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Coccospheres confer mechanical protection: New evidence for an old hypothesis

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ABSTRACT

Emiliania huxleyi has evolved an extremely intricate coccosphere architecture. The coccosphere is comprised of interlocking coccoliths embedded in a polysaccharide matrix. In this work, we performed in-situ scanning electron microscopy based compression tests and conclude that coccospheres have a mechanical protection function. The coccosphere exhibits exceptional damage tolerance in terms of inelastic deformation, recovery and stable crack growth before catastrophic fracture, a feature, which is not found in monolithic ceramic structures. Some of the mechanical features of the coccospheres are due to their architecture, especially polysaccharide matrix that acts as a kind of bio-adhesive. Our data provide strong evidence for the mechanical protection-hypothesis of coccolithophore calcification, without excluding other functions of calcification such as various biochemical roles discussed in the literature.

Statement of Significance

Although bio-mechanics of shell structures like nacre have been studied over the past decade, coccospheres present an architecture that is quite distinct and complex. It is a porous cell structure evolved to protect the living algae cell inside it in the oceans, subjected to significant hydrostatic pressure. Despite being made of extremely brittle constituents like calcium carbonate, our study finds that coccospheres possess significant damage tolerance especially due to their interlocking coccolith architecture. This will have consequences in bio-mimetic design, especially relating to high pressure applications.

1. Introduction

Organisms produce extraordinary crystals in terms of shape and properties. Echinoids for example are able to regenerate their spines, while maintaining the original pattern of the macro-structure [1]. The greatest number of biologically formed crystals belong to the classes of sulfides, sulfates, phosphates and carbonates [2]. Coccolithophores are unicellular haptophyte algae, which produce calcium carbonate platelets (coccoliths) and assemble them into a hollow sphere, the so called coccosphere. Since the hard inorganic parts of coccolithophores are built inside the cell, they differ from those of most other calcifiers.

The most common species of the approx. 200 extant species is *Emiliania huxleyi* (*E. huxleyi*) [3]. The coccospheres of this species are ca. 5–10 μm in diameter and consist of several layers of coccoliths that are interlocked and glued to each other by means of polysaccharides (Fig. 1a, [4,5]). The strain RCC1238 used in this study possesses on average 2–3 coccolith layers and 20 coccoliths per cell [4] (Fig. 1b). The coccoliths are similar to a cable reel, consisting of a central tube that connects the lower proximal shield and the upper distal shield and encloses the central area (Fig. 1c and d) [6]. This morphology enables the coccoliths to interlock closely on the coccosphere and form a robust structure [6]. A single segment of an *E. huxleyi* coccolith comprises of two crystal units, the radial R-unit that is built up of a single crystal with the c-axis oriented parallel to the coccolith plane and the c-axis of the vertical V-unit is perpendicular to the coccolith plane (Fig. 1d grey arrow) [7,8]. This differs from other biological crystals like the ones of bone, corals, sea urchin and mollusk shells, which show a mosaic like crystal assembly [9–14], i.e. consisting of co-oriented crystallites in the mesoscopic size range (1–1000 nm) [15]. It was suggested that these mesocrystal-composite structures, e.g. mollusk shells, have a high fracture toughness compared to the one of single crystalline structures [14].

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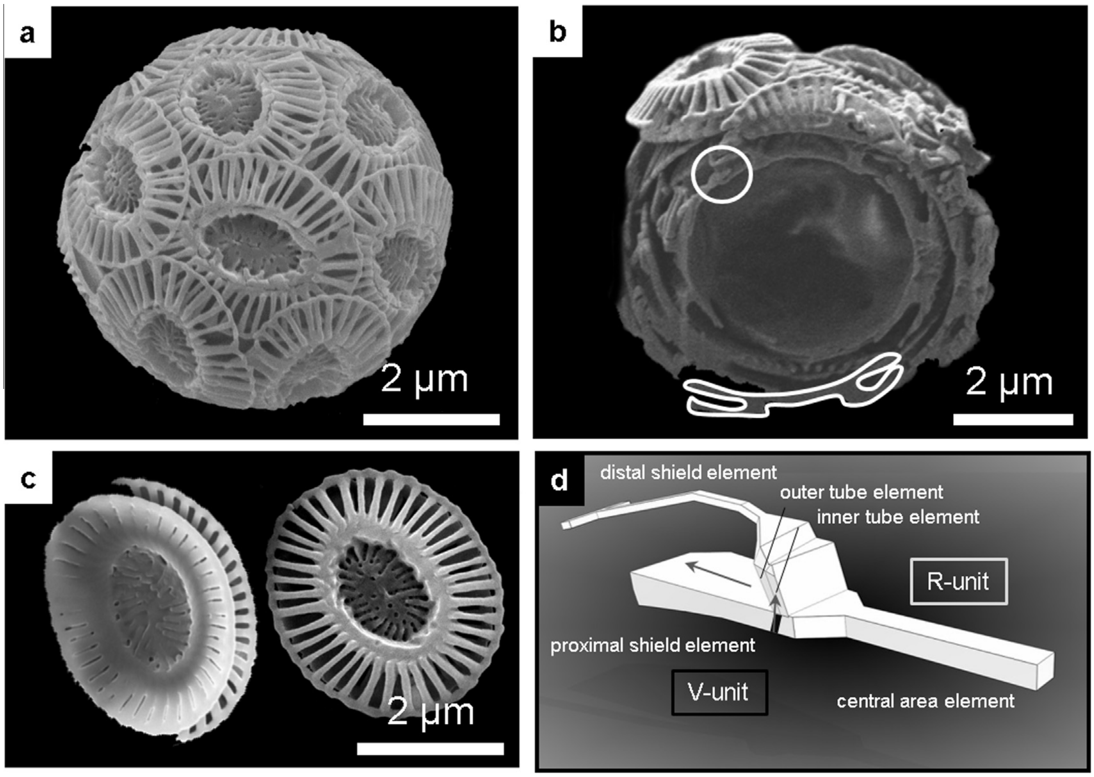

Fig. 1. SEM micrograph of (a) a complete and (b) a FIB cut coccosphere of the investigated *E. huxleyi* strain RCC1238. An individual coccolith and the interlocking of coccoliths are marked with white lines. In (c) secondary electron images of a coccolith viewed from top and bottom are shown. The model of a single discrete element of a coccolith is displayed in (d) (modified after [34,8]), showing the morphologically complex bi-crystalline calcite units (primary R and interstitial V whose orientations are shown by arrows) [7].

Despite decades of research the function of calcification in coccolithophores is a matter of debate, with the seminal discussion by Young [16] (see also the extended version, Young [17]) still being the central work of reference. Among the many hypotheses put forth, a mechanical/protective role of coccospheres was suggested more than a century ago [18], and remains one of the most plausible ideas [16]. The type of protection provided by coccospheres is not identical to the one provided by diatom frustules. While the latter protect the algae from macro-grazers such as copepods, coccospheres do not seem to have this function [19,20]. This, however, is not due to the mechanical properties (i.e. stability) of the coccosphere, but merely reflects the difference in prey/predator size ratio. While copepods, more often than not, have to destroy the diatom frustule in order to eat the diatom, coccolithophores are swallowed whole and coccoliths are only accidentally crushed by copepod mandibles [21,22]. Coccospheres most likely confer a general mechanical stability to the algae. This hypothesis has traditionally been based on morphological observations, but still awaits corroboration by micro-mechanical measurements [18,23,16]. In other words, how much load is needed to break a coccosphere? The mechanical stability of diatom frustules is regarded as being instrumental in the evolutionary success of diatoms [24,25,20]. The average force needed to break a diatom frustule ranges from 0.2 to 0.8 mN [20]. If the coccospheres also showed the ability to accommodate increasing loads by inelastic dissipation before undergoing fracture, this would be strong evidence in favor of the mechanical protection role of coccospheres.

In this study, we performed in-situ compression measurements on coccospheres of *E. huxleyi* by means of a flat punch indenter acting as a micro-crusher inside the scanning electron microscope (SEM). This allows us to determine the loads corresponding to the initial deviation from elastic deformation and the maximum load before fracture and collapse of the coccosphere occurs. Simultaneously, the deformation process was closely monitored for signs of failure of coccospheres, individual coccoliths and coccolith segments.

2. Experimental procedure

Coccospheres cultured in aged, sterile-filtered (0.2 μm pore-size cellulose-acetate filters) North Sea seawater enriched with 100 μmol L^{-1} nitrate, 6.25 μmol L^{-1} phosphate, trace metals and vitamins as in f/2 medium [26] were filtered onto an Omnipore polycarbonate membrane filter by using a vacuum pump. The filter was dried at 60 °C and the material was afterwards removed with a spatula and dissolved in ethanol. The sample was then dropped on a silicon wafer and dried. In the end a thin carbon film was deposited to avoid charging effects in the SEM. For more details on the coccolithophore culturing see Langer et al., [27] and Hoffmann et al., [4].

Loading was carried out in situ using the ASMEC Unat II indenter (ASMEC GmbH, Radeberg, Germany) inside a JEOL-JSM 6490 tungsten filament SEM. Individual coccospheres were loaded in compression under displacement control at a rate of 10 nm/s. This represents a uniaxial testing condition, which is different from the hydrostatic (tri-axial) stress state normally experienced by the coccospheres under water. Uniaxial compression of the sphere in the vertical direction leads to tensile opening stresses in the perpendicular direction, in addition to flexural (bending) loading of individual coccoliths. This is a more potent stress state to cause fracture compared to hydrostatic stress state. Therefore the failure loads obtained from these tests represent the lower limit of what the coccospheres can sustain in its underwater environment. The