate-buffered saline; sGAG, sulfated glycosaminoglycan.



SUPPLEMENTARY FIG. S2. KLD delivers HB-IGF-1 to cartilage. KLD premixed with 615 nM HB-IGF-1 or IGF-1 was cast into cartilage annuli (as in Fig 1A), and cultured in basal medium for 9 days. Following 9 days in culture, the gel and explant were separated and each incubated in 10×PBS for 48 h to desorb the GF by disrupting any non-specific electrostatic interactions. HB-IGF-1 and IGF-1 released to the medium and found in the desorption baths were assessed by ELISA (Cat. No. DY291, which binds IGF-1 and HB-IGF-1; R&D Systems[S6]). Approximately 92% of the HB-IGF-1 recovered was found in the cartilage after 9 days, while ∼90% of the IGF-1 recovered was lost to the medium. These results indicate strong delivery of HB-IGF-1 premixed in KLD to surrounding cartilage *in vitro*. ELISA, enzyme-linked immunosorbent assay; GF, growth factor; HB-IGF-1, heparin-binding insulin-like growth factor-1; IGF-1, insulin-like growth factor-1.



SUPPLEMENTARY FIG. S3. Viability staining of cartilage explant annuli following 4 weeks of culture in ITS/FBS medium using FDA (live cells, *green* channel) and propidium iodide (dead cells, *red* channel). For viability imaging, samples were sectioned by hand with a scalpel perpendicular to the axis of the top flat surface of the explant rings. **(A)** Untreated control; **(B)** trypsin pretreatment alone; **(C)** HB-IGF-1 alone; **(D)** trypsin+HB-IGF-1. All conditions showed strong viability. Scale bar = 100 μm. FDA, fluorescein diacetate.

Supplementary References

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