

Acicular building blocks in the corallites of *Porites lutea*

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Abstract. A detailed study of the crystal building blocks of *Porites lutea* corallites was performed using transmission electron microscope (TEM) and applying a preparation method that preserved the original microstructure of the skeleton. Bundled acicular aragonite crystals could be identified and were found to be of high structural quality. These tens of μm long needle shaped crystals were in general electron transparent - indicating a thickness well below 500 nm - and covered by an amorphous, possibly organic matrix. The growth of the orthorhombic crystal needles was found to proceed preferentially along $\langle 100 \rangle$ directions with the $\langle 010 \rangle$ and $\langle 001 \rangle$ growth directions being inhibited. The width distribution of these needles shows a multimodal behaviour, which could indicate a multi-stage nucleation process throughout the skeleton formation.

1. Introduction

Living coral polyps build reefs by laying down a calcium carbonate skeleton. Combining bioorganic material and inorganic matter in this way is termed biomineralisation, a process that is common to many marine organisms, particularly molluscs [1]. Biomineralisation is of particular interest to materials scientists because the minerals grow more efficiently (at lower temperatures and pressures) than their geological counterparts [2]; coral skeletons have recently been proposed as a basis for low-cost, self-regenerating coatings [3] and as a matrix for bone grafts [4]. Investigations into biomineralisation in corals have previously focused on the mesostructure of the skeleton [5], but a full understanding of the mechanism behind the nucleation and growth of the mineral phase requires a thorough study of the relevant features of the skeleton microstructure. Key features that have been identified are the centres of calcification (COCs), which are widely considered to be sites of crystal nucleation [6]. It is assumed that acicular (needle-like) crystals are forming the building blocks of the skeleton [7] by radiating out from the COCs and intergrow with those from neighbouring COCs to form the bulk skeleton. However, the detailed microstructure of the crystals is largely unknown. Successive studies have debated their symmetry [8,9], preferred growth orientation [1,10] and the importance of organic material (about 10% wt [1]) in the matrix; the combination of diffraction and high resolution imaging possible with TEM makes it an ideal tool to answer these questions.

TEM analysis of coral skeletons has previously been accomplished with the use of replicas [11] and, more recently, powdered skeleton has been successfully analysed [3]. These techniques are not completely satisfactory: The information provided by replicas is topographical and inverted, whilst laser ablation causes microstructural detail to be lost and introduces artefacts. Also the application of focused ion beam thinning was reported for red corals [12] but the actual building blocks of the coral skeleton could not be retained. Hence, manufacture of a TEM sample where structural features are

preserved has rarely been reported. Therefore we developed a thorough preparation method for this purpose and carried out the structural characterization by TEM.

2. Method

Coral specimens of the genus *Porites lutea* were obtained ready-prepared from the Centre for Tropical Marine Ecology (CTME), Bremen. The polyps were soaked in fresh water and removed from the skeleton by brushing. The skeleton was air-dried, with no subsequent chemical treatments being employed. A reliable TEM sample preparation technique allowing the preservation of structural features was developed, based on the established tripod polishing technique. The skeleton was stabilised by soaking it in Gatan G1 epoxy resin and curing it at less than 150 °C. A slice perpendicular to the skeletal growth direction was taken using a diamond-coated saw and single layers of glass and silicon were fixed to either end using M-bond epoxy resin. The sandwich was sliced into samples using a diamond-coated saw. A single sample was temporarily fixed onto a tripod holder using a thin layer of Crystalbond 509 and polished to $\sim 20\ \mu\text{m}$ and in the final stage of thinning a bevel was applied. The sample was then ion milled using a Precision Ion Polishing System (PIPS), to produce an electron transparent area. The sample morphology was studied using a scanning electron microscope (FEI Sirion). Phase contrast and bright field imaging as well as diffraction imaging was performed using a JEOL 2011 TEM at an acceleration voltage of 200kV with an ultimate point resolution of 1.9 Å.

3. Results and discussion

An optical micrograph of coral skeleton sliced perpendicular to the growth direction is shown in figure 1. The marked dark spot is a COC with the crystals of interest in this paper radiating out from it.

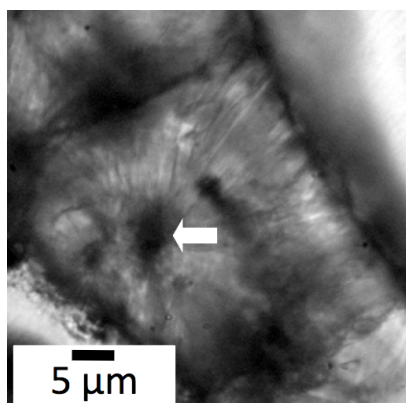


Figure 1. Optical micrograph of a slice of coral skeleton. The dark spot marked with an arrow is a COC, with needle-like crystals arranged in a fan-shaped structure.

An SEM image of the overall structure is displayed in Fig. 2 (a) revealing stacks of aragonite platelets forming the corallite microstructure. Figure 2 (b) shows a bright field image of needle-like crystals which can be attributed to neighbouring COCs. There is clear evidence of intergrowth, which lends weight to competitive growth theories that have previously been discussed [13]. There is evidence of amorphous material surrounding the needles, which may have an organic origin. A study of nacre [14] has indicated the presence of voids of organic material within the aragonite platelet structure. There is no evidence of such voids in the high quality coralline aragonite needles, suggesting that a different growth process for the coral skeleton formation. The sample was tilted into the [010] zone axis and a diffraction pattern of a single needle was obtained (figure 2 (b) inset). Analysis indicates that the needles are monocrystalline and are made of aragonite (a polymorph of calcium carbonate) with orthorhombic symmetry. Some forbidden reflections were observed in the experimental diffraction pattern; this is likely to be a result of multiple scattering as the sample is still relatively thick, but some authors have indicated that forbidden reflections in aragonite may be

indicative of a lower triclinic symmetry [9]. From the SEM and the TEM investigations we conclude that the crystals are flat in [010] direction, which indicates that crystal growth in this direction is inhibited compared to the [100] direction.

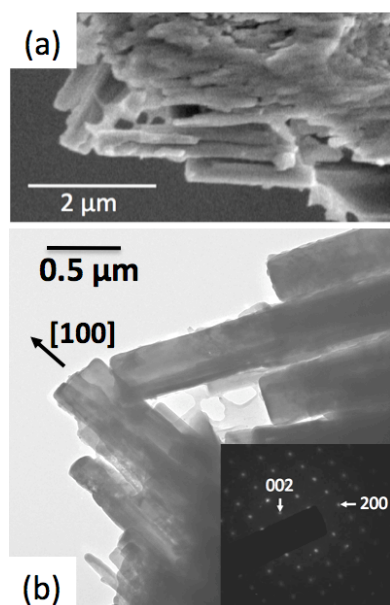


Figure 2. (a) Scanning electron micrograph showing bundled layers of aragonite crystals. (b) Bright field transmission electron micrograph of these needle-like crystals. **Inset.** Selected area diffraction pattern from a single needle tilted into the [010] zone axis. The black arrow indicates the [100] growth direction of some of the needles.

Figure 3 shows a magnified image of a single aragonite needle. High resolution TEM (HRTEM) was performed (inset), which show a high crystalline quality. Again, an amorphous phase surrounding the needle is evident.

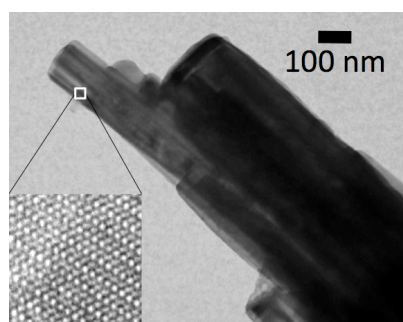


Figure 3. Bright field transmission electron micrograph at higher magnification. **Inset.** HRTEM image taken from an area similar to the one indicated by the box on the main image (not to scale).

An organic amorphous phase has previously been identified as a key component of the biomineralisation process and is known to be a source of lattice distortions [8]. In corals, a variety of roles have been suggested for organic components at various stages of skeletal growth: as a barrier to prevent damage to the coral polyp [5] or as a mucus containing seed crystals for nucleation [11]. The width-distribution in $\langle 001 \rangle$ directions of individual crystalline needles was determined from the TEM images. The resulting distribution is shown in figure 4; it is interesting to note that there is a strong multi-modal distribution. This could be indicative of a multi-stage nucleation process with the wider needles originating at COCs and the narrower needles originating from secondary, unidentified nucleation sites. Alternatively, needles of different widths could originate from separate calcification centres.

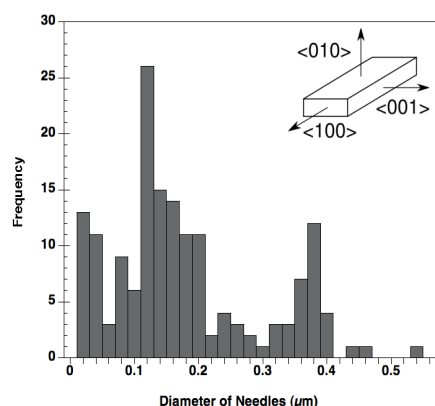


Figure 4. Histogram showing the distribution of aragonite needle diameters, d . A multi-modal distribution is evident. **Inset.** Schematic of a simplified aragonite needle showing the main identified crystallographic orientations.

The main crystallographic directions on an aragonite crystal are sketched in figure 4 (inset). A cuboid-shaped crystal has been used for simplicity, but the probability of faceting should not be discounted. The fastest growth is seen along the $\langle 100 \rangle$ direction; a result that has also been reported for nacre [15]. However, the strong growth inhibition observed along the $\langle 010 \rangle$ direction that causes platelet-shaped crystals to form is not observed in nacre and also voids that can be observed in the nacre structure could not be identified in the corallites crystals.

4. Conclusion

The microstructure of high quality crystalline needles considered to be building blocks of the coral skeleton was successfully analysed with TEM using a sample preparation method based on the tripod polishing technique. The needles were found to be platelets encased in an amorphous phase and were formed from orthorhombic aragonite with a preferred growth direction along $\langle 100 \rangle$. It is suggested that the amorphous phase plays an important role in promoting or inhibiting growth along particular crystallographic directions.

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