

PATTERNS OF BIOMINERALS DEPOSITION (CONTRIBUTIONS FROM ENERGY FILTERING ELECTRON MICROSCOPY), Marcos Farina, Laboratório de Biomineralização, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, 21941-590, Rio de Janeiro, RJ, e-mail: mfarina@anato.ufrj.br

Biomineralization concerns to the formation of amorphous and crystalline minerals under the control of organisms [1]. These minerals, exhibit in most of cases, specific morphologies which are different from their inorganic counterparts. In this work we present the organization patterns of some biomineralized tissues, from nanometer to micrometer range. We also show the influence of polysaccharides from the cell wall of brown algae in the morphology of calcite crystals grown in the presence of these molecules *in vitro*.

The enamel of rodent incisor is hierarchically organized when observed in different length scales. In sub-millimeter range, it is possible to observe by scanning electron microscopy of longitudinally polished teeth, a periodicity in contrast distribution (Fig. 1a). Higher magnification shows that this is due to the organization of the enamel prisms (Fig. 1b). In sub-micrometer range it is observed that each prism is composed of myriads of single crystals originated at the enamel-dentin-junction (Fig. 1c). Otoliths are calcium carbonate containing structures present in the inner ear of vertebrates that participate in the sound perception (fish) and equilibrium (fish and other vertebrates). Otoliths of fish are composed of aragonite (sacculle and utricle organs) and vaterite (lagena organ). Polished sections of otoliths from the sacculle show that each otolith is composed of nanocrystals oriented regularly and in parallel. In figure 2a we see that the lines of growth (parallel to the dark lines in the figure) of the structure are disposed perpendicularly to the direction of crystals deposition, while in figure 2b a growing layer of otolith is depicted, in a view perpendicular to the previous figure. It is known that the gaps formed by the 3-D organization of tropocollagen molecules inside fibrils are very important in the context of biomineralization of vertebrates, as in several tissues the first crystalline calcium phosphate to be mineralized appears inside the gap region [2]. Figures 3a and 3b show small crystals of calcium phosphate inside the gaps of a collagen fibril, from the integument of the toad *Bufo marinus*. This fact confirms the control exerted by the collagen itself on the mineralization process [3].

In vitro studies concerning the precipitation of calcium carbonate in the presence of polysaccharides (alginates and fucans) extracted from the cell wall of the brown alga *Padina gymnospora*, were performed in order to assess their possible role in the determination of the calcium carbonate crystals morphologies (Fig. 4a and 4b). This alga accumulates high amounts of heavy metals from water in the form of crystalline minerals, associated to the polyanionic polysaccharides of the cell walls. It is shown that, in spite of the appearance of new faces in the calcite crystals precipitated in the presence of alginates (Fig. 4a) and fucans (Fig. 4b), there was neither a modification of the calcium carbonate polymorph nor the presence of a different morphology (e.g. a chiral structure), showing that the molecules adsorbed to the regions with higher surface areas (corners and edges of the original rhombohedral morphology) slowing down growth in corresponding direction, thus causing the appearance of new faces.

In conclusion, electron microscopy methods of imaging and analysis are very important to the understanding of the morphology, composition, and growth of biominerals; particularly when inorganic crystals are grown in the presence of soluble molecules extracted from the organic matrices containing the same minerals that undergone biological control during deposition.

References

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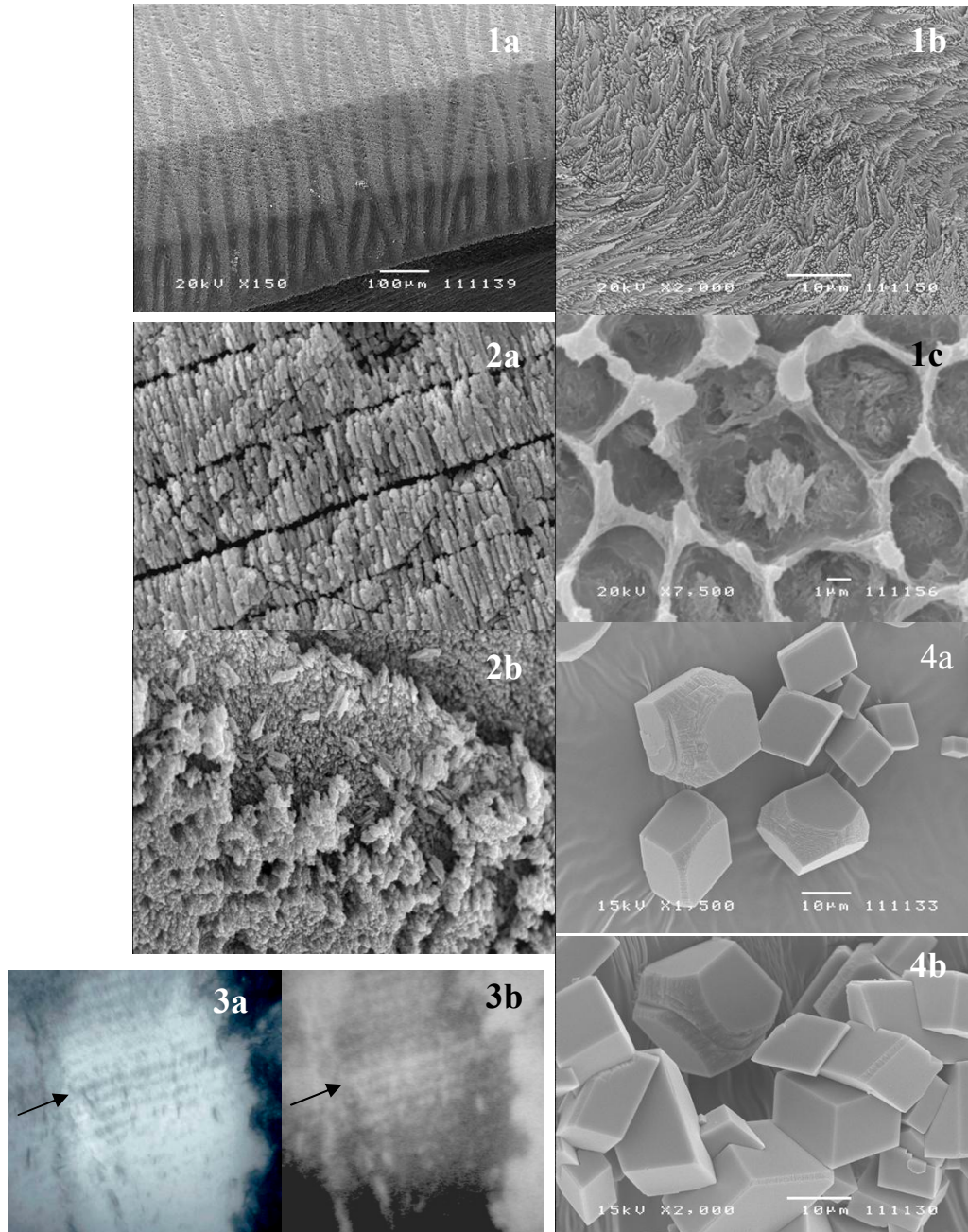


Fig. 1a: Polished section of the enamel from the rodent *Cuniculus paca*. **Fig. 1b:** Enamel prisms are seen. **Fig. 1c:** Crystals at the enamel-dentine-junction. **Fig. 2a:** Polished section of a saccule's otolith showing growth lines (parallel to the dark regions) and rows of crystals running perpendicular to these lines. Bar in Fig. 1b = 2 μm in this figure. **Fig. 3a:** Zero-loss image ($\Delta E = 0\text{eV}$) of a type I collagen fibril from the integument of the toad *Bufo marinus* depicting the very small crystals inside the gap regions of the fibril. **Fig. 3b:** Structure-sensitive image ($\Delta E = 260\text{eV}$) from the same region, showing that crystals do not contain carbon (in fact they contain O, P, Ca; data not shown). White region indicated by the arrow correspond to the region that appears dark in the previous figure (arrow in figure 3a). **Fig. 4a:** Calcite crystals grown in the presence of alginates. **Fig. 4b:** Calcite crystals grown in the presence of fucanes.