

New morphotypes, cell-wall structures, and elemental distribution of the Gunflint microfossils

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Precambrian microfossils have been studied by previous investigators for decades to constrain their microbial activity, while their ultrastructure and chemical composition have been poorly understood. Since microbial metabolism is performed on the cell surface, the elemental compositions, ultrastructure and coexisting minerals of microfossils can be a strong clue to understand their microbial metabolisms. In the present study, in situ analysis on individual microfossils by high spatial resolution Secondary Ion Mass Spectrometry (NanoSIMS) was performed together with other standard geochemical and mineralogical methods on the Gunflint microfossils, well known as great preservation, occurred in the 1.9 Ga Gunflint Formation, Canada.

Morphological observation revealed that some new morphotypes of microfossils having morphological affinities to cyanobacterial akinete (*archeaoellipsoides*), eukaryotes and fungi, which had not been reported in this age. Bilayered cell-wall structures were also newly discovered in thick-wall spherical microfossils. Having multi-layered wall structure are essential for modern-style microbial life, such as cyanobacteria, to promote complex metabolism and the storage of organelles. These findings indicate that advanced microbes and modern-style cell wall structures had already evolved in the Paleoproterozoic ocean, earlier than thought in previous studies.

Secondary ion mapping for $^{12}\text{C}^-$, $^{12}\text{C}^{14}\text{N}^-$, and $^{32}\text{S}^-$ were newly obtained on 30 individual Gunflint microfossils with 10 morpho-types. They showed mostly similar elemental distribution patterns among different morpho-types. A unique elemental distribution was observed in globular type microfossils, showing that heterogeneous N and S distribution between the cell wall and inner structure. This indicates that this type of microfossil possibly had a special function, such as production of baeocytes during cell differentiation like modern cyanobacteria (e.g., *Pleurocapsales*).

Outer parts (i.e., walls) and inner parts of examined microfossils often showed contrast chemistries. Inside organic matter contained microcrystals of iron-bearing minerals and trace amounts of Ca or Mo. They imply that successful detection of minerals and elements, which are possibly related to cyanobacterial enzyme. Outer parts were generally depleted in N and enriched in S. In addition, Na, K, and Cl were often detected from cell wall indicating deposition of chloride crystals with organic matter of microfossils, which were suggested that the Gunflint microbiota were active in the evaporitic environment.

This research does not only constrain the microbial activities indicating the early rise of advanced microbes and modern like biological functions in the Gunflint ocean, but also provides new keys to understand the evolution of early life and their living environments in the Paleoproterozoic era.

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