LIFE SCIENCES

Cathodoluminescence Analysis of Extra-Cellular Matrix

Spectral quantitative CL analysis of tissue isolated ECM

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Abstract

Thanks to its nanometer scale resolution, high-sensitivity and quantitative label-free capabilities, Cathodoluminescence (CL) analyses were performed for the first time in a soft biological derived tissue. Due to a short carrier diffusion, a 40nm-resolution was demonstrated allowing isolated collagen tissue CL analysis. Distinction of spectral signatures of two different Extra-Cellular Matrix (ECM) networks had been performed. This reveals how CL can track differences in proteins distribution and monitor cell-modelling process by revealing the modifications along isolated ECM matrix.

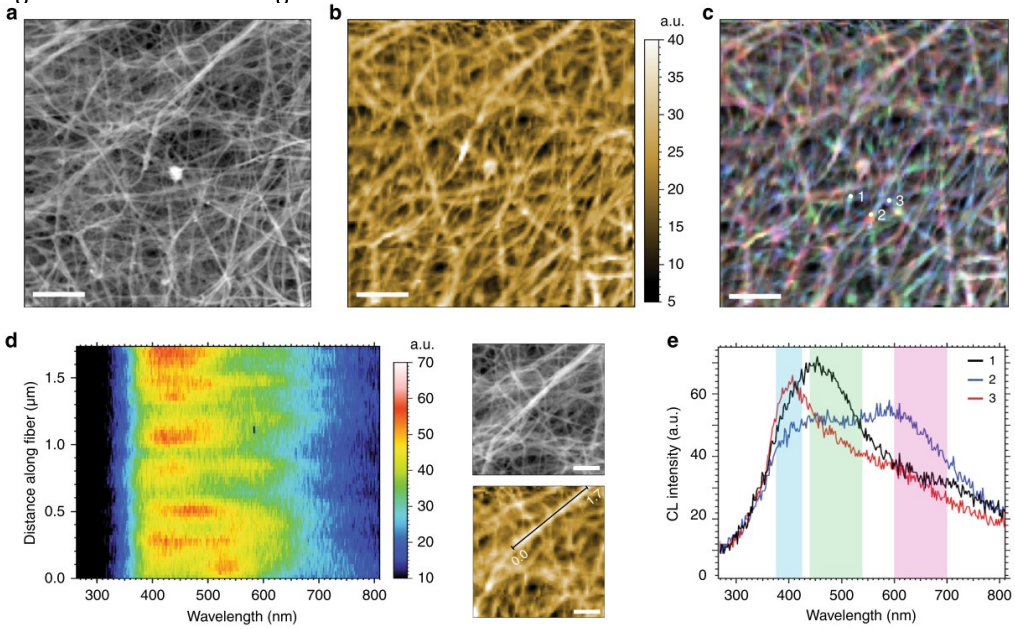

Fig. 1: High resolution SEM and spectrally resolved auto-cathodoluminescence images of a bovine collagen gel.

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Introduction

One of the main challenges in the life sciences area is the deeper understanding of complex biological structures. For that, non-destructive, label-free and nanometer spatial analysis tool is required but so far no such tool is present on the market. By combining the nanometric spatial resolution of an electron beam with the simultaneous spectroscopic analysis of the materials, Cathodoluminescence (CL) appears as a new and innovative way to reach these specificities. This application note illustrates how CL uncovered differences in proteins distribution in complex and heterogeneous biomaterials.

Experimental part

CL analyses were performed on bovine collagen gels prepared from 5mg/ml stock of liquid bovine type I collagen and on compressed rat collagen sheets laminated with fibrin (CFC gel). Samples were fixed in 4% PFA and embedded in paraffin. 80µm-tick sections were placed on clear silicon substrates then deparaffinized using xylene solvent and finally sputtered with a 3-4nm thin Au-Pd layer to ensure no surface charging.

CL-SEM analyses were performed at 8keV acceleration voltage, 10nA probe current and at room temperature. CL hyperspectral maps were recorded with 43.2nm pixel size, achieved by focusing a ~4nm tight beam. Data were recorded at 20ms/pixel providing enough signal-to-noise (SNR) ratio. Post-data treatment was done with Attomap software.

More details of the sample preparation and CL analyses can be found in the published paper.

Results

Spectrally resolved CL

Due to their fragility and their sensitivity to vacuum and e-beam irradiation, biological are hardly analysable in such conditions. In addition, CL analyses of organic and biological samples are challenging due to the very low light emission of these latter after electronic excitation. Thanks to the high optical collection efficiency offered by the integrated reflective objective on Attolight systems, CL signals have been successfully detected for the studied samples.

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The examination of the surface morphology by SEM reveals nanometer size isolated extracellular fibrils (Fig. 1a). Highly efficient optical collection allowed to perform label-free auto-CL hyperspectral imaging at 550nm spectral bandwidth (Fig. 1b) and at three monochromatic wavelengths (overlay of RGB components 400/490/650nm; Fig 1c). These CL images in good correlation with the SEM image, revealed complex spectral information from the individual fibers. To our knowledge, this first time a technique allows the access to nanometric hyperspectral information in a complex biological sample network.
Figure 1c reveals the heterogeneity of the molecular composition in the material. Figure 1e exhibits the different CL emissions from 3 different locations on the sample. The 2D projection of the CL signal evolution along a collagen fibril is shown fig. 2d. Each pixel row is obtained by probing every 43.2nm along the selected fibril (1.73µm distance, marked in the zoomed SE and auto-CL images). Clear spectra evolution along the fiber demonstrates very short carrier diffusion lengths in this type of bioorganic materials. In addition, the high spatial precision enables to access to precise spectral information and thus to the clear identification of the molecular composition variation.

CL characterization of engineered collagen-fibrin-collagen gel

Human smooth muscle cells (hSMC) were added during the gelation phase of the collagen then cultured for 2-4 weeks. A decellularization treatment was then done to lead to humanized CFC (collagen-fibrin-collagen) gels. Whereas the presence of fibrin layers within the CFC gel is not observed in SE image (Fig. 2c), blue-shifted auto-CL emission on CL spectra reveals significantly (Fig. 2b) their presence. Fibrin CL emission was also detected on the left-side facet of the studied CFC gel, visible in Fig. 2c as a side wall of the cross-section in the bottom left corner of the SE image. This thin fibrin layer is present due to expansion of the fibrin excess in a mold during the CFC gel lamination process. Identification of such a thin coating on a topographically-similar collagen would not be possible by means of SEM without CL.

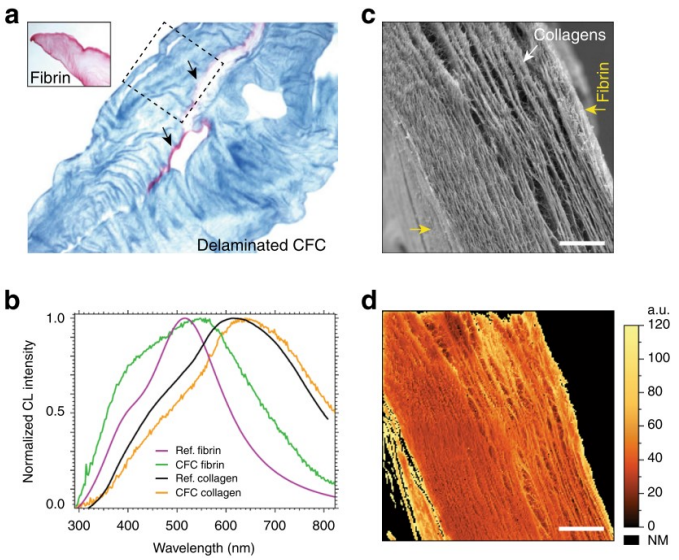

Fig 2: Optical histology SEM and auto-CL images of bio-engineered hybrid rat CFC gel

Figure 2b shows a slight spectral shift as well as a difference in full width at half maximum (FWHM) between the black and orange curves. These evolutions indicate an increased heterogeneity of the studied sample. The green curve referred to CL signal from fibrils sites. Compared to the reference spectrum (purple curve), the CFC fibrin curve (green curve) displays a slight red-shift as well as a higher FWHM, suggesting a more complex molecular composition, likely due to diffusion at the collagen–fibrin interface during the CFC preparation process.

Conclusion

The capability of spectrally resolved CL microscopy in the identification and localization of ECM protein complexes was demonstrated. To our knowledge this is the first time, label-free CL imaging is performed on a complex biological sample. Thanks to the small beam size (4nm) and high optical collection efficiency, Attolight tools were able to detect and provide new information on the material composition moreover over a 180 µm FOV (max FOV in the CL mode of the microscope can reach 300 µm), with a lateral resolution of only a few nanometres.

Reference

Quantitative intrinsic auto-cathodoluminescence can resolve spectral signatures of tissue-isolated collagen extracellular matrix, M.S. Zielinski, E.Vardar, G. Vythilingam, E-M. Engelhardt, J. A. Hubbell, P. Frey, H. M. Larsson, Nature Communications Biology (2019) 2:69

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