Supporting Information

Cellular and Chemical Gradients to Engineer the Meniscus-to-Bone Insertion

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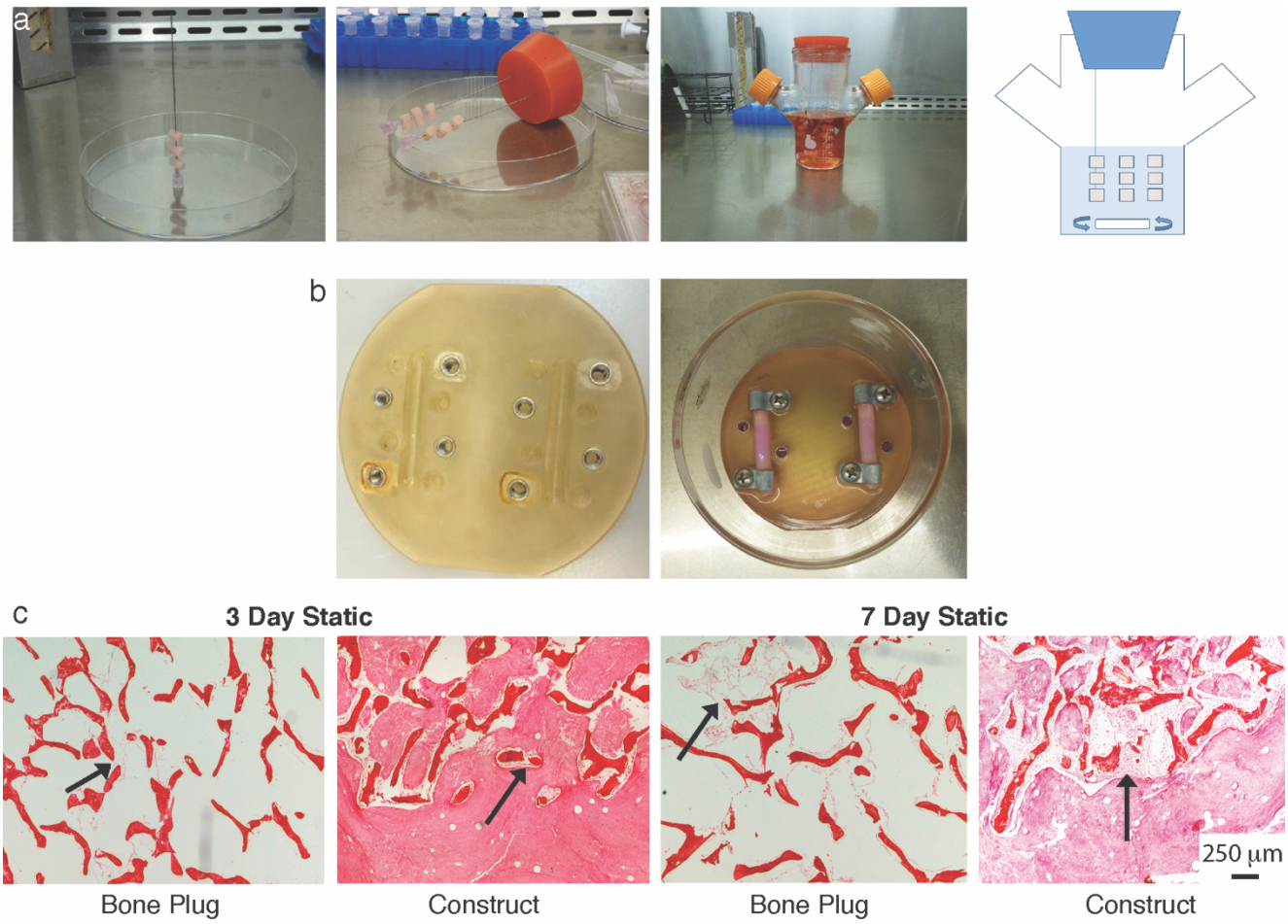

Figure S1. (A) Bone plug reseeding. Decellularized bone plugs were attached to spinal needles, hung from bottom of a silicone rubber stopper, and submerged in MSC suspension. **(B)** Single media culture system. Constructs sat in troughs on polysulfone mold and were clamped down on either end at the bony attachment before being submerged in meniscal media. **(C)** Picrosirius red images of seeded bone plug and construct generated with bone plugs in to static culture for 3 or 7 days. Constructs created with bone plugs cultured for seven days had built up dense MSC matrix (→) prohibiting the injected collagen from

infiltrating the trabeculae. This effect was mitigated by culturing bone plugs for 3 days prior to making constructs, and was used as the culture duration for all experiments.

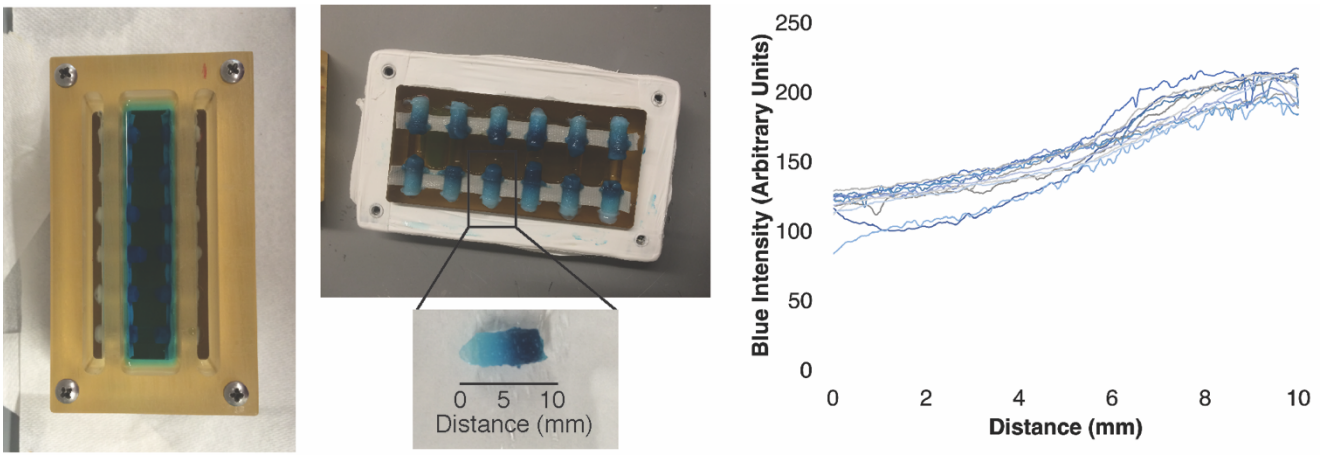

Figure S2. Diffusion of blue dye was used to characterize the chemical gradient produced by the diffusion bioreactor. Bone plugs infiltrated with collagen were placed in bioreactor for simulated culture for 11 days. Blue dye in center chamber diffused to outer chambers. Bone plugs were sectioned axially after culture period and analyzed in ImageJ for blue intensity and plotted as a function of axial distance. Plots of dye intensity for n=12 samples showed the establishment of a stable solute gradient across the bony portion of the construct over extended time in culture.

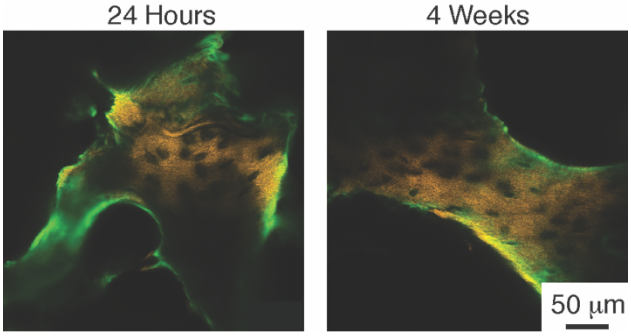

Figure S3. Combined second harmonic generation and two photon excited fluorescence images of bone plugs submerged in Calcein (green) at 24 hours and 4 weeks after labelling. Calcein labelling maintained intensity over the course of the experiment, indicating a lack of photobleaching over this time scale.

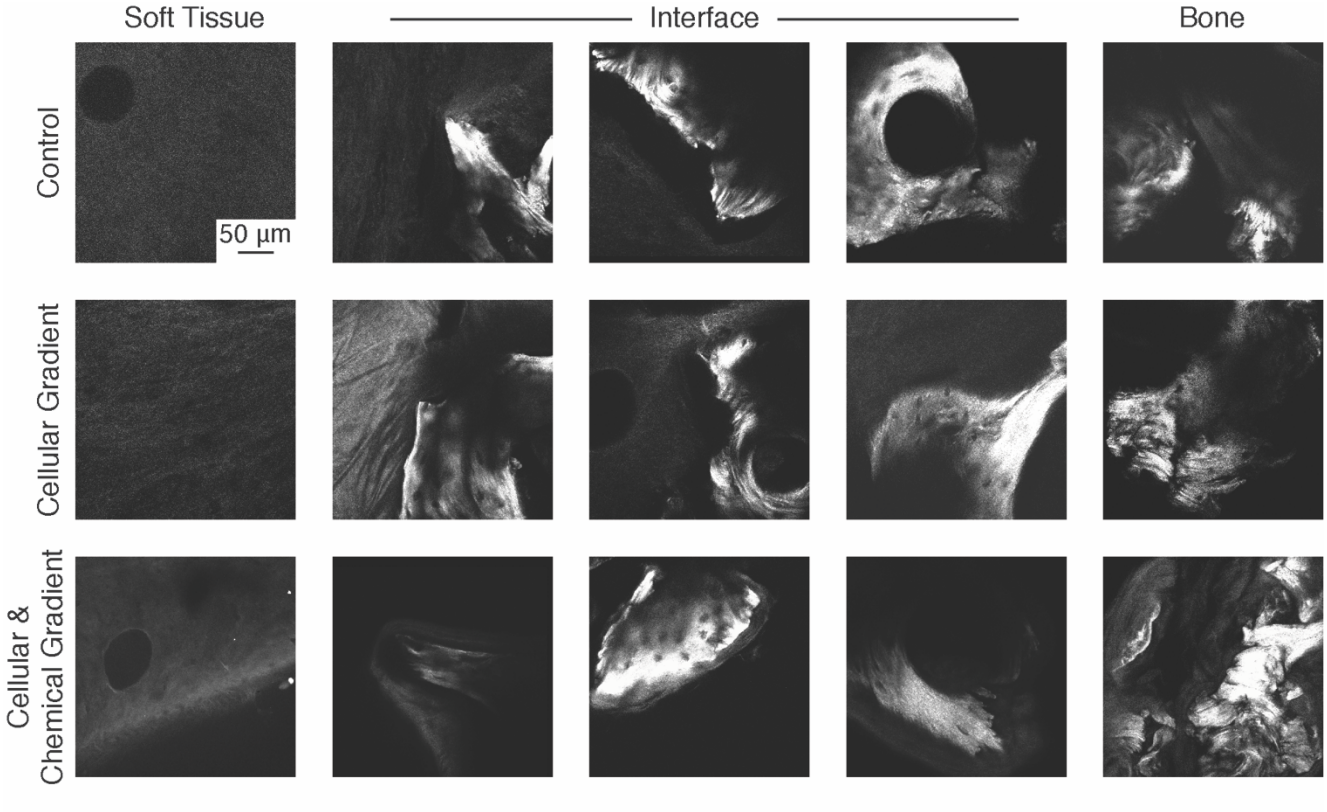

Figure S4. SHG-only images of Calcein/Calcein Blue labelled tissue engineered meniscal enthesis constructs corresponding to the images of Figure 7.

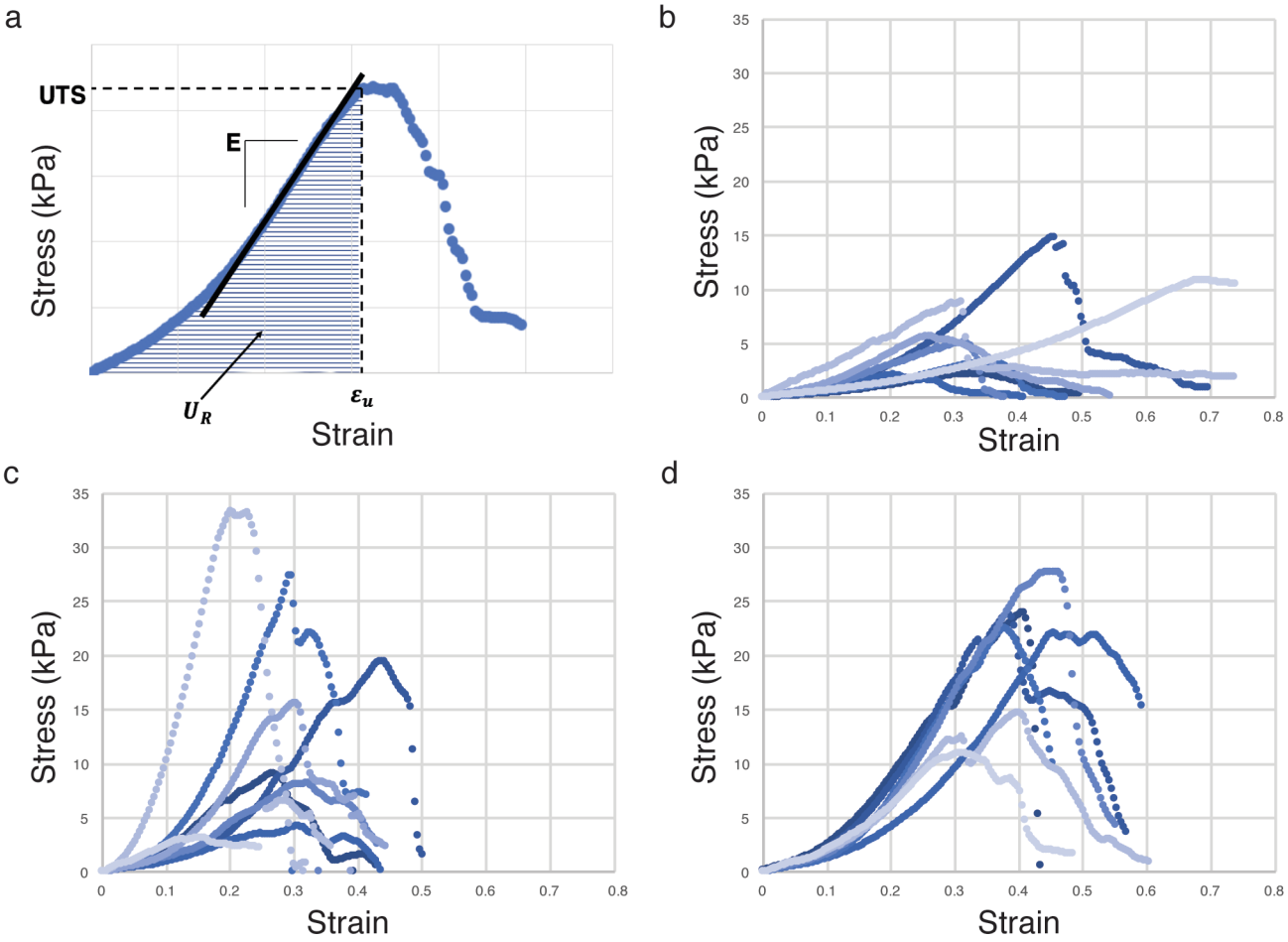

Figure S5. (A) Sample mechanical testing analysis of a representative stress-strain curve, demonstrating the calculation of Young's modulus (E), ultimate tensile strength (UTS), ultimate strain (ε_u), and modulus of resilience (U_R). **(B)** Stress-strain curves for Control (n=8). **(C)** Stress-strain curves for Cellular Gradient (n=8). **(D)** Stress-strain curves for Cellular & Chemical Gradient (n=8).