doi:10.1017/S143192761501123X Paper No. 1044 Microsc. Microanal. 21 (Suppl 3), 2015

Solving the Controversy of Earth's Oldest Fossils using Electron Microscopy

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Filamentous microstructures in 3.46 billion-year-old rocks (Apex chert) from Western Australia have been claimed to represent the oldest morphological evidence of life on Earth [1]. However, the biological nature of these filaments has been questioned on numerous occasions [e.g., 2] and this has led to one of the longest running and most controversial debates in palaeontology. Here we use a combination of focused ion beam (FIB) milling, TEM and SEM to decode the detailed morphology and chemistry of the Apex filaments and determine their formation mechanism.

Critical to the claims of a biological origin for the Apex filaments is a suggested presence of cells, having three-dimensional wall compartments made of carbon [3]. In contrast, our TEM analyses of ultrathin wafers through four representative filaments reveal filament morphologies that are characteristic of a mineralic origin, with complex nano-scale intergrowths of mineral phases (Fig. 1a). Each filament is made up of multiple plate- or sheet-like grains of phyllosilicate (Fig. 1b, green), sitting within a matrix of microcrystalline quartz. Occasionally quartz is also seen inter-grown with the phyllosilicate within a filament. ChemiSTEM mapping shows that the phyllosilicate mineral(s) contain the elements K, Al, Si, O, plus variable amounts of Ba and minor Mg. Electron diffraction patterns of this mineral obtained in the TEM possess d-spacings consistent with a 2:1 layered phyllosilicate crystal lattice structure. This structure is found both in micas such as muscovite and some clay minerals. The nano-morphology of the phyllosilicate, appearing as a worm-like stack of crystals, closely resembles vermiculite, a common alteration product of mica. However, the chemical composition is spatially heterogenous on the nano- to micro-scale. This, together with the presence of barium, suggests that the phyllosilicate is likely a complex hydrothermal association of mica alteration products that are best termed vermiculite-like.

Further ChemiSTEM mapping shows that carbon (Fig. 1b, yellow) and iron (Fig. 1b red) are closely associated with the phyllosilicate filaments. Both carbon and iron are seen interleaved between sheets of phyllosilicates within the body of the filaments, and also coat the outer margins of some parts of the filaments. In addition, carbon occurs away from the filaments within the quartz matrix where it forms a boundary phase between quartz grains (Fig. 1a). These data indicate significant redistribution of carbon both within and around the Apex filaments, in marked contrast to patterns found by us in *bona fide* fossil microbes from younger rocks [4]. Carbon interleaved between phyllosilicates within the filaments may resemble 'cellular compartment walls' when investigated with lower spatial resolution (for example in optical work). Our higher spatial resolution analysis of supposed 'cellular compartments' instead reveals very inconsistent compartment lengths (<50 nm up to c. 1 μm), with length/width ratios that match crystal growth patterns and are unlike any known microbial cells.

3D FIB-SEM data reveal further complexities to the filaments and additional insights into carbon distribution in their vicinity. These data demonstrate how the morphology of the filaments changes quite

significantly over spatial scales of only a few micrometres along the length of a filament. In some FIB slices, their filamentous nature is clear and books of phyllosilicate crystals appear neatly stacked, while in other slices the filaments are seen to branch, suddenly thicken or be joined by additional microstructures. Furthermore, SEM highlights a number of nano-cracks within the chert matrix; these often feed right into the filaments and are filled with carbon. Like the TEM data, the SEM data are incompatible with these filaments being fossils of primitive filamentous organisms.

Carbon distribution in Apex 'microfossils' is, therefore, not comparable with true cellular morphology. Our non-biological formation model is: 1, Hydration of mica flakes (abundant in the country rock) during widespread hydrothermal activity resulting in vermiculite-like phyllosilicate formation. 2, Continued heating plus expulsion of water from phyllosilicate crystal lattices, causing exfoliation (i.e., accordion-like expansion at right angles to the cleavage plane), and creating the initial worm-like filamentous morphological expression of microfossil-like artefacts. 3, Adsorption of later hydrocarbons (and locally additional iron) onto the phyllosilicate, mimicking cell walls. We note that exfoliated vermiculite has high adsorption capacity for hydrocarbons resulting from the strong capillary action of slit-like pores between plate-like grains, encouraging its use for cleaning up oil spills [5].

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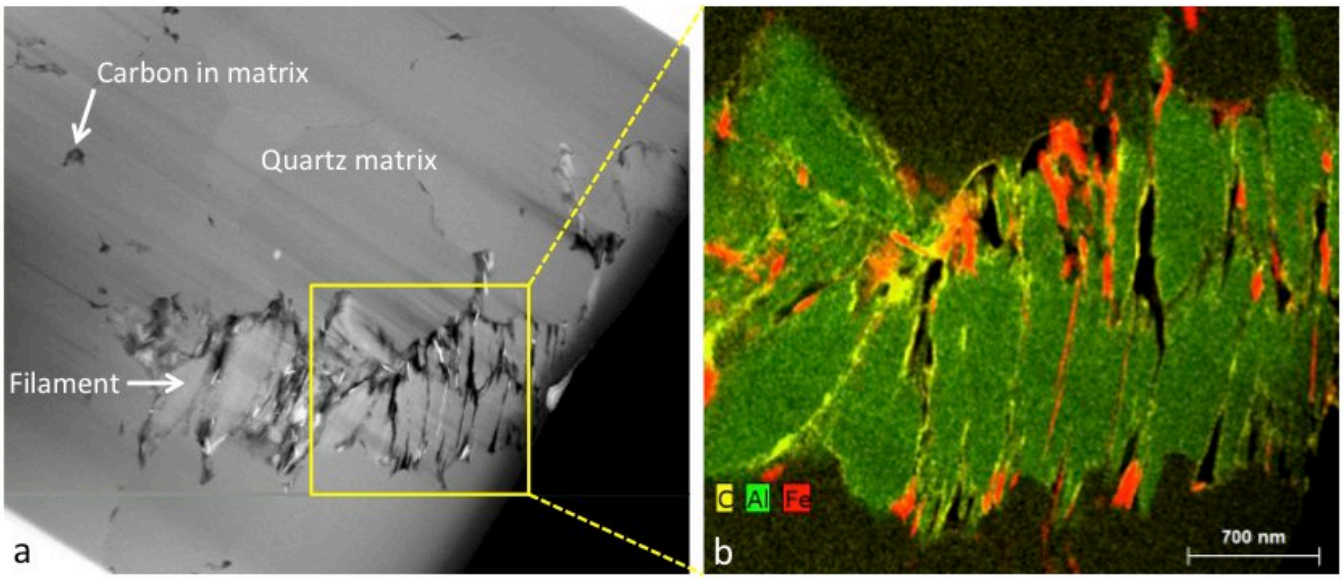

Figure 1. a) HAADF-STEM image of a filament from the 3.46 billion-year-old Apex chert. b) False colour ChemiSTEM three-element overlay map of area boxed in (a). The filament comprises stacks of sheet-like phyllosilicate grains (green) with carbon (yellow) and iron (red) interleaved between some of the sheets and around some of the filament margin. This distribution of phases is incompatible with a biological origin for the filament.