Electronic Supplementary Material (ESI) for Soft Matter.

Supplemental Material

Methods

Femur head and neck were excised from a 0.5-day old mouse, embedded in the polyvinylpyrrolidone solution and cryo-sectioned as described in Methods. A frozen 12 μm section was picked up on a glass slide and air-dried. A 300 x 400 μm cartilage region was cut from the middle of the section, hydrated in CNC solution (2 mM $CaCl_2$, 150 mM NaCl, pH~7.5), transferred to CNC solution, washed, mounted on a 2mm BaF_2 window and air-dried quickly, so that the cutout region spent 2 minutes in the solution. The section was covered by another 2 mm BaF_2 with 30 μm spacers between the windows, and, sealed. The sealed windows were mounted into flow-through high-definition infrared (HDIR) chamber[1] thermo-stated at 20.0 °C and placed under Vertex 70 / Hyperion 3000 infrared hyperspectral imaging system (Bruker Optics, MA).

The sample was hydrated by flushing CNF solution (2 mM $CaCl_2$, 150 mM NaCl, 10 μM NaF, pH~7.5) between the windows, and transmission spectra of the section were recorded using with a focal plane array detector at variable time intervals at 8 cm^{-1} spectral resolution, and, 2.79 μm pixel and 6 μm optical resolutions. Each recording accumulated the number of scans increasing from 100 to 1000 (collection time increasing from 3 to 30 min) and was followed by flushing fresh CNF solution. Analyses of visible images and HDIR spectra indicated that the section did not move along the windows and the gap between the windows near the section remained fixed at 12 μm based on the intensity of the water absorption band at 1645 cm^{-1}.

Results

We acquired infrared absorption spectra, Fig. S1C, of a mouse cartilage section, Fig. S1A,B, at different time intervals after hydration in physiological-like solution using a focal plane 128 x 128 array detector covering a 355 x 355 μm^2 area of the section. Spectra from each detector pixel, Fig. S1C, were corrected for water contribution and decomposed onto individual components dominating in cartilage matrix as described in Ref. 2. The apparent density of the components was calculated by dividing their contribution to the spectra by the nominal section thickness of 12 μm. The resulting density distributions were plotted as greyscale images in Fig. S1D.

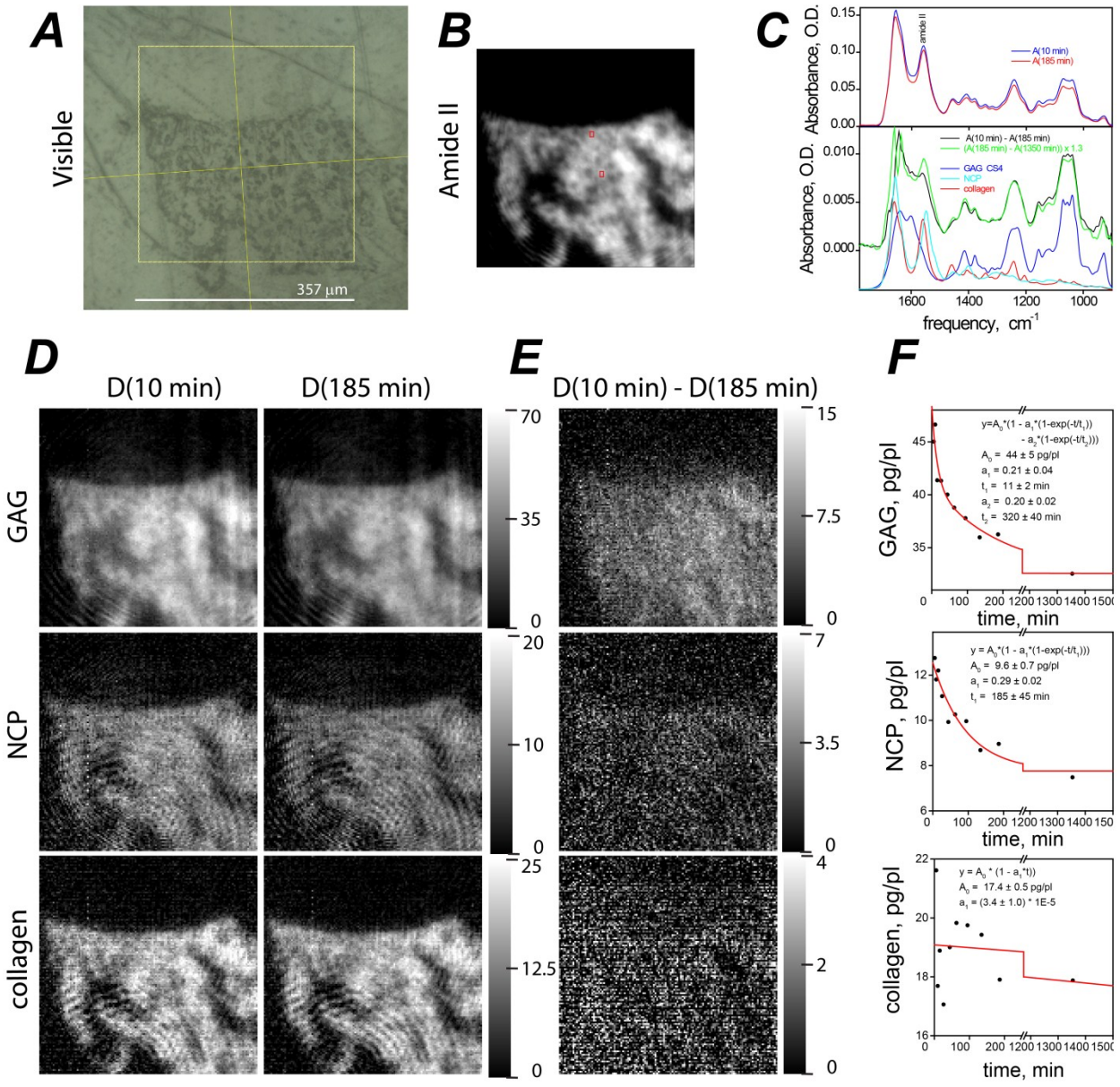
We observed a decrease in absorption intensity with increasing time, Fig. 1C, at pixels corresponding to extracellular matrix in the section, Fig. 1B. These data showed increasing loss of molecular components of the matrix. This was indicated by the difference of spectra between different time points and the spectra of compounds modeling major matrix components at physiological conditions, Fig. S1C. The corresponding images of the loss of the components is shown in Fig. S1E. The loss of individual components as a function of time were quantified by exponential decay fitting with the minimal number of exponents needed to describe the data, Fig. S1F. The loss of glycoseaminoglycans (GAG) exhibited at least two exponential decay components, while non-collagenous core proteins (NCP) exhibited a single exponential decay in the measured 23-hour interval, Fig. S1F. Collagen component exhibited slight loss close to the detection limit. Note that the fitting parameters were similar for matrix areas near and away from section edges (cf. red squares in Fig. S1B), suggesting that the components losses were not limited by lateral diffusion along the section sandwiched between HDIR chamber windows.

The lost components were dominated by aggrecan, the major proteoglycan of cartilage matrix, and by other non-collagenous proteins. This was indicated by the mass ratio of the lost components, GAG-to-protein molecular mass ratios of individual proteoglycan molecules, known approximate matrix content of proteoglycans and the lost GAGs being dominated by chondroitin sulfate, Figs. S1C,F.

Supplemental References

1 E. L. Mertz, J. V. Sullivan, Flow-through, thermal expansion-compensated cell for light spectroscopy, 2008, *United States patent* 7,355,697.

2. E. L. Mertz, M. Facchini, A. T. Pham, B. Gualeni, F. De Leonardis, A. Rossi and A. Forlino, *The Journal of Biological Chemistry*, 2012, **287**, 22030-22042.



Supplemental Fig. S1. HDIR hyperspectral imaging of loss of extracellular matrix components from a 12 μm section of unfixed cartilage of a 0.5-day old mouse. ***(A)*** Visible transmission image of a dehydrated cartilage section loaded into HDIR chamber and viewed via infrared microscope objective. The yellow square marks region captured by the infrared array detector. ***(B)*** HDIR transmission image of the section after hydration. The image is based on integral intensity of amide II spectral band shown in C. Pixel brightness is proportional to the amide II intensity. Note that darker "holes" in this image correspond to cell lacunas because almost all cells fell off during loading of the section. Red squares away and near the section edge illustrate 2x3 pixel areas used to monitor matrix content after section hydration. ***(C)*** HDIR spectra of a representative matrix area shown in B and of model compounds representing the major matrix components. The top panel shows spectra corrected for water contribution at 10 and 185 minutes after hydration. Bottom panel shows differences between matrix spectra at given time points and spectra of model compounds showing substantial loss of glycoseaminoglycans (GAG) and non-collagenous core proteins (NCP) from this unfixed section. The shown model compounds are

chondroitin sulfate 4 (CS4, the dominating GAG in mouse cartilage), type II collagen (the dominating collagen type in cartilage), and, NCP represented by bovine serum albumin recorded previously[2]. The model spectra are vertically offset for clarity. ***(D,E)*** Images of apparent densities *D* of matrix components at given time points and their differences obtained by decomposition of array detector spectra onto model NCP, collagen and sulfated and unsulfated GAGs compounds as described in Ref. 2. Vertical greyscale bars relate pixel brightness to the apparent densities in units of pg/pl. The density differences in panel (*E*) are between 10 and 185 min time points for GAG and NCP, and, between 185 and 1350 min for collagen. ***(F)*** Representative time dependences of matrix contents measured at a 2x3 pixel matrix area and their fitting curves. Fitting models of exponential decay with minimal number of parameters were used, and, their formulas are given in the plots. The resulting fitting parameters averaged over 10 matrix areas and their standard errors are shown in the plots.