Supplementary Data

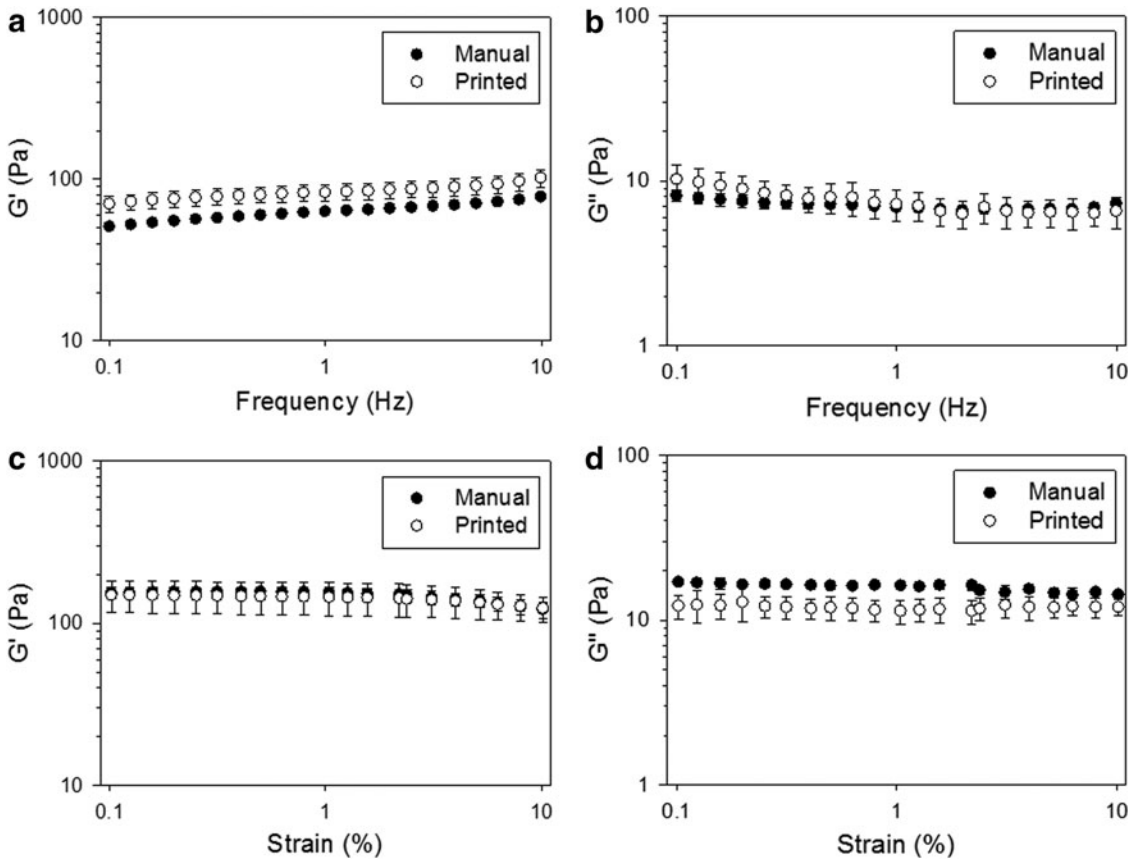
Supplementary Methods

Rheology of collagen gels

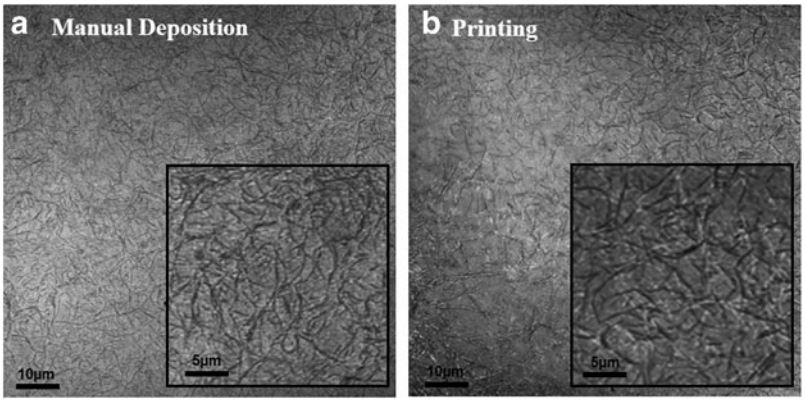
The mechanical properties of collagen structures were measured using a parallel-plate rheometer (AR G2; TA Instruments). Two types of collagen structures (20 mm in diameter, 1 mm in thickness) were prepared on a petri dish by manual deposition or printing with a final concentration of 3.0 mg/mL. A solution of 0.8 M sodium bicarbonate was used as the cross-linking agent. The collagen samples were incubated at 37°C for 3–4 h. Before the measurements, the samples were carefully removed from the petri dishes and transferred to the bottom plate of the rheometer. Frequency sweep test was performed with 1% strain at room temperature. Strain sweep test was performed with 0.1 Hz frequency at room temperature. Three samples were assessed for each test.

Confocal reflectance microscopy

Confocal reflectance microscopy (CRM) was performed to visualize the microstructure of collagen scaffolds. Two types of collagen structures were prepared by manual deposition or printing as described earlier. The collagen samples were incubated in optimal cutting temperature-Dulbecco's phosphate buffered saline (OCT-DPBS) mixture overnight, then embedded within OCT for cryosectioning. Fifty-micrometer-thick collagen slices were prepared, and CRM was performed using a confocal microscope (Zeiss LSM 510). Collagen specimens were illuminated with a 543 nm laser, and the reflected light was detected using a 420 nm-long pass filter. 63× and 100× objective lenses were used to acquire the images.



SUPPLEMENTARY FIG. S1. Dynamic storage moduli (G′) and loss moduli (G″) of collagen constructs. Only subtle differences were observed between samples prepared by manual deposition or printing in frequency sweep tests **(a, b)** and strain sweep tests **(c, d)**.



SUPPLEMENTARY FIG. S2. Confocal reflectance microscopy of collagen constructs. Images comparing the microstructure of collagen scaffolds prepared by **(a)** manual deposition and **(b)** printing. Inset shows magnified images. No significant differences in diameter, length, density, or orientation of collagen fibers were observed.