

Supporting Information

Young et al. 10.1073/pnas.1313561110

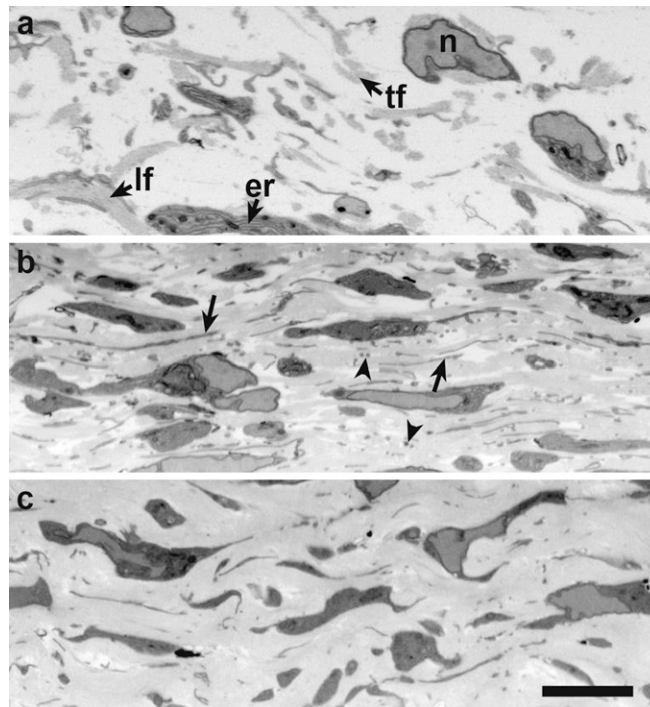


Fig. S1. Backscatter electron images from focused ion beam scanning electron microscopy (FIB SEM) datasets after contrast reversal in developing chick cornea at embryonic days 10 (A), 14 (B), and 18 (C)—still images for Movie S1, Movie S4, and Movie S7, respectively. At 6,000 \times magnification, resolution attained precludes observation of individual collagen fibrils, but fibril bundles [in transverse (tf) and longitudinal (lf) section] and cell organelles [nucleus (n), endoplasmic reticulum (er)] are clearly visible. Cells appear rounded at day 10, with scattered collagen fibril bundles and large areas of fibril-free extracellular space. In condensing stroma, spaces are reduced at day 14, cells appear more flattened, and cell processes are abundant in longitudinal (arrows) and transverse (arrowheads) sections. At day 18, cells follow contours of compacted lamellae with few spaces remaining. (Scale bar, 0.5 μm .)

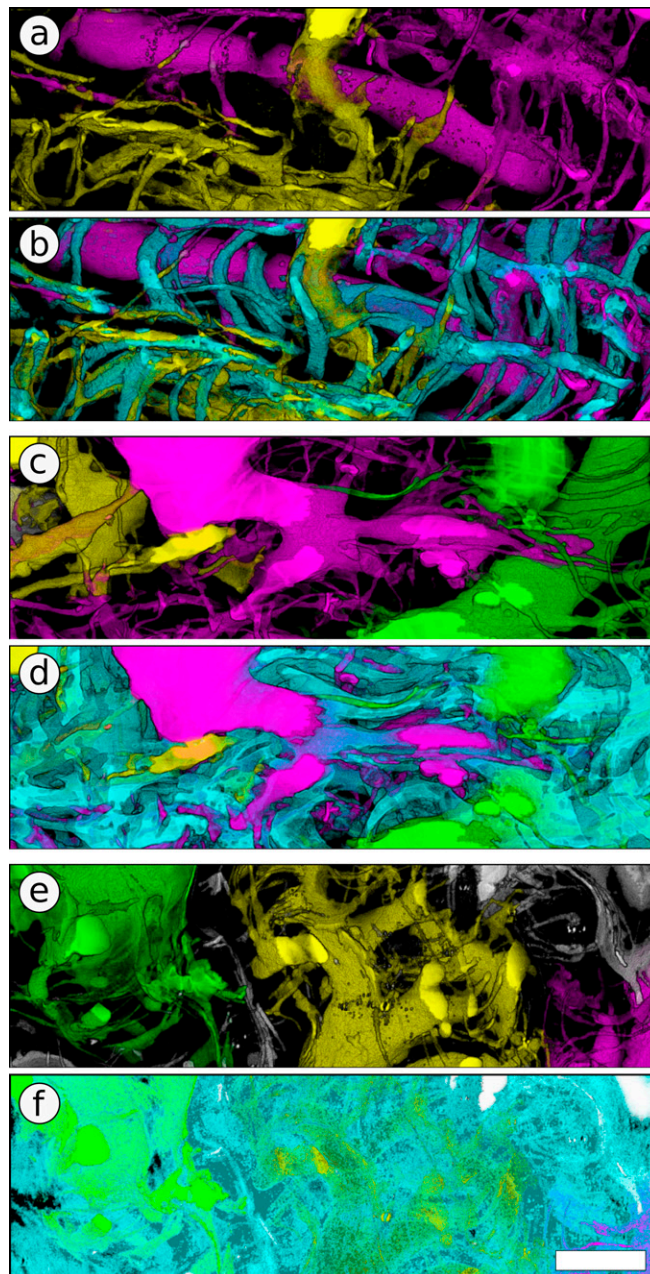
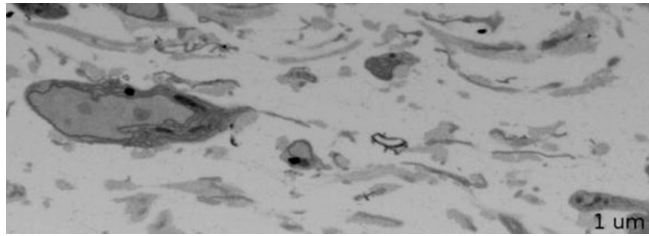
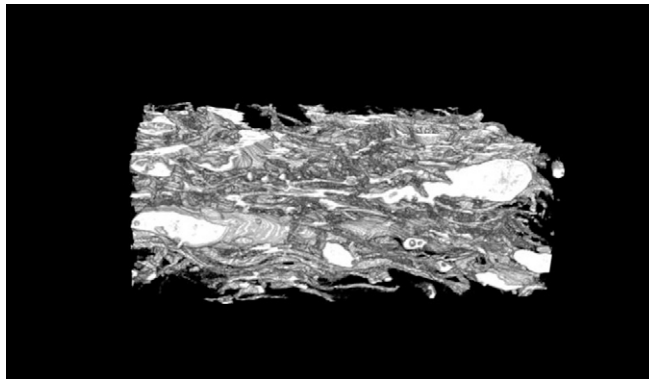


Fig. S3. 3D reconstructions in ImageJ from FIB SEM datasets of chick corneal stroma at 10 (*A* and *B*), 14 (*C* and *D*), and 18 (*E* and *F*) d of development. Reconstructed volumes are all 2.1 microns thick. *A*, *C*, and *E* show separate cells in yellow, green, magenta, and white, and in *B*, *D*, and *F*, the same cells surrounded by collagen (cyan). (Scale bar, 5 microns, applicable to all panels.)



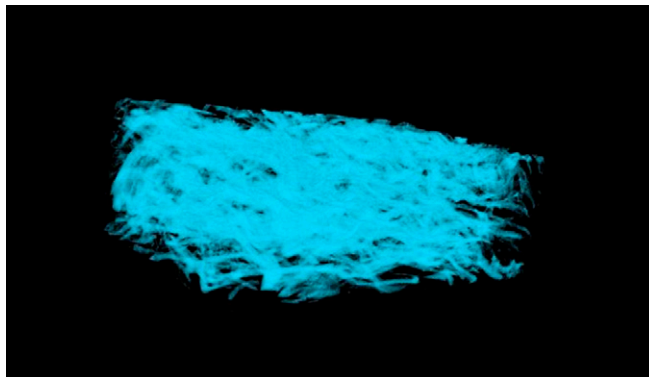
Movie S1. Fly-through of 464 image sequence from volume electron microscopy of embryonic day 10 chick cornea, viewed in sagittal section, showing $30 (x) \times 11 (y) \times 23 (z) \mu\text{m}$ tissue volume. Rounded cells with irregular processes and collagen bundles are suspended within a fluid milieu appearing as clear structureless background. (Scale bar, $\sim 2.5 \mu\text{m}$.)

[Movie S1](#)



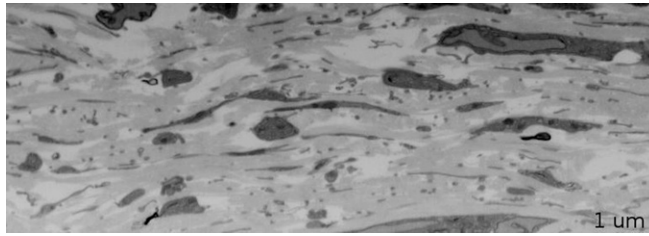
Movie S2. 3D reconstruction of cellular component from day 10 dataset. Rounded cells express processes of diverse shapes and occupy approximately the same proportion of the sample as that occupied by matrix. (Scale bar, $\sim 5 \mu\text{m}$.)

[Movie S2](#)



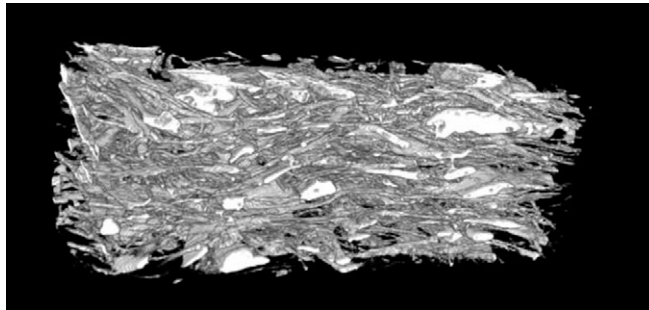
Movie S3. 3D reconstruction of extracellular matrix from day 10 dataset. Presumptive lamellae are represented by loose collagen bundles, occupying only 20% of the sample volume, which, although not yet connected, already show orthogonal arrangement. (Scale bar, $\sim 5 \mu\text{m}$.)

[Movie S3](#)



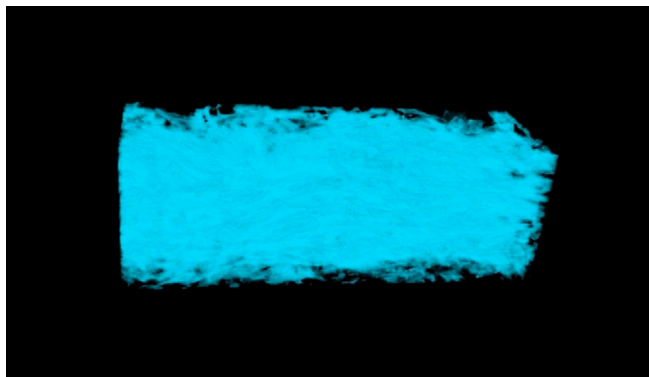
Movie S4. Fly-through of 231 image sequence from volume electron microscopy of day 14 chick cornea, viewed in sagittal section, showing $30 (x) \times 11 (y) \times 11.5 (z) \mu\text{m}$ tissue volume. Cells appear flattened, with cytoplasmic processes, which appear as elongate tubes or rounded spots, in x/y or x/z planes, respectively. Fluid spaces are reduced and collagen fills more of the extracellular space. (Scale bar, $\sim 2.5 \mu\text{m}$.)

[Movie S4](#)



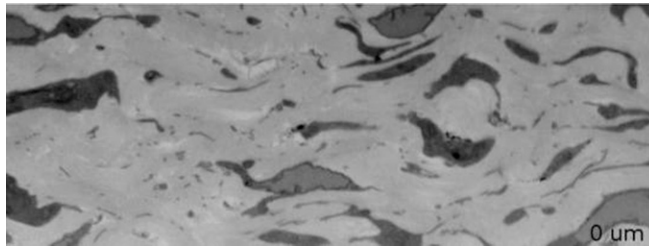
Movie S5. 3D reconstruction of cellular component from day 14 dataset. Cells are flattened with prominent filopodia, some of which traverse the entire $30 \mu\text{m}$ width of the sample. (Scale bar, $\sim 5 \mu\text{m}$.)

[Movie S5](#)



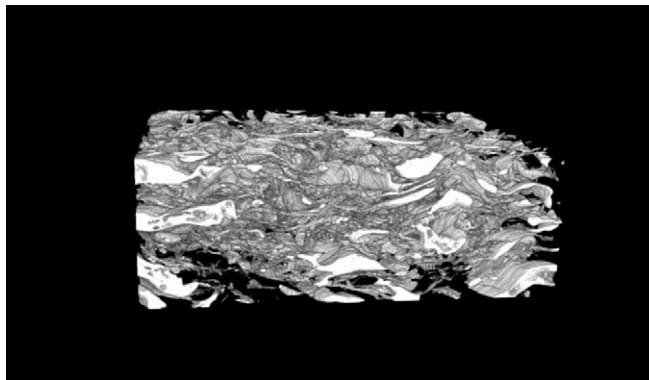
Movie S6. 3D reconstruction of collagenous matrix from day 14 dataset. Matrix constitutes 50% of total sample volume. (Scale bar, $\sim 5 \mu\text{m}$.)

[Movie S6](#)



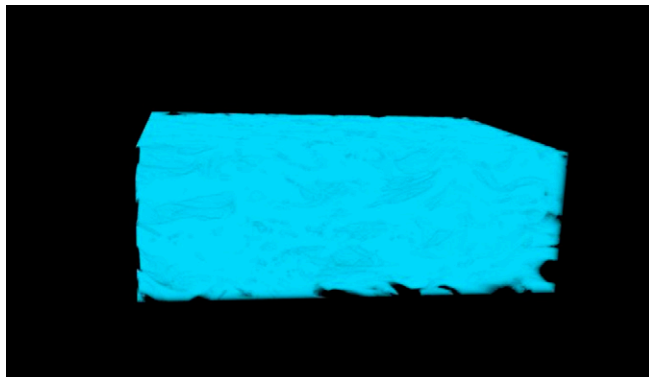
Movie S7. Fly-through of 570 image sequence from volume electron microscopy of day 18 chick cornea, viewed in sagittal section, showing $30 (x) \times 11 (y) \times 28.5 (z) \mu\text{m}$ tissue volume. Flattened cells follow an undulating course demarcating the borders of collagen lamellae; filopodia are less conspicuous. Few fluid spaces remain in the condensed extracellular matrix. (Scale bar, $\sim 2.5 \mu\text{m}$.)

[Movie S7](#)



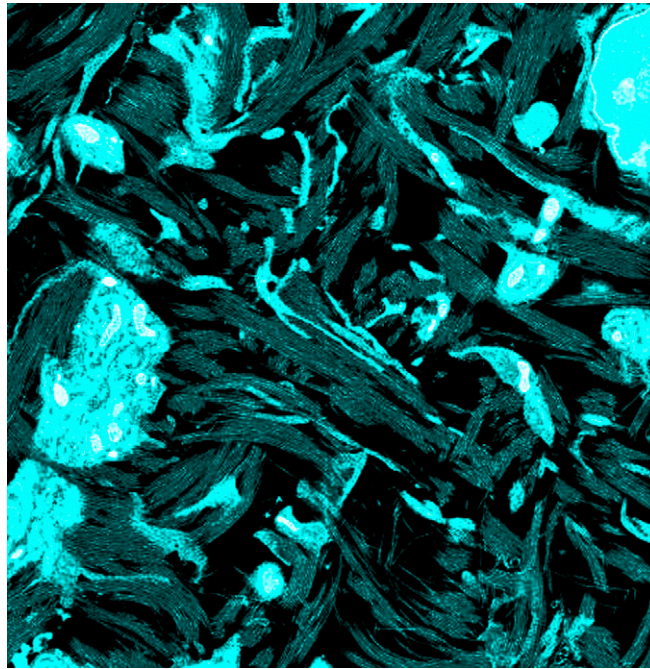
Movie S8. 3D reconstruction of cellular component from day 18 dataset shows undulating contours of keratocytes and less prominent of filopodia. (Scale bar, $\sim 5 \mu\text{m}$.)

[Movie S8](#)



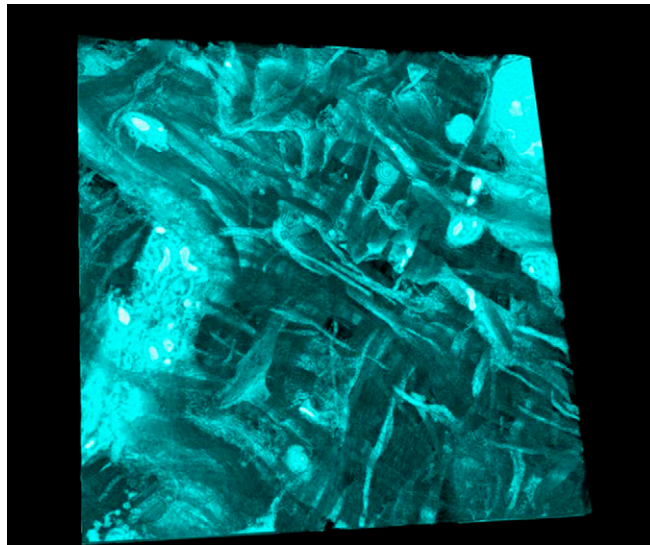
Movie S9. 3D reconstruction of collagenous matrix from day 18 dataset with condensed lamellae constituting 70% of total sample volume. Openings represent locations of cells as matrix spaces are virtually absent. (Scale bar, $\sim 5 \mu\text{m}$.)

[Movie S9](#)



Movie S10. Fly-through view of the 12-d chicken embryo cornea volume used to create Fig. 4. This image stack can be visualized as a 3D reconstruction by importing it as an animated gif into the open source image analysis software Fiji (<http://fiji.sc>). Once opened, the file can be displayed using the plugin 3D Viewer with threshold value 0 and resampling factor value 1. (Scale bar, $\sim 1 \mu\text{m}$.)

[Movie S10](#)



Movie S11. 3D reconstruction of a randomly selected region from a 12-d chicken embryo cornea as imaged by SBF SEM (3View). Lighter tones correspond to increased protein density (often corresponding to cellular mass). Darker tones show collagenous material. The volume reconstructed in this figure corresponds approximately to $4.74 \times 4.84 \times 3.0 \mu\text{m}$. (Scale bar, $\sim 1 \mu\text{m}$.)

[Movie S11](#)