Methods of Electron Microscopy of Biological and Abiogenic Structures in Artificial Gas Atmospheres

O. V. Gradov\textsuperscript{a},\textsuperscript{b} and M. A. Gradova\textsuperscript{b}

\textsuperscript{a}Tal’roze Institute of Energy Problems of Chemical Physics, Russian Academy of Sciences, Leninskii pr. 38, Moscow, 119334 Russia

\textsuperscript{b}Semenov Institute of Chemical Physics, Russian Academy of Sciences, ul. Kosygina 4, Moscow, 119991 Russia
e-mail: o.v.gradov@gmail.com

Received October 13, 2014; in final form, March 2, 2015

Abstract—This paper reviews opportunities for using electron microscopy in various gas atmospheres for the analysis and morpho-physiological modification of biological structures. The approaches that allow varying the gaseous phase content, as well as temperature, humidity, and pressure, are considered. The applicability of both kinetic and dynamic approaches to the tissue and bioinorganic structure manipulations is pointed out. The possibility of simulation of the beam-induced formation and disintegration of abiogenetic molecular structures is also mentioned as a particular case of the electron beam influence and treatment of the precursor medium in an artificial atmosphere.

Keywords: electron microscopy, atmospheric scanning, electron beam processing, artificial atmospheres, gas microchambers, abiogenesis

DOI: 10.3103/S1068375516010063

1. PRINCIPLES OF ELECTRON MICROSCOPY IN A GAS ATMOSPHERE

In the last quarter of the 20th century, the ultimate problem and exceptional necessity for experimental morphological studies in situ under an electron beam affecting singles cells [1] and tissues [2] was to create a gas atmosphere in a chamber suitable for the intravital observation and retention of physiological functions of the object under study, which conflicted with the accessibility of the quality of detection of the image or analytical response from the experimental tissue because of the inevitability of electron diffusion in the gas [3], from which followed not only the interaction of the irradiating beam with the atmosphere surrounding the biological object but also the interaction of the signal beam, i.e., the beam being detected, with the gas [4], which made senseless any attempts to acquire analytical information from the registered signal as a result of the effects of electron–ion recombination [5] and background problems with cathodoluminescence and scintillations in the gas [6]. From these prerequisites, there was the critical requirement in practice to overcome the conflict between the intravital character of the physiological study of the object in a natural environment that to this end was to be had in the electron microscope chamber (the composition of the gas, variable temperature and pressure, possibility for the partial replacement of the composition of the gas for the purposes of preconditioning [7–9], among other things, in the case of the change in the pressure in the chamber and partial pressure of the gas [10, 11], etc.) and needs of registration, which were integral features of the method itself for physical reasons (or, more properly, principles).

In addition, in the absence of the possibilities for microscopy in various gas atmospheres, there was also no possibility for the intravital dynamic study of the structure of cells and tissues depending on the change in the composition of the experimental “atmosphere” (which provided the basis for such a currently promising field as gas biology [12, 13]) despite the fact that methods for dynamic and, in particular, intravital structural study have been known in electron microscopy since the mid-1960s (so-called stroboscopic electron microscopy [14–17], which is currently successfully replaced by the methods of four-dimensional electron microscopic analysis [18–20], although many such methods of microscopy, in particular, dynamic cryoelectron microscopy and microprocessing, which is a novel approach in structural, systemic, and synthetic biology [21], retain the elements of a classic stroboscopic technique [22]).

The solution was reached in the pioneer works by G.D. Danilatos, who laid down the principles of a new method, so-called ESEM (environmental scanning electron microscopy), which allowed introducing samples into a controlled gas atmosphere, including natural atmosphere. Omitting the intermediate phases of the publication activities of the aforesaid author, who has over fifty works on ESEM, one can cite a
series of works describing a generalized form of equipment of this type, which is suitable for both atmospheric scanning microscopy and similar electron microscopy in a controlled gas atmosphere, for the sake of completeness of information [23–26]. A little later, similar units became quite common in various laboratories, which resulted in the creation of many techniques for fine ultrastructural analysis or cytomorphological analysis under atmospheric pressure. Today, there are many methods and techniques of this type, including atmospheric pressure scanning transmission electron microscopy [27], atmospheric correlative scanning electron microscopy and atmospheric correlative immunoelectron microscopy [28, 29], and technologies for atmospheric scanning electron microscopy for the registration of dynamic phenomena in liquids and gases on the corresponding interphase boundaries [30]. These technologies are suitable for the analysis of single cells in a gas atmosphere [31, 32], cell cultures and tissues [33, 34], and visualization and systematization of biologically relevant objects of the natural environment [35] and protein microcrystals [36] (lately, effective visualization down to the atomic level has been achieved under normal pressure of the natural environment [37]).

2. BIOPHYSICAL AND BIOCHEMICAL PROBLEMS OF ELECTRON MICROSCOPY IN A GAS ATMOSPHERE

Two fundamental elements of atmospheric electron microscopy and electron microscopy in a gas atmosphere for biological applications can be distinguished: (a) achieving variable pressure of the atmosphere being pumped and (b) selecting a biologically optimum chemical composition of the atmosphere. The first attempts at creating electron microscopes with controlled pressure of the gas date back to the early 1960s [38], but their actualization did not take place until the creation of ESEM in 1980s, while currently variable pressure electron microscopes are heavily used for charge contrast mapping of biological samples [39] and similar tasks of biological visualization [40], as well as for ultramicroscopic sample preparation and processing of samples at a nanostructured level [41]. The second problem is somewhat more complicated because, if electron microscopy is considered in a reactor approximation [42], it is necessary to take into account the inevitable interaction of the gas being pumped into the chamber with the gaseous components of biological tissues, which is particularly relevant in the case of objects popular for electron microscopic visualization such as gas vacuoles of single-celled animals [43] or lung tissues [44], as well as in experiments on the study of the secondary structure of protein in gas vesicles [45] and applied test measurements on gas permeable lenses [46]. Since numerous myohistological studies and interdisciplinary biochemical works are also conducted in electron microscopy chambers with a controlled composition of the gas [47, 48], this statement can be extended to this field as well, and taking into account the catalytic character of interactions and enzymatic properties of myosin [49], this line of research can be interpreted as an analog of catalytic electron microscopic studies in a gas atmosphere [50]. It is clear that, since catalytic processes in a biological medium occur under the principles of biophysical chemistry [51] and enzyme kinetics [52], the transition to studying the microstructure and ultrastructure in naturally occurring atmospheric conditions that preserve the dynamics is equivalent to the emergence of electron microscopic dynamic biochemistry, not any longer in that quite simplified interpretation that existed in the 1940s–1950s [53, 54] and affected the scientific thought up to the late 1980s [55], but in that more modern sense that is associated with chemical and macromolecular (even phase supramolecular) analyses [56], compartmentalization, and chemical kinetics, in particular, modern enzyme kinetics [57].

Dynamic physicochemical processes can be studied under the thermostatting of the experimental atmosphere at a specified level. Biophysical and physiological processes also require thermostatting (if we are not referring to cryobiology and extremophiles). Accordingly, electron microscopic accessories and tools for the preparation and management of a sample in situ in a chamber with thermostatting/cryostatting are required.

Differentiated tools for the monitoring of the atmosphere in a gas chamber are currently developed: gas injection holders with thermal heating in transmission microscopy [58], special-purpose cells for in situ observations [59, 60], multifunctional scanning electron microscope systems for work in a controlled atmosphere [61], which progressively differ from earlier constructions [62] (in particular, by the wide-range temperature control with the retention of the content of gases in an analytical chamber [63]). Multiple metrological procedures applicable also in the case of work with biological or hybrid objects have been developed specifically for this equipment. This refers to the methods of semiquantitative X-ray microanalysis with digital data processing which evens out the aberrations introduced by the gas [64], specialized atmospheric technologies that correct aberrations [65], techniques for measuring the effective path length of charge carriers of a scanning beam in a gas atmosphere [66], gas cascade amplification in high-resolution electron microscopy [67], and methods for real-time control of the composition of a gas in a chamber during low-energy electron spectroscopy or chemical visualization performed on the basis of equivalent principles [68]. The highest accuracy of metrology is achieved in the position-sensitive analysis of such a type when using ideal experimental units with electron emission from carbon tubes excited.
under gaseous and atmospheric conditions by atmospheric-pressure plasma [69].

3. PROBLEMS OF HUMIDITY OF BIOLOGICAL SAMPLES AND OXIDATIVE NATURE OF THE ATMOSPHERE

In all the biological works on atmospheric-pressure electron microscopy, there is a common problem, humidity of samples (similar to how the quality of the surface depends on the wettability of a crystal and can be qualitatively determined by it in X-ray structural and X-ray diffraction analyses [70]). As is apparent from psychrometric principles [71], the rate of evaporation of a liquid increases with the decrease in the pressure and relative humidity; hence, it is necessary to use a chamber that maintains a certain humidity, but this may conflict with the parameters of a gas column and/or chamber which are optimum for the registration. At the same time, the need for the psychrometric optimization for cells, tissues, or isolated fragments can be considered to be proven (at least, long and thoroughly investigated for plants [72, 73] and postulated on the basis of obvious considerations for the climatic stabilization during the cultivation of animal tissues in thermostatted incubators with a controlled gas atmosphere [74–76]).

Since the early 1970s [77, 78], works on the constructions of atmospheric chambers for the analysis of wet biological samples have appeared. Currently, this trend continues to develop but already with a focus on nanostructured materials science [79]. Recent advances in the field of microstructural aquaometry are associated with cryoelectron microscopy [80], especially with the correlative analysis of induced changes under nucleolar stress [81]. When natural atmosphere or artificial gas filling of chambers is present, aquaometry differs from known thermal vacuum methods of aquaometry [82] because, from the psychrometric perspective, it possesses higher complexity and dimension of curves depending on the content of gases. The correlation of this characteristic with the wettability of the surface and its sorption characteristics is an interesting problem because hydrophobicity affects the formation of the condensate and the results of the electron microscopic measurement of the dew point in the absence of other parameters [83].

Sorption and filtration specimens and chromatographic stationary phases obtained as a result of biochemical and biomolecular research are some of the particular cases of such difficulties. In particular, changes in the structure of sorbents during gas chromatography [84, 85] and changes in analytical plates, which are based on reactions with a solid surface, are known [86]. Moreover, there are specialized electron microscope units for obtaining isotherms of adsorption [87] and data on structural changes at an atomic level during the interaction of a gas with a solid surface [88] and for observing processes of interaction in real time [89, 90]. Given that most cytomorphological and ultrastructural studies are generally conducted on support and electron microscopy grids, the need for the registration of aforementioned phenomena in atmospheric electron microscopy with the use of wet specimens is obvious.

Another biological fine point for conducting electron microscopy in gas atmospheres is the oxidative character of many atmospheres, including those belonging to standard atmospheres for gas pumping. Thus, effects of gas polymerization during electron or other charge-corpuscular bombardment of surfaces resulting in morphologically detectable changes have been known since the 1940s [91], while technologies for the ablation of biological surfaces using activated oxygen were developed for electron microscopy in the 1970s [92]. In addition, despite a clear focus on nanostructured materials science, the range of problems of synthesis or processing of matter (cells, tissues, biological materials, etc.) with a focused electron or ion beam [93, 94], which results in the preparation of many exotic intermediates and assembly of exotic structures, continues to remain relevant. Multiple attempts to minimize and reduce interactions of the beam with the gas in controlled-pressure electron microscopes [95] ultimately lead to the reduction in chemical processes in the gas atmosphere and on the surface but do not exclude certain induced structural transformations in the substance itself.

Physical optimization using Monte Carlo methods, which is often used by experts in electron microscopy when simulating electron beam dispersion [96], where the sample acts as a target on the propagation path of electrons, with which exactly the beam interacts, does not provide an answer to the question on the structural—chemical transformations in the atmosphere if they are not associated with the elementary chemism (isotopy and nuclear physical processes). Generally, such simulations affect the improvement of the quality of the image [97] but not the biologically orientated optimization of the physics of the process of interaction of the beam with the tissue, although it is known in biomedicine that the rational configuration of an electron beam provides the optimization of the structural—biological effect [98], while methods for site-specific beam focusing have long been used in cryoelectron microscopy [99]. A so-called surface skirt, beam broadening during its dispersion in a gas atmosphere [100], can be a partial solution in the method of electron microscopy of biological specimens, although the decrease in focusing causes the deterioration of the quality of the image, and the latter is fraught with a well-known effect on the results of semi quantitative X-ray microanalysis in a column [101]. In turn, problems with distinguishing the components of the signal in scanning electron microscopy under the conditions of an atmosphere (a gas) or natural atmosphere and at “natural” temperature have been known since the 1970s [102], while methods for their
distinguishing emerged only in the early 1990s—no sooner than the tools of computer automation made it possible to do so [103].

4. APPLICABILITY OF GAS ELECTRON MICROSCOPY IN THE STUDY OF ABIOGENESIS

From the viewpoint of the corpuscular effect on biological and abiogenic media [104, 105], a synchronized synthesis and chemical analysis of protobiopolymers under a beam directly in a gas column, which simulates the conditions for abiogenesis and formation of prebiological compounds under the action of known corpuscular factors in various gas atmospheres, can be of interest. In this case, real-time analysis can be conducted according to the principles of position-sensitive electron microprobe analysis/characteristic X-ray secondary-emission spectrometry (energy-dispersive or wavelength-dispersive spectrometry depending on whether photons are scaled by energy or by wavelength). Also, despite the fact that most protobiopolymers formed in thin layers or films are “soft matter,” micro X-ray structural analysis of formed entities is implementable [106]. From the chemical perspective, the task of simulating abiogenetic processes in artificial gas atmospheres is determined by the fact that known or proposed compositions of prebiological and xenobiotic atmospheres, in particular, reducing methane-containing and hydrogen-containing atmospheres [107], volcanic eruptive and fumarole gases [108], nitrogen-containing atmosphere in a series of exotic exoplanetary concepts [109], and a whole range of others, contain various combinations of simple organogen compounds such as ammonia, methane, hydrogen, nitrogen, carbon monoxide, carbon dioxide, sulfur dioxide, and formaldehyde required for the abiogenic synthesis of organic matter. In a general thermodynamic case, an abiogenic atmosphere is an ideal gas with which a protocol membrane or lipid bilayer can “work” as Maxwell’s demon [110]. From the perspective of physics of abiogenesis, this problem statement is determined by a known role of cosmic rays [104, 105], in particular, charged particles [111, 112], in the formation of prebiological organic compounds in gas atmospheres [113], which is fundamentally different from the principles of cosmic formation of the same compounds under the action of cosmic radiations [114]. Much the same is true for the emission of particles by radioisotopes [115], which, taking into account the presence of the phenomena of cluster radioactivity, emission of one or two protons, positron decay, etc., makes it possible to bombard a “precursor” for abiogenic synthesis with modes of radiations which are quite differentiated for the synthesis of various products. Also, because of the possible catalytic role of the mineral support in the processes of prebiological synthesis and structure formation, it is essential to take into account the chemical interaction of the atmosphere with the support when studying such processes in an electron microscope column. However, this aspect of the problem is not included in the goals of this paper; therefore, it is not considered further in more detail. As a result of the reductive character of the primitive atmosphere (which corresponds to the necessary conditions for the abiogenic synthesis of organic matter), there should be no problems related to the fixation of samples in a gas atmosphere associated with gas oxidation (see i. 3) during the simulation of abiosynthesis in a gas atmosphere in an electron microscope column.

NECESSARY TERMINOLOGICAL REMARK

In conclusion, it should be noted that, while not being a tool for nondestructive control, a method of gas electron microscopy operates as an analytical method only to the limits determined by the quantum structure of possible precursors and protobionts [116, 117], after which it starts to directly affect the sample itself, thus causing organization or transformations in its structure. That said, we would also like to warn readers of careless citation without using an original work, which is common for periodical publications in Russian, when microscopes capable of registering the behavior of a quantum gas [118, 119] are “intermixed” with gas electron microscopes similar to the microscopes considered in this work, as a result of which statements are made about the achievability of quantum resolution on gas electron microscopes, which is not implementable in practice because of the interaction with the atmosphere in the propagation path of the beam to the object or else, it is more correct to say, when referring to the action by the beam on the precursor specimen, the target.

CONCLUSIONS

(1) The problem of environmental condition microscopy can be divided into two parts, namely, microscopy at natural (room/laboratory) temperature and microscopy in a natural atmosphere. Technically, both problems can be solved in one instrument combined for these purposes. The latter problem can also be extrapolated to other atmospheres that are not equivalent to Earth’s atmosphere with respect to the composition. Under such conditions, abiogenesis can be directly simulated under a beam of an electron microscope, or xenobiological systems, chemotrophic bacteria (including anaerobic bacteria), lability of microbial ecosystems for atmospheres with various compositions of the gas, etc., can be studied. The possibility for the occurrence of compartmentalized structures from abiogenic material as early as the early stages of formation of protobiologic systems under the corresponding conditions of the primitive atmosphere of Earth makes the morphostructural study of
the products of abiogenic synthesis a necessary addition to the data on their chemical composition.

(2) The problem of deep environmental electron microscopy in liquid media has not been solved and, probably, is unsolvable at this stage (although, e.g., on accelerators, sources of synchrotron radiation, corresponding leads are possible; however, these are not electron leads but leads of the “optical” beam).

(3) It is possible to combine morphological (“localomics”), dynamic (“dynamomics”), spectral (X-ray secondary-emission spectrometry or microprobe in a scanning mode, which belongs to localomics with respect to the time scale), and structural (electron diffraction and X-ray diffraction methods in situ; in general, structural analysis in situ of soft matter structures such as biological systems) analyses in the case of work and manipulations in a gas atmosphere.

(4) The combination of localomics and dynamomics results in synchronous morpho-physiological/functional-morphological and morpho-biochemical/histochemical analyses in situ under specified conditions of the atmosphere, which creates prerequisites for conceptually new 4D ultramorphology on the basis of gas electron microscopy.

(5) It is possible to combine synthesis or induced self-assembly of organic structures and compounds under a beam in an atmosphere, which makes it possible to apply the method of gas electron microscopy for simulating chemical stages of abiosis and other beam-induced processes using a gas-filled chamber as an experimental reactor.

ACKNOWLEDGMENTS

We are grateful to the members of the Department of Metrology and Measuring Instruments, Vernadsky Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, who provided us with the documentation for unique experimental equipment that offered an opportunity to start works in this direction.

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Translated by E. Boltukhina