



Exploiting algal mineralization for nanotechnology: bringing coccoliths to the fore

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Complex mineral structures are produced by many microalgal species. Pioneering work on diatom silica has demonstrated the potential of such structures in nanotechnology. The calcified scales of coccolithophores (coccoliths) have received less attention, but the large diversity of architectures make coccoliths attractive as parts for nano-devices. Currently coccolith calcite can be modified by the incorporation of metal ions or adsorption of enzymes to the surface, but genetic modification of coccolithophores may permit the production of coccoliths with customized architectures and surface properties. Further work on the laboratory cultivation of diverse species, the physicochemical properties of coccoliths and on genetic tools for coccolithophores will be necessary to realize the full potential of coccoliths in nanotechnology.

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Current Opinion in Biotechnology 2018, 49:57–63

This review comes from a themed issue on **Plant biotechnology**

Edited by **Alisdair Fernie** and **Joachim Kopka**

<http://dx.doi.org/10.1016/j.copbio.2017.07.013>

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Introduction

Unicellular algae have been much lauded as environmentally friendly production systems for organic molecules including lipids for use in biofuels, therapeutic proteins and bioactive small molecules [1]. In contrast, much less attention has been paid to the biotechnological potential of inorganic minerals produced by algae. This is surprising as intricate mineralized structures with complex 3D architectures and nanoscale patterns are prominent features of many groups within the Ochrophyta (Figure 1). For instance, members of the Synurophyceae, Chrysophyceae, and the Palmales produce silica scales, while the Dictyochophyceae (Silicoflagellates) and Bacillariophyceae (diatoms) produce complex silica (SiO₂) skeletons [2]. Within the Haptophyta, coccolithophorid algae produce intricate scales made of calcite (CaCO₃) called coccoliths [3**].

Diatom silica and coccolithophore calcite are available in abundance in diatomite and chalk deposits respectively and so have found a range of uses including silica supports for catalysis and calcite filler in paint formulations. The use of algal biominerals in nanotechnology however is a much more recent development. Work in this field has focused on diatom silica, which has a large surface area and is readily functionalized, leading to potential applications as an enzyme immobilisation substrate, as optoelectronic components and drug delivery vehicles. Some excellent reviews have been published summarising the work on diatom silica, to which we refer the reader for further information [4–8].

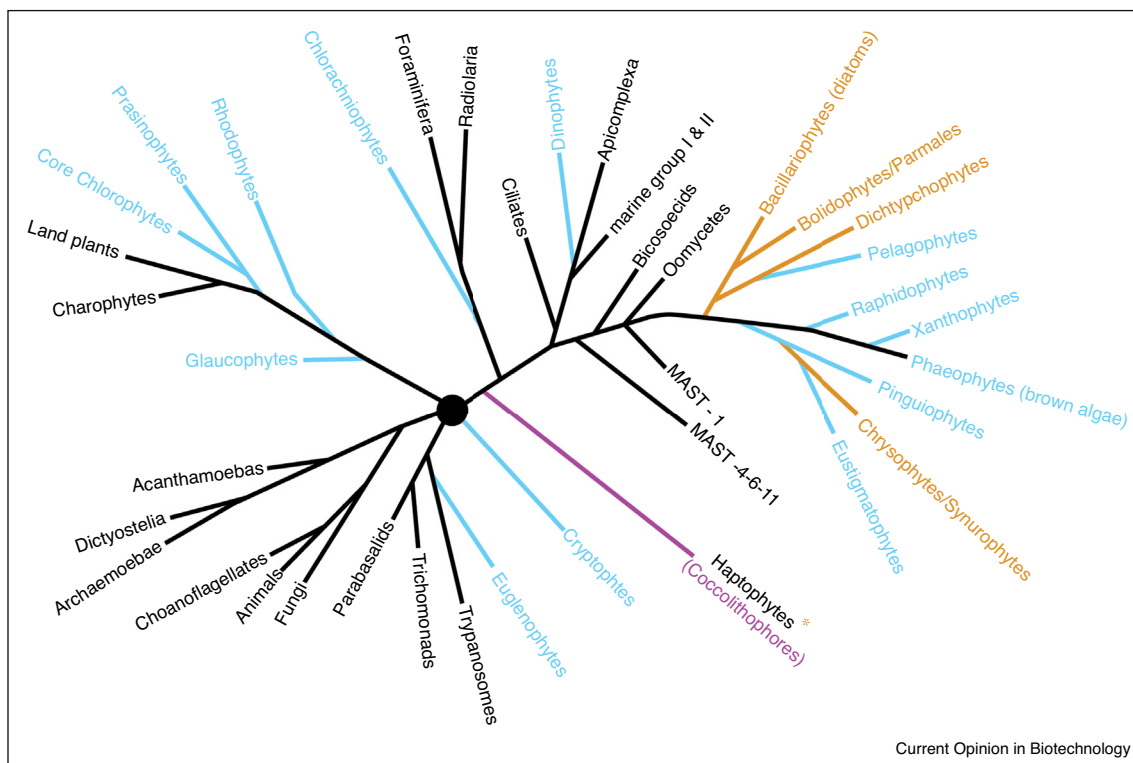
The large diversity of coccolith architectures found in nature (Figure 2), and the fact that synthetic minerals of similar complexity cannot yet be reproducibly fabricated at this scale, makes them attractive for various applications in nanotechnology. A key advantage of at least some coccolithophore species is that coccoliths are not essential for cell growth and survival in culture, as is evidenced by the many strains that have lost the ability to calcify [3**,9]. Thus, the bioengineer wishing to manipulate coccolith form will be less constrained than when working with diatom silica which appears to be essential for cell survival. The flexibility of the calcification machinery is well illustrated by members of the genus *Scyphosphaera* which produce two distinct coccolith morphologies on the same cell (Figure 2d) [10,11].

This review will focus on the potential of coccolithophores as a source of mineralized materials for nanotechnology. After a brief summary of the process of coccolithogenesis we will discuss the properties of coccoliths which make them suitable as components in nano-devices and micro-devices. We will then outline methods by which coccoliths could be modified and functionalized and discuss the practicalities of coccolith production before highlighting the challenges and opportunities ahead.

Coccolithogenesis

There are two main types of coccolith. Heterococcoliths are produced by diploid cells, and are made of calcite crystals with complex shapes (Figure 2h, inset). The calcite crystals nucleate intracellularly on an organic baseplate within a specialised vesicle, in which they develop to their final morphologies before being secreted to the cell surface and incorporated in the shell of coccoliths surrounding the cells, called the coccosphere [10]. The coccoliths are loosely connected to the cell surface

Figure 1



Schematic showing the phylogenetic relationships of phytoplanktonic (colored) and other eukaryotic taxa (black). Phytoplankton groups producing silica based biominerals are colored in orange and those producing calcium carbonate in violet. The Haptophytes include calcifying species (coccolithophores), a silicifying species (*) [12], and non-mineralizing species. The phylogenetic tree was adapted from [13] with permission.

and are probably held together by sticky macromolecules and, in some species, through interlocking of the coccoliths [14,15]. In many species it has been found that coccoliths are coated with an acidic polysaccharide which may be involved in calcium delivery [16,17] or crystal growth and morphogenesis [18] and which partially protects the coccoliths from dissolution [19].

The second type of coccolith are produced by the haploid phase of some, but not all, coccolithophore species and are known as holococcoliths. The crystals comprising holococcoliths are typically smaller than those in heterococcoliths and have simple shapes (Figure 2c). They appear to be formed extracellularly, but within an outer organic layer [20]. For both holo- and heterococcoliths, the molecular detail of how crystal nucleation and growth is so tightly controlled remains elusive.

Coccoliths as parts for micro-devices and nano-devices

Devices constructed on sub-micron scales are expected to be a transforming technology in a wide range of fields, from environmental sensing to advanced electronics, medical diagnostics and drug delivery. In the last decade substantial progress has been made in the development of

nanoactuators, which can power the movement of objects at this scale [21–23]. However, inexpensive and flexible methods to generate parts of micro-devices and nano-devices in a high throughput manner are still lacking. For example, the fabrication of nanopores is often carried out by expensive and time-consuming focused ion beam milling [24].

One field in which coccoliths have clear potential is nanofluidics. When nanoscale pores and channels interact with a fluid, physical phenomena which do not apply at the microscale become relevant. For example, the surface charge of a pore can govern the transport of selected ions and asymmetric pore geometries and surface charges can lead to a net flow of certain ions in one direction (ion current rectification) [24]. Coccoliths with hollow funnel and tubular shapes such as those of *Discosphaera tubifera* (Figure 2g) and *Rhabdosphaera clavifera* [25] seem to be particularly relevant to this area, as do coccoliths that have nanoscale pores or slits such as those of *Pontosphaera japonica*, *Michaelsarsia elegans* or the holococcoliths of *Calyptrolithophora papilifera* (for pore sizes and coccolith dimensions see [3•, 11, 26, 27]) (Figure 2d, c, e). The high pore density of the latter may also allow efficient encapsulation of selected molecules by suitable control of pore

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