Chemical composition and sulfur speciation in bulk tissue by X-ray spectroscopy and X-ray microscopy: corneal development during embryogenesis

Supporting Material

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Table S1 Energies of K-alpha fluorescence emission lines for distinct elements.

Element	**K-alpha edge energy (eV)**
Phosphorus (P)	2015
Sulfur (S)	2308
Chlorine (Cl)	2622
Potassium (K)	3313
Calcium (Ca)	3691

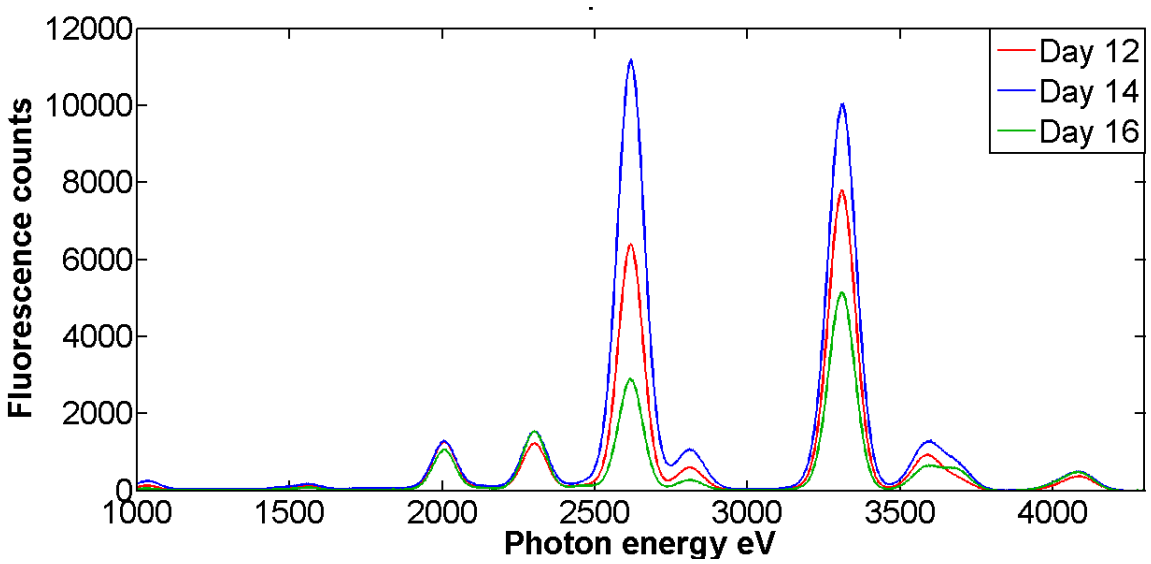

FIGURE S1 Elemental alterations in the developing chick cornea. XRF microscopy average spectra of developing chick corneas at days 12 (red), 14 (blue) and 16 (green). Four corneas per embryonic day were analyzed using the scanning X-ray microscope at the endstation ID21 at the ESRF.

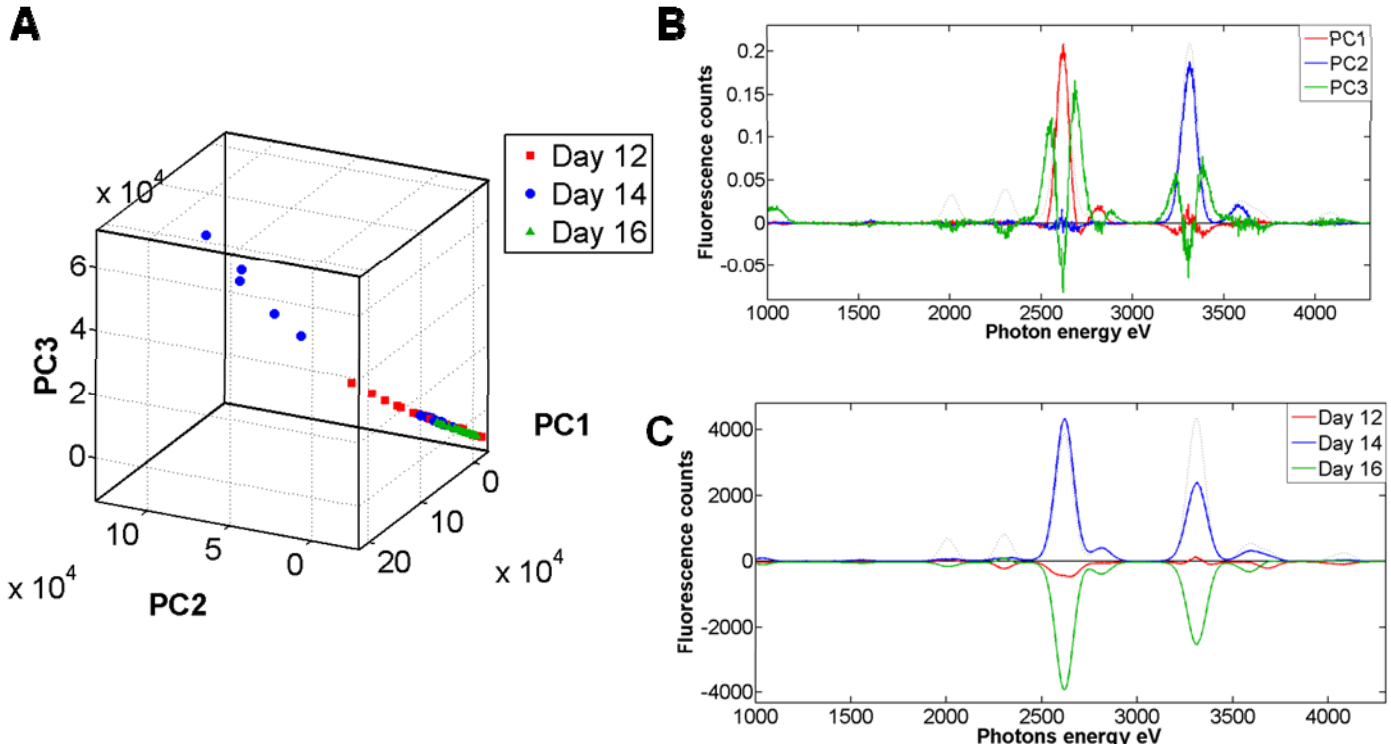

FIGURE S2 Identification of the chemical composition associated with spectral clusters of distinct developing chick corneas using XRF microscopy with PCA. **(A)** 3-D PCA scores plot of chick corneas at different developmental days: day 12 (red squares) *vs.* day 14 (blue circles) *vs.* day 16 (green triangles). **(B)** PCA loadings plot of developing chick cornea at day 12 *vs.* chick cornea at day 14 *vs.* chick cornea at day 16 with principal components (PCs) as follows: PC1 (red), PC2 (blue), PC3 (green). **(C)** Corresponding PCA cluster vectors plot of developing cornea at day 12 *vs.* cornea at day 14 *vs.* cornea at day 16 (cornea at developmental day 12 as comparator).

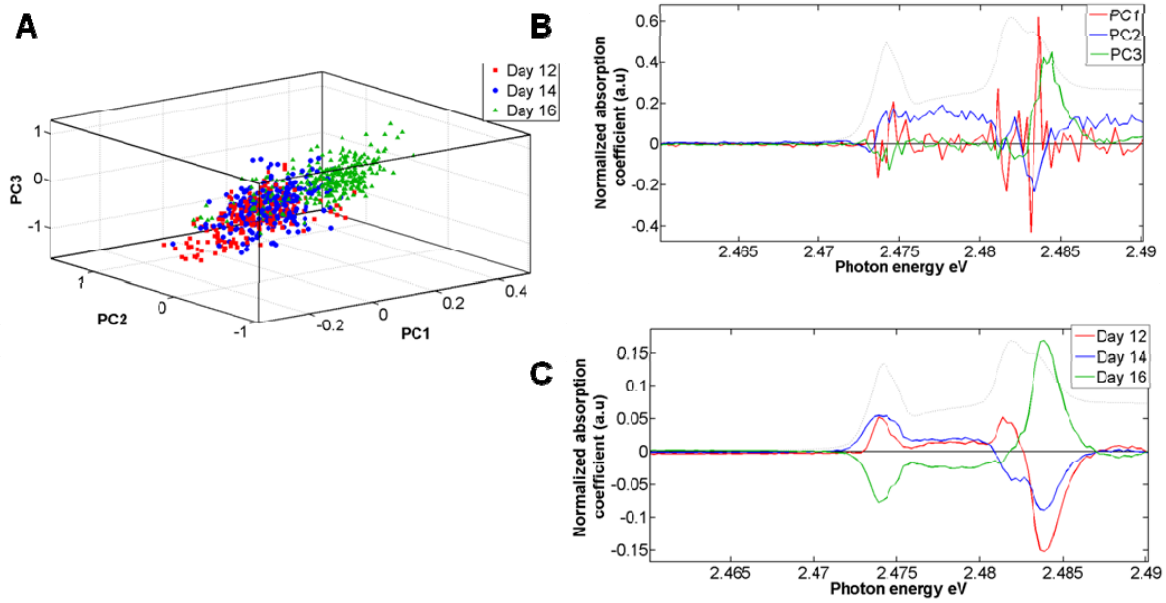

FIGURE S3 S speciation in the developing chick cornea. S XANES spectra of developing chick corneas at days 12, 14 and 16 were obtained and data subjected to PCA. **(A)** 3-D PCA scatter plot of the S speciation of developing chick corneas at day 12 (red squares) *vs.* day 14 (blue circles) *vs.* day 16 (green triangles). **(B)** Corresponding PCA loading plot of developing chick corneas with principal components (PCs) as follows: PC1 (red), PC2 (blue) and PC3 (green). **(C)** PCA clusters vector plot of developing chick cornea at day 12 *vs.* day 14 *vs.* day 16 (chick cornea at day 12 as comparator).