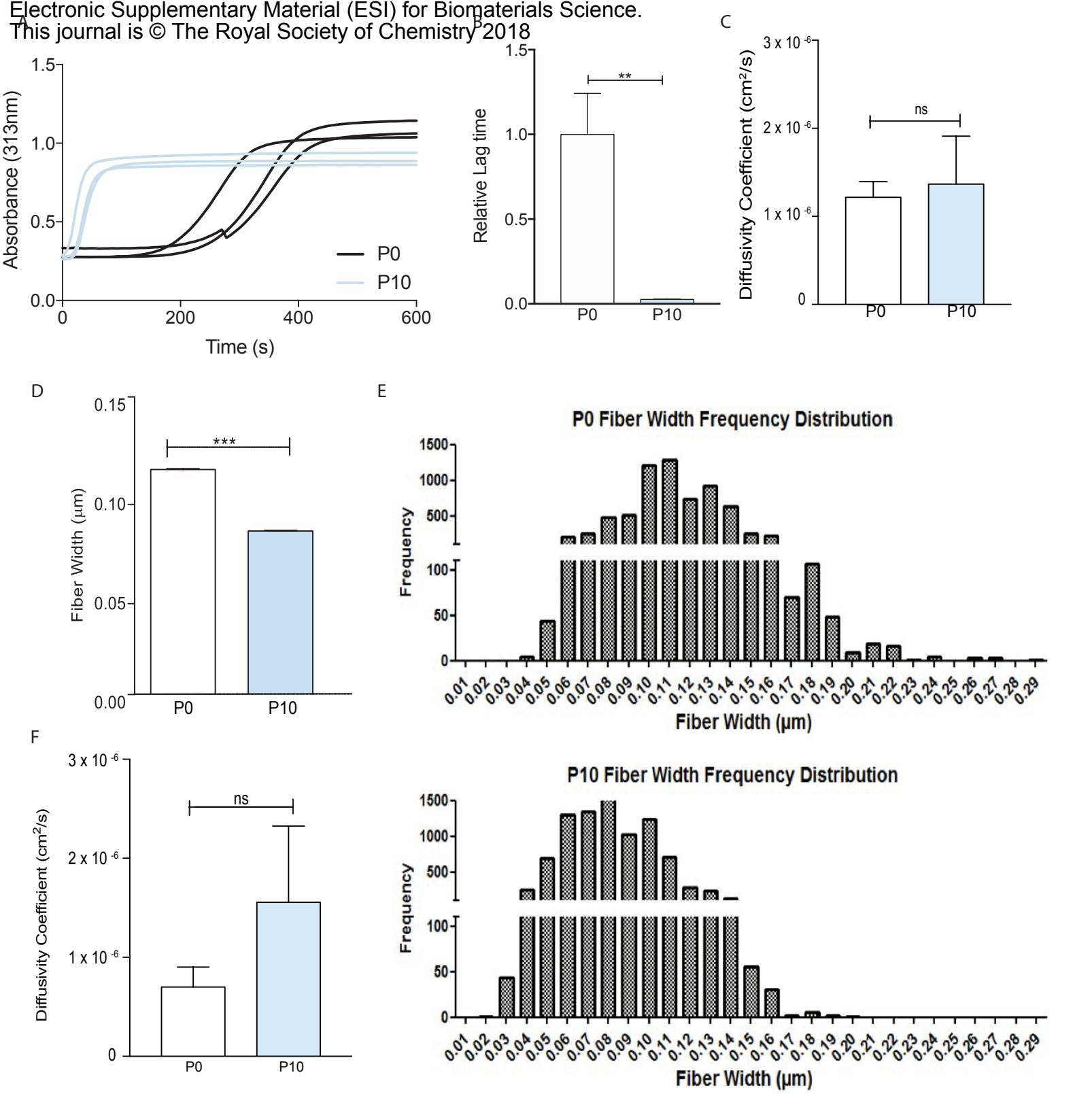
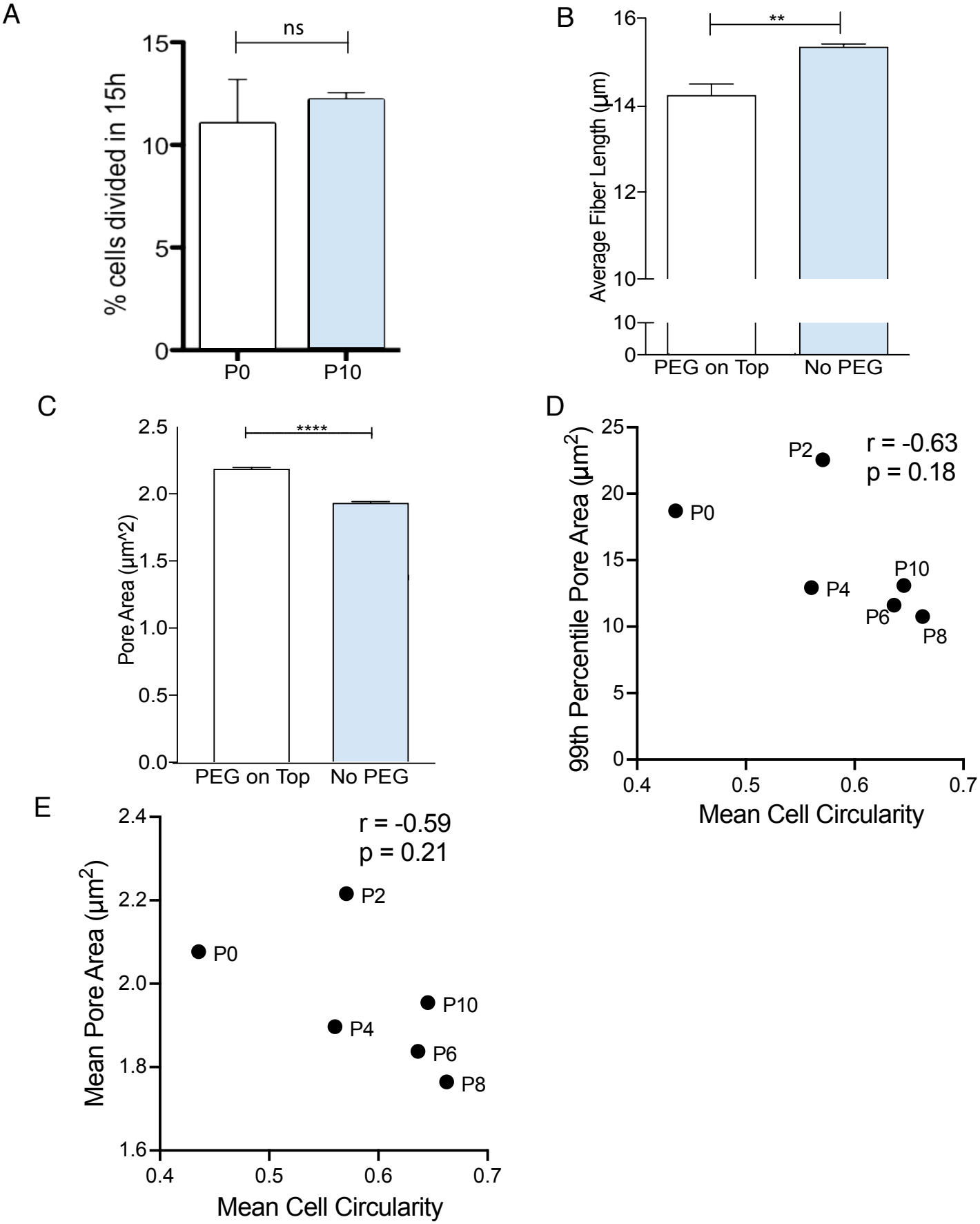
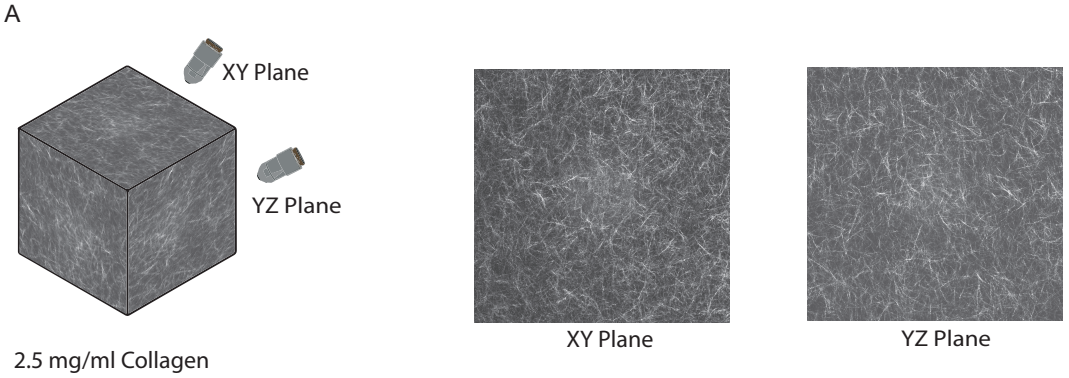
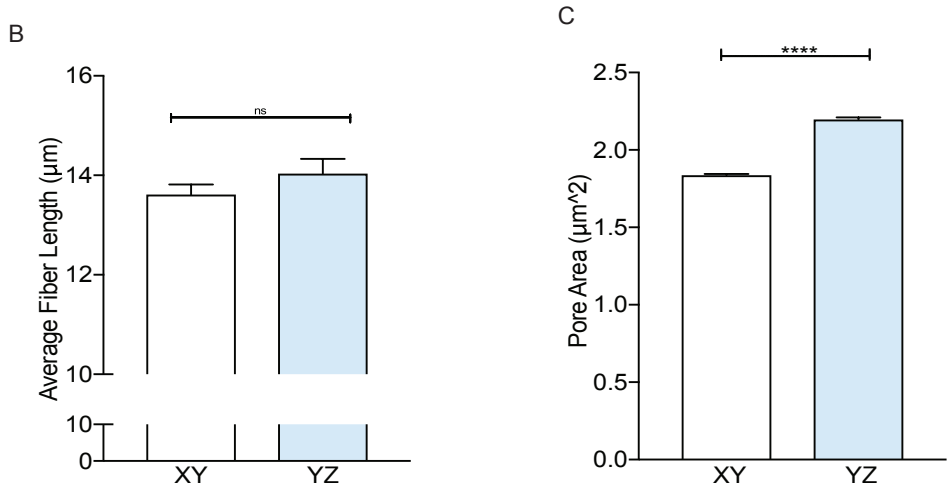


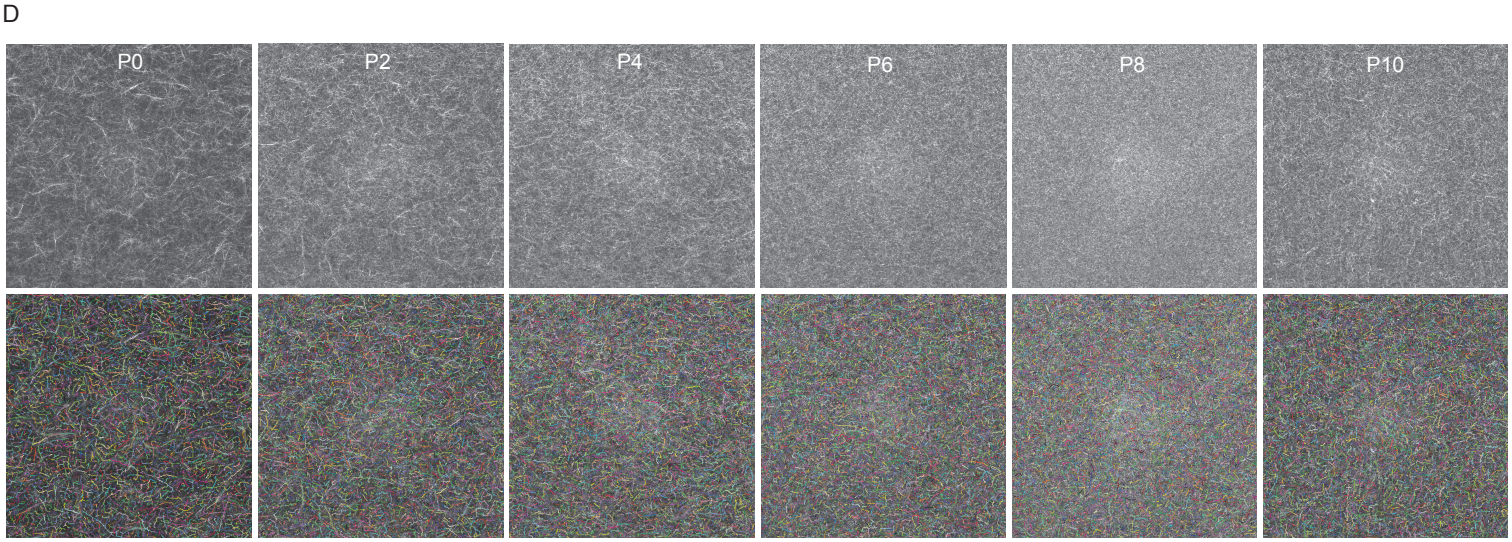
Supplementary Figure 1. (a) Collagen assembly as shown by turbidimetry (b) Relative lag time before Collagen nucleation of 2.5 mg/ml gels with and without 10 mg/ml PEG added before polymerization (c) Diffusivity of fluorescently labeled PEG through 2.5 mg/ml gel and 2.5 mg/ml gel with 10 mg/ml PEG. (d) Average width of collagen fibers in 2.5 mg/ml collagen polymerized without a molecular crowding agent, P0, and with 10 mg/ml of PEG as a crowding agent added during polymerization, P10. Widths measured by analysis of scanning electron microscopy. (e) Histogram of fiber width measurements in the P0 and P10 conditions show overlapping distributions. (f) Diffusivity of fluorescent dextran through 2.5 mg/ml gel and 2.5 mg/ml gel with 10 mg/ml PEG.



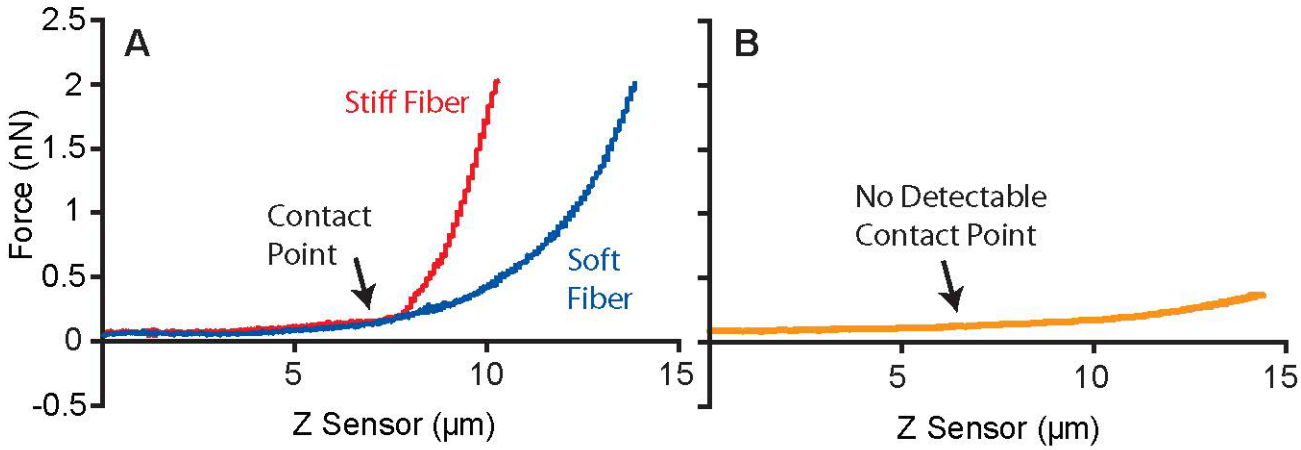
Supplementary Figure 2. (a) Percent of cells that underwent division during the first 15 hrs of culture in the P0 and P10 conditions. (b) Mean fiber length in 2.5 mg/ml and 2.5 mg/ml with 10 mg/ml of PEG added on top after polymerization. Lengths measured by analysis of reflection confocal micrographs. (c) Pore size in 2.5 mg/ml and 2.5 mg/ml with 10 mg/ml of PEG added on top after polymerization. Pores measured by analysis of reflectionconfocal micrographs. It is important to note that the large number of pores and fibrils analyzed tends to generate statistical significance between conditions, even when differences are small[82]. (d) The 99th percentile of pore sizes and (e) mean of all pore sizes plotted against mean cell circularity in each matrix condition. N=3 replicates for each condition. At least three fields of view were analyzed per replicate. At least 300 cells were analyzed in each condition. Bar graphs show the mean and standard error of measurements. Statistical significance tested by ANOVA and reported as $p<0.001$, ***; $p<0.01$, **; $p<0.05$, *.







Supplementary Figure 3. (a) Reflection confocal micrographs of the X-Y plane and Y-Z plane of 2.5 mg/ml collagen polymerized without a molecular crowding agent. Characterization of (b) mean fiber length and (c) pore size as a function of orientation. (d) Visual overlay of fiber analysis conducted in CT-FIRE. N=3 replicates for each condition. At least three fields of view were analyzed per replicate. Bar graphs show the mean and standard error of measurements. Statistical significance tested by ANOVA and reported as $p<0.001$, ***; $p<0.01$, **; $p<0.05$, *.



Supplementary Figure 4. AFM measurements of local fibril stiffness. (a) Force-position plots of raw data where the calculated indentation points are indicated and aligned between soft (blue) and stiff curves (red). When the indentation force trigger was met, e.g. when the probe is centered over a pore, data is not collected. (b) An example of the force curve (orange) that would be obtained from a pore, noting that there is no calculable contact point and tip deflection is only the result of drag on the cantilever during tip approach.