The Effect of Ashing on Cells: Spectromicroscopy of Physiological Elements


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We analyzed the effects of cold oxygen plasma ashing of neurobiological specimens on different elements with synchrotron spectromicroscopy. Our results demonstrate that while carbon is almost completely removed, phosphorus, calcium, potassium, sulfur, and, to some extent, nitrogen are retained and their relative concentration is enhanced. © 1997 Academic Press

Ashing is a procedure used to reduce the thickness of tissue sections or to investigate the chemical properties of biological specimens (1–3). This procedure, in particular, is supposed to eliminate certain elements (carbon, nitrogen, and hydrogen) and enhance the relative concentration of others—thus, making it easier to investigate these latter. The effects of ashing on the structure of specimens have been extensively analyzed (1). On the other hand, the chemical effects are much less known.

Our present study analyzes these effects, by comparing ashed and nonashed neurobiological specimens with synchrotron spectromicroscopy (4–8).

MATERIALS AND METHODS

Synchrotron spectromicroscopy yields information on the microchemical composition of the specimen (4–8). It can study biological or materials science samples as long as they are sufficiently conductive, flat, and UHV compatible. The technique consists of sending onto the system monochromatized X rays emitted by a synchrotron light source (the Wisconsin Synchrotron Radiation Center storage ring Aladdin in our case) and detecting the photoelectrons thus produced. The photoelectron intensity vs photon energy curves reproduce the optical absorption coefficient of the system, and from characteristic spectroscopic features one can extract information on the presence and chemical status of each element (9).

We studied ashed and nonashed neurobiological specimens with the recently commissioned MEPHISTO spectromicroscope (from the French acronym “Microscope à Emission de Photoélectrons par Illumination Synchronique de Type Onduleur”), with a spatial resolution of 0.2 μm.

The specimens were primary cultures of rat cerebellar granule cells. A detailed description of our specimen preparation process can be found in Refs. (7) and (8). In short, cells extracted from rat cerebellum were allowed to grow for 8 days on gold-coated silicon substrates, pretreated with 10 μg/ml of poly-L-lysine solution. Selective techniques were used to obtain a prevailing population of granule cells (10–12).

At the end of the growth period, the cultures were washed, fixed with paraformaldehyde, and dehydrated. Part of the cultures were analyzed in this form, and others were first ashed with a cold plasma (150 C, Plasma-Processor 300E, Techn. Plasma GmbH, München) in the presence of oxygen for ≥24 h.

RESULTS AND DISCUSSION

Figure 1 shows an ashed granule cell culture imaged by MEPHISTO. In these micrographs the contrast is originated by the different photoelectron emission
yield of different elements. In the present case, the images were acquired at 140 eV photon energy, where phosphorus is photoemitting more electrons than gold; therefore, the phosphorus-containing cells appear brighter than the surrounding gold substrate.

Note that after ashing the cell thickness is reduced and the cell structures appear flat. Carbon constitutes the backbone of the macromolecules supporting the cytoarchitecture of cells; therefore, by removing carbon by ashing, the cells lose their skeleton and consequently their three-dimensional structure.

With the MEPHISTO microscope one can select microscopic areas on micrographs like those of Fig. 1 and acquire X-ray absorption spectra scanning the photon energy, thus revealing the chemical composition of specific features such as cell structures.

Figures 2–8 show results obtained in the spectral regions of the carbon 1s, nitrogen 1s, oxygen 1s, calcium 2p, phosphorus 2p, potassium 2p, and sulfur 2p edges. All spectra are normalized to the monochromator yield curve.

A comparison between the spectra for ashed and nonashed specimens, reported in all figures, shows the results of our study.

Specifically, as shown in Fig. 2, carbon is almost completely removed by ashing. We also note that the traces of carbon left after ashing appear in a different chemical status with respect to the nonashed sample. The first spectral feature, in fact, occurs at 285 eV (aliphatic carbon) before ashing and at 286 eV after ashing. Such a difference may be related to a more oxidized C state formed during ashing with oxygen plasma.

On the other hand, Fig. 3 shows evidence of the presence of nitrogen in the nonashed sample. The nitrogen peak is shifted to higher energy after ashing, suggesting a change in its chemical environment. This shift may be due to a more oxidized state of nitrogen in the ashed sample.
ence of nitrogen even after ashing, with no significant difference in N concentration. This somewhat surprising result can be explained by the probable differences between the carbon-removal process and the nitrogen-removal process.

The chemical state of N is also modified by ashing: the first peak at 400 eV is more intense than the edge at 403 eV in the ashed sample, and vice versa in the nonashed sample. This feature at 403 eV may correspond to the aminic group –NH₂ present in basic amino acids, and –CONHC– group in peptide bonds (13), removed by ashing. Also, the spectral “shoulder” at >410 appearing after ashing can be interpreted as –ONO₂, NO₂, and –ONO (13), most likely being formed during the oxidation procedure.

Figure 4 shows that oxygen, as expected, is not removed by the ashing process, and yet its concentration is enhanced. We also observe that ashing induces the
CONCLUSIONS

We analyzed the effects of cold oxygen plasma ashing on neuron cells by synchrotron spectromicroscopy. By comparing spectra taken on ashed and nonashed cell cultures we conclude that: first, carbon is almost completely eliminated by the ashing process, as expected. Second, we found that nitrogen is not removed, although its chemical status is altered by ashing. Third, we found that the relative concentrations of oxygen, calcium, phosphorous, potassium, and sulfur are dramatically increased. Altogether our results indicate that the ashing procedure applied to biological samples greatly facilitates the study of fundamental elements present in small amounts or trace concentrations.

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