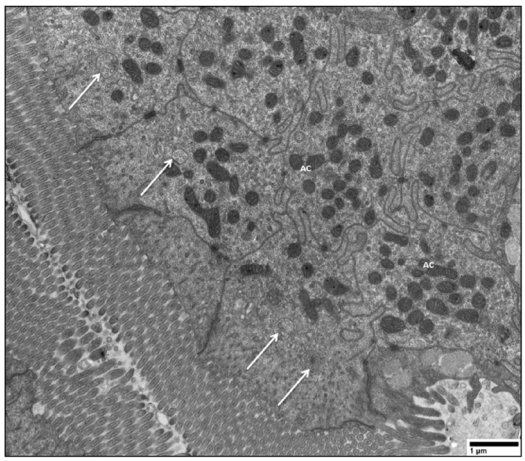
Highlights from Microscopy AND Microanalysis

Biological Applications

In Vivo Multivesicular Body and Exosome Secretion in the Intestinal Epithelial Cells of Turtles During Hibernation by WA Vistro, Y Huang, X Bai, P Yang, A Haseeb, H Chen, Y Liu, Z Yue, I Tarique, Q Chen, *Microsc Microanal* | doi:10.1017/S1431927619015071

We investigated the *in vivo* biological processes of multivesicular bodies (MVBs) and exosomes in mitochondria-rich cells (MRCs), goblet cells (GCs), and absorptive cells (ACs) in turtle intestines during hibernation. The exosome markers cluster of differentiation 63 (CD63) and tumor susceptibility gene 101 (TSG101) were positively expressed in intestinal villi during hibernation. The distribution and formation processes of MVBs and exosomes in turtle MRCs, GCs, and ACs were further confirmed by transmission electron microscopy (see figure). During hibernation, MRCs and ACs abundantly secreted early endosomes (ees), late endosomes (les) and many "heterogeneous" MVBs. Interestingly, the ees, les, and MVBs were detected in the cytoplasm of GCs during hibernation; however, they were absent when turtles were not hibernating. In addition, the number of different MVBs with intraluminal vesicles and heterogeneous endosome–MVB–exosome complexes was significantly increased in MRCs, GCs, and ACs during hibernation. These findings indicate that intestinal epithelial cells potentially perform a role in the secretion of MVBs and exosomes, which are essential for mucosal immunity during hibernation.

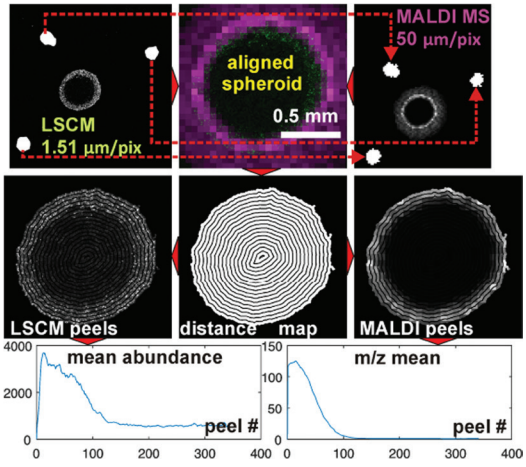


Absorptive cells (ACs) secreted more multivesicular bodies (arrows) in the intestine of Chinese soft-shelled turtles during hibernation period. The most MVBs were localized near the apical cytoplasmic region of ACs in the preparation for fusion with the plasma membrane and the release of exosomes. Scale bar=1 micron.

Techniques

Quantitative Assessment of Anti-Cancer Drug Efficacy from Coregistered Mass Spectrometry and Fluorescence Microscopy Images of Multicellular Tumor Spheroids by J Michálek, K Štěpka, M Kozubek, J Navrátilová, B Pavlatovská, M Machálková, J Preisler, A Pruška, *Microsc Microanal* | doi:10.1017/S1431927619014983

Spheroids—three-dimensional aggregates grown from a cancer cell line—represent a model of living tissue for chemotherapy investigation. Distribution of chemotherapeutics in spheroid sections was determined using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI). Proliferating or apoptotic cells were immunohistochemically labeled and visualized by laser scanning confocal microscopy (LSCM). Drug efficacy was evaluated by comparing coregistered MALDI MSI and LSCM data of drug-treated spheroids with LSCM data of untreated control spheroids. We developed a fiducial-based workflow for coregistration of low-resolution MALDI MS with high-resolution LSCM images (see figure). To allow comparison of drug and cell distribution between the drug-treated and untreated spheroids of different shapes, we introduced a common diffusion-related coordinate, the distance from the spheroid boundary. In a procedure referred to as "peeling," we correlated average drug distribution at a certain distance with the average reduction in the affected cells between the untreated and the treated spheroids. This approach makes it possible to differentiate between peripheral cells that died due to therapy and the innermost cells that died naturally.



(Top row) *Fiducial markers* (i.e., three white dots) are registered onto MALDI MS images (red dashed arrows) to align the LSCM and MALDI spheroid images (center). (Middle row) Spheroid "peels" denote regions over which average drug concentration (right) and cell abundance (left) will be calculated. (Bottom row) Mean values of drug concentration and proliferating cell abundance.

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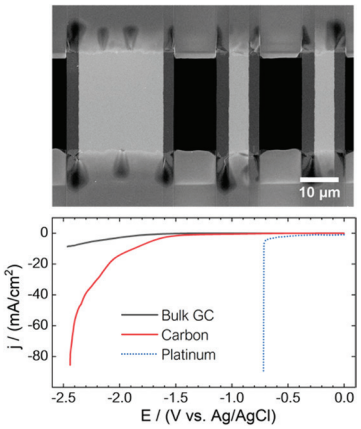
Materials Applications

Electrochemical Behavior of Carbon Electrodes for *in situ* Redox Studies in a Transmission Electron Microscope by R Girod, N Nianias, V Tileli, *Microsc Microanal* | doi:10.1017/S1431927619015034

Implementation of liquid cell transmission electron microscopy (TEM) toward the study of electrocatalytic reactions is hindered by the reactivity of metallic substrates. Carbon electrodes are used for their inertness in bulk electrocatalytic measurements, however, their performance can be significantly modified when they are microfabricated on chips for TEM. Here, we characterized the electrochemical behavior of carbon-coated electrodes in a microfluidic cell. We first used cyclic voltammetry to study the effects of scan rate, flow rate, and liquid layer thickness on the electrochemical response of the substrates. To relate the potentials with bulk values, we then implemented a system using a bulk reference electrode in series. We used this configuration and an open-cell setup to study the capabilities of the substrates for a range of energy conversion reactions, including CO_2 reduction reaction (see figure). Our results demonstrate that, compared to platinum electrodes, the carbon-coated ones systematically exhibit a wider inert potential window. This work provides a basis for interpretation of microscale electrocatalytic redox reactions in the TEM.



Scanning electron micrograph showing the scale of carbon-coated band electrodes on the electron transparent window of a chip (top). Comparison of electrochemical activities of carbon-coated and platinum microelectrodes with a bulk glassy carbon (GC) (bottom).