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Review

Coral biomineralization: A focus on intra-skeletal organic matrix and calcification

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ABSTRACT

In the recent years several papers and some reviews have dealt with characterization, localization and influence on the precipitation of calcium carbonate, of the organic matrix from scleractinian corals. In fact, it has been well established that coral calcification is a biological controlled process orchestrated in space and time by the organism also trough the secretion of organic matrix molecules because it has been well established that coral calcification is a biological controlled process, and thus is orchestrated in space and time by the organism also through the secretion of organic matrix molecules. In this review is presented a scientific path on the biomineralization of corals having as focusing point the intra-skeletal organic matrix, the molecules that are associated with mineral (aragonite). The review starts with a an overview on coral tissue, skeleton and tissue skeleton interface, describes the intra-skeletal organic matrix putting attention mainly on the proteins associated to aragonite and finally describes the *in vivo* and *in vitro* calcium carbonate precipitation experiments carried out aimed to evaluate the role of the organic matrix. The last paragraph reports studies on the role of the organic matrix in controlling calcification when corals are subject ocean acidification effects. The readers are expected to find a source of inspiration for new studies on the biomineralization of corals that are organic matrix addressed and merge diverse scientific disciplines.

Contents

1. Overview

Biomineralization is the science that studies the formation, structure and properties of minerals deposited by organisms, usually referred as biominerals [1–3].

Corals play an important role among mineralizing organisms (Fig. 1). They lead to a production of calcium carbonate ($CaCO_3$) of about 10^{12} kg year^{-1} [4]. Beside this, coral calcification is a globally important biological and geochemical process as it allows the tiny polyps of coral colonies to build the most important bioconstruction of the world, coral reefs [5]. Coral skeletons not only serve as the 3D-framework for reef building, but may also play indirect physiological roles such as light scattering [6]. In addition, coral skeletons are used for paleoclimate reconstruction [7] and as bone implants [8].

Hard corals, the Scleractinian order, which accrete exoskeletons are distinguished from soft corals (*Octocorallia* and *Antipatheria*). Scleractinian corals can host symbiotic algae, or zooxanthellae, in

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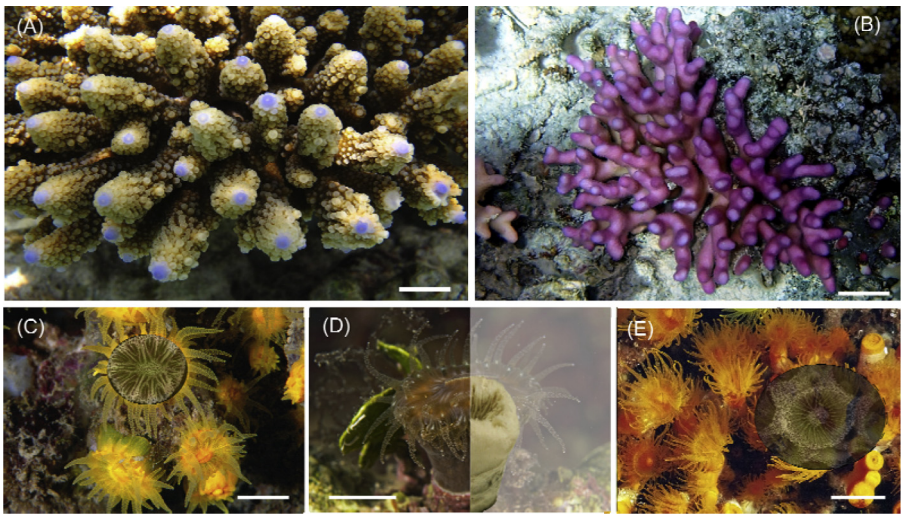

Fig. 1. *In situ* camera pictures of the scleractinian coral. *A. digitifera* (A) and *S. pistillata* (B) are among the most common studied species from the reef. *L. pruvoti* (C), *B. europaea* (D) and *C. caespitosa* (E) are Mediterranean species for which the role of OM in calcification was recently investigated. A shadowed image of the skeleton is inserted in the pictures (C)–(E). Scale bar: 1 cm.

the polyp tissue, or not have zooxanthellae. Coral can be also colonial, the reef building, or solitary.

The reef building corals are hermatypic on the opposite of the ahermatypic ones. In several reviews details on the structure of scleractinian corals are available [9–15].

The study of coral calcification started more than 150 years ago [16]. Nevertheless, the knowledge still remains patchy and multiple studies have involved only few species. Skeleton formation was initially considered as a mineralogic process where the basic element of the skeleton – that is, the fiber – was described as a single orthorhombic crystal of aragonite formed without biological involvement [17]. Proposed biomineralization mechanisms in scleractinian corals range from biologically induced, that is, precipitation regulated primarily by physico-chemical and environmental parameters [18–22], over combinations of both abiotic and biotic processes [23–31], to strict biological control highly regulated with an organic matrix component [*e.g.* 32–47]. This debate, which is not restricted only to coral biomineralization [48,49], is partly the result of difficulties in defining clear criteria (morphological, structural, crystallographic and chemical) for addressing biomineralization pathways.

2. Tissue and skeleton

The scleractinian corals are organisms composed of polyps covering the skeleton. In colonial corals the polyps are linked together by a tissue, the coenosarc that is missing in solitary corals. The exoskeleton (extracellular) is located at the base of the coral tissues. In this section a brief, and not exhaustive, introduction to tissue, skeleton and tissue skeleton interface features is presented.

The oral tissues are in contact with seawater, whereas the aboral tissues are facing the skeleton and cover it almost completely. Tissues consist of two epithelial layers, an ectoderm and an endoderm, separated by a connective layer called mesoglea [50,51]. The aboral ectoderm in contact with the skeleton, the calicoblastic ectoderm, has a topography that exactly complements the growth surface of the skeleton [32,52]. Calicoblastic cells are long (10–100 μm), highly interdigitated, and overlap each other, their form is species specific and changes during the diverse stages of the skeletongenesis. The calicoblast cell secretes the organic matrix (OM) and control the flux of ions leading to the formation of the aragonitic skeleton [*e.g.* 37,39,51,53].

Regarding the coral skeletal microarchitecture the main building units are the Early Mineralization Centers (EMCs) and the fibrous aragonite crystals that radiate from around these centers (Fig. 2). These basic building blocks are structurally similar in hermatypic (reef building) and ahermatypic corals [54–58]. The spatial arrangement of the centers and the incremental zonation of the fibers vary among taxa [59] (Fig. 2). The EMCs have a high organic content [60] in which $CaCO_3$ grains are embedded and the crystallinity of the $CaCO_3$ within the EMZ is lower than in the aragonitic fibers [61]. The fibrous aragonite crystals comprise the bulk of the coral composition and, in contrast to the EMCs, have a low organic concentration of about 1% by weight [62] while the entire skeleton contains at least 3% by weight organic material [63]. Also the distribution of trace and minor elements is not uniform throughout the skeleton [64]. The EMCs contain higher concentrations of magnesium [40], strontium, and barium than the fibers [41]. Heterogeneity in strontium concentration occurs both within and between the EMCs and the fibers [54,65–67]. The distribution of trace and minor elements and stable isotopes [42–45,68,69], has been used to evaluate the degree of biological control and contributed to the interpretation of climate proxies [*e.g.* 70]. This information has strongly contributed to the (sometimes contradictory) debate on the degree of biological control over the calcification process and the influence of environmental parameters.

The skeletal microarchitecture of EMCs, and mainly of the fibers, ends up in a diversity of skeletal morphologies that is species specific. This has been historically used for classification of scleractinian corals, [71–73] and recently it has been generally confirmed by modern molecular methods [54,74]. Thus, skeletal morphology is a characteristic of species and is under a genetic control and needs to be dictated by the organism during the skeletongenesis. However, despite this biological control, coral morphology can vary with environmental parameters [*e.g.* 75].

The appearance of coral skeleton morphology is due to the mineral growth process, for which diverse mechanisms, associated to the extent of biological control over calcification, have been proposed [11,14 and references therein]. A well accepted mechanism of growth is that *via* a two-step matrix-mediated process [38]. Such growth process is based on cyclic secretion of mineralizing compounds by the tissue basal ectoderm. These biochemical components are repeatedly produced resulting in a stepping growth mode of fibers and a layered global organization of coral

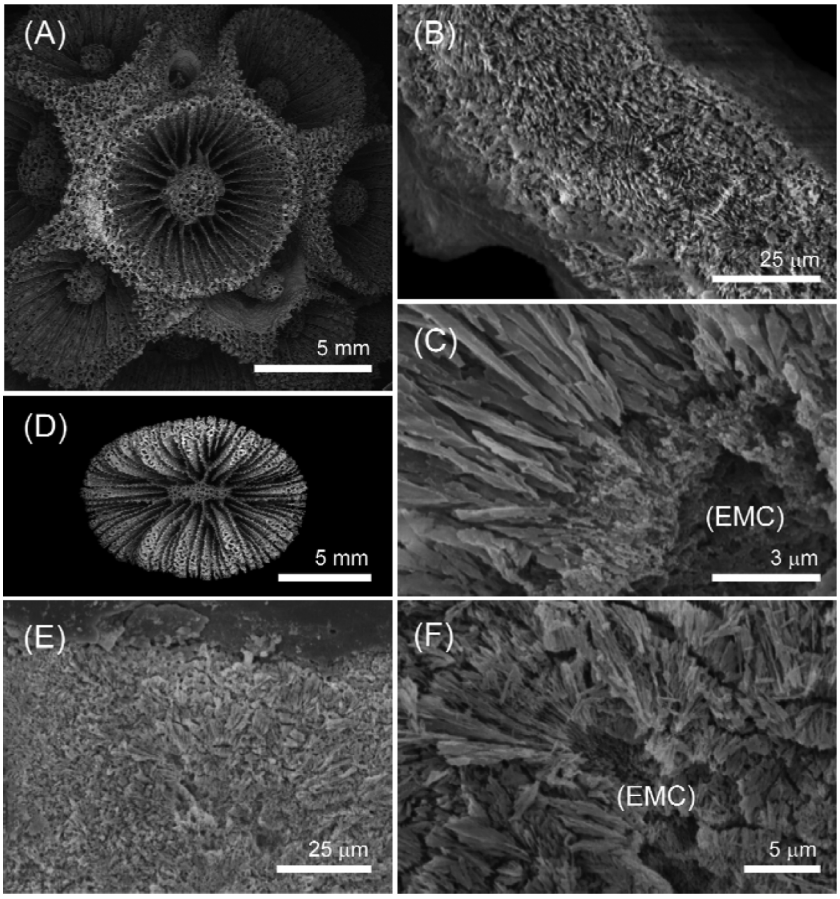

Fig. 2. Scanning electron microscopy images of the ultra-structural features of *C. caespitosa* (A–C) and *B. europaea* (D–F). The EMC is indicated. Despite the different growth form, colonial *vs* solitary, respectively, and the different morphology of the skeleton, a similar microarchitectural organization made of aragonitic fibers and EMCs is observed. The distribution of the EMCs as well the textural organization of the fibers varies among taxa [59].

skeletons. The crystal-like fibers are built by superimposition of few micron-thick growth layers. A biomineralization cycle starts by the secretion of a mineralizing matrix and the final step is the crystallization phase, during which mineral material grows onto the organic framework. The initial mineralization is characterized by randomly oriented microgranular components and the second step is characterized by consistent crystallographic alignment that determines the crystallography of the aragonite fibers [38].

The space between the skeleton and the calicoblastic cells has a species-specific size and does not exceed a few nanometers in thickness [51]. One of the first researches on this issue was carried out on the branching coral *Pocillopora damicornis* [76]. Microscopic observation showed vesicles rich in calcium hosting fine granular particles in flattened and interdigitated calicoblast cells after the settling stage. However, the narrow space separating the calicoblast epithelium from the underlying skeleton was devoid of any substructure. Differently, the scleractinian coral *Galaxea fascicularis* revealed the presence of sulfur rich organic fibrils located between the calicoblast epithelium and the skeleton [77]. On these fibrils small nodular structures (30 nm) rich in calcium were observed. These regions appeared associated with the nascent crystals of $CaCO_3$. The ultrastructural nature of the calcifying interface in *G. fascicularis* has been further investigated [35]. Two distinct types of vesicles (380 and 70 nm in diameter), were predominant throughout the calicoblastic cells, but these were never seen to be entering, or to be contained within, sub-skeletal spaces, nor did they contain any crystalline material. In this study a network of organic filaments (26 nm in diameter) extended from the apical membranes of calicoblast cells into sub-skeletal cavities was observed.

A more detailed, and consistent, information was obtained through the study of the tissue/skeleton interface using the hermatypic coral *Stylophora pistillata* as a model [39]. The results showed that: (i) a morphological correspondence between the tissues and the skeleton was present and that the calicoblast cell layer was in direct physical contact with the skeletal surface; (ii) the distribution and density of desmocyte cells, which anchor the calicoblastic ectoderm to the skeletal surface, vary spatially and temporally during skeletal growth; (iii) the tissue above the coenosteal spines lack endoderm and consists only of ectodermal cell-layers separated by mesoglea.

The occurrence of a highly viscous macromolecular layer in the mineralizing space between tissue and skeleton has been reported in several studies [78–82]. Its presence could have relevance in the control of diffusion process of ions and macromolecules involved in the nucleation and growth stages of the calcification process, as already reported for other calcifying organisms [49]. In gels, or gelling environments, ionic and molecular transport occurs only by diffusion and convection is neglectable.

Seawater is the main source of ions used for calcification, thus to reach the calcifying site ions have to cross tissue layers [13,83and references therein]. This can occur through a paracellular pathway driven by diffusion, a transcellular pathway driven by active transporters or both. The intercellular ion permeability determines the fraction of paracellular ionic trafficking. The research was focused mainly on the transport through the calicoblastic layer, being the one above the skeleton. The transport of Ca^{2+} was reported to occur mainly by a transcellular pathway, although the presence of a paracellular route was revealed through

the use of a fluorescent dye (calcien) [13]. The latter was confirmed by recent research showing also that the intercellular junctions control and restrict the diffusion of molecules through pores of defined sizes [84]. The trancellular pathway, beside its relevance in the ionic trafficking, involves Ca^{2+} channels/carriers, localized by immunohistochemistry, in the calicoblastic cells [85]. Among them, a plasma membrane calcium ATPase has been identified [86] and suggested to play in the regulation pH in the calcifying medium, while removing Ca^{2+} [11].

Metabolic CO_2 is the main source of inorganic carbon for calcification [87], although CO_2 is obtained also from seawater [88]. Its speciation, which determines the saturation state of calcium carbonate, is mainly related to the activity of carbonic anhydrase [*e.g.* 89,90] and to the pH of the calcification fluid.

The ionic transfer to the calcification site, the metabolic activity of the calicoblast cells and pH determine the molecular and ionic composition of the calcification media, being a fluid or a highly viscous sol. The pH of the calcifying media is increased with respect to seawater pH during the calcification process [91,92], shifting the equilibrium composition of inorganic carbon in favor of CO_3^{2-} relative to HCO_3^-, promoting the reaction precipitation of $CaCO_3$. It was observed that scleractinian corals up-regulate pH at their site of calcification such that internal changes are approximately above one-half of those in ambient seawater [93]. This phenomenon has been recently discovered to favor the transport of molecular CO_2 into the calcification site [94].

3. Intra-skeletal organic matrix components

The complete description of the organic matrix (OM) should include molecules both into the skeleton and at the interface between tissue and skeleton. Those found into the skeleton, though initially present at the interface, can be not necessarily representative of those initially at the interface. However, the majority of knowledge on the OM comes from the organic molecules extracted from the skeleton. On the other hand, only the molecules able to interact with the growing $CaCO_3$ can be likely entrapped into the skeleton, giving strength to the use of the intra-skeletal OM as model of the entire OM. In this section only the intra-skeletal OM (below indicated as OM) will be considered.

In pioneering *in vitro* experiments the OM excretion was shown [95] using primary tissue cultures of both soft (*Xenia elongata*) and hard (*Montipora digitata*) corals and these OMs showed structural differences. Using the coral *Mycetophyllia reesi* it was shown that some components of the OM come from the extracellular macromolecular material associated the epithelium of the calicoblast cells [51]. A study using antibodies raised against OM demonstrated that even if other cell types, including zooxanthellae, can supply precursors for OM synthesis, however, only calicoblast cells facing the skeleton are directly responsible for the synthesis and secretion of the OM components [96].

The composition of OM has been investigated through diverse analytical techniques, it has been shown the OM contains proteins, polysaccharides and lipids (see below). The distribution among these components changes among species and recently it was evaluated for Mediterranean corals showing lipids and saccharides are the major components [97]. OMs from zooxanthellate and nonzooxanthellate scleractinian corals showed compositional differences correlated with the symbiotic or non-symbiotic character in both proteic (*via* Asp, Glu, Ala and Ser) and glucidic phases (*via* GAIN, GIcN and Gal) [98].

The OM components are usually divided on the basis of their solubility in water upon skeleton extraction. The majority of the studies concern the soluble OM (SOM), while so far much less attention has been dedicated to the insoluble OM (IOM).

The biochemistry of the whole SOM from several species has been characterized. A high content of sulfur (mainly as sulfate) containing proteoglycans has been found in the SOM of *Montastrea curta*, *Favia stelligera* and *Lophelia pertusa* [55]. The mapping of the sulfated proteoglycans showed that this fraction of the OM is dominant within both EMCs and the surrounding fibrous tissues [55]. An additional evidence of the relevance of sulfated proteoglycans in the OM comes from the observation that the adsorption ability of glycosaminoglycans on coral surfaces depends on the charge density due to sulfate groups [99]. Newly synthesized SOM components were studied in microcolonies from *S. pistillata* [100]. The presence of low molecular mass matrix components (<3.5 kDa), but no free amino acids in the SOM was observed. These molecules represented the bulk of SOM components. High-performance liquid chromatography investigations of the SOM from *Monsastrea curta* and *Porites australiensis* showed one protein (160 kDa) for *M. curta* and a main one (200 kDa) and a less abundant one (25 kDa) for *P. australientis* [63]. In both instances, high molecular weight acidic sulfated polysaccharides were found as well. The combination of several nucleic acid resources to a recent proteomic analysis of the *Acropora millepora* SOM enabled the identification of several SOM proteins. These proteins showed a large range of isoelectric points, compositional patterns and signatures. Besides secreted proteins, there were proteins with strong adhesion properties and polysaccharides rich in arabinose [101].

The proteic component of the SOM, and of the OM, has been deeply investigated and the studies on the proteins represent the bulk of the SOM studies for corals. This is probably due to the general observation that proteins control the biomineralization process in the majority of the organisms [1–3], despite the fact that in corals, and not only, proteins are not the main molecular components by mass of the OM.

Scleritin was the first protein isolated and characterized from the OM of the sclerites of the *Corallium rubrum* [102]. It is a secreted basic phosphorylated protein which exhibits an amino acid sequence of 135 amino acids and a signal peptide of 20 amino acids.

Galaxin (53 kDa) from *G. fascicularis* is the most complete studied protein from coral skeleton [103,104]. The primary structure has a tandem 30 residue repeat and is glycosylated, but with no apparent calcium-binding activity. There are two conserved cysteines in each of the 9 repeats that may invoke cross-linking to form a polyprotein network.

A number of proteins were isolated from the skeleton of *S. pistillata* and *P. cactus* [105]. *S. pistillata* yielded three acidic proteins (55, 47, and 37 kDa) of which only the 55 kDa had calcium-binding properties. In the skeleton of *P. cactus* five acidic proteins (68, 50, 47, 37, and 33 kDa) were observed. The proteins of 68, 50, and 47 kDa stained as calcium binding proteins. Of particular interest was the long polyaspartate (36 Asp) domain of the 55 kDa protein from *S. pistillata*. The presence polyaspartate sequences is a common feature of many proteins associated to $CaCO_3$ skeletons [106–108]. This feature has been long proposed to denote control of over the forming $CaCO_3$ polymorph [1–3].

Drake et al. [109] using liquid chromatography–tandem mass spectrometry analysis of SOM of *S. pistillata*, combined with a draft genome assembly from the cnidarian host cells of the same species, identified thirty-six proteins. The proteome contained an assemblage of adhesion and structural proteins as well as two highly acidic proteins. From this information the authors identified a biomineralization "toolkit," an organic scaffold upon which aragonite crystals can be deposited in specific orientations to form a phenotypically identifiable structure.

In the staghorn coral *A. millepora* thirty-six OM proteins were identified as well, by using a combination of proteomics and transcriptomics [110]. Besides secreted proteins, extracellular regions

of transmembrane proteins are also present, suggesting a close control of aragonite deposition by the calicoblastic cells. In addition to the expected soluble OM proteins (Asp/Glu-rich, galaxins), the OM repertoire included several proteins containing known extracellular matrix domains. This study showed few coral-specific proteins, many proteins having counterparts in the non-calcifying cnidarians.

Four highly acidic proteins from the coral *S. pistillata* were obtained from the expression of genes [111]. These proteins showed convergent sequence evolution among calcium carbonate-precipitating organisms, suggesting a common underlying mechanism for biomineralization. Recently, the ultrastructural mapping of individual SOM proteins in the calcification site or the skeleton of the coral *S. pistillata* was investigated [112]. The spatial arrangement at the nanoscale of key SOM proteins revealed that they are embedded within the aragonite crystals in a highly ordered arrangement consistent with a periodic calcification pattern. In the tissue, these proteins are not restricted to the calcifying epithelium, suggesting that they also play other roles in the coral's metabolic pathways. Most importantly, these results show that that the biomineral is produced in discrete nanoscale packages in which the secreted organic matrices remain entrapped within the crystalline units whose growth they control, leading to the formation of highly ordered, microscopic, heterologous structures, which are aggregated to form a macroscopic skeleton, as proposed for other calcifying organisms [113].

A recent review on the comparison among proteins intimately associated with the $CaCO_3$ skeleton in corals (Cnidaria), mollusks (Mollusca) and sea urchins (Echinodermata) suggests that there are few sequence similarities across all three phyla [114]. However, there are conserved motifs that include acidic proteins, structural and adhesion proteins and signaling proteins. Based on this analysis and the fossil record, in metazoans biomineralization appears as a robust and highly controlled process. It is important to note that the activity of the acidic proteins should be not affected by predicted pH values for the coming century. This implies that biomineralization could tolerate future scenario of ocean acidification (see Section 5).

Among the OM components lipids have been poorly investigated. Only recently the lipids extracted from the skeletons of seven modern coral species have been characterized [115]. Lipids differed in quantity and composition among the species. Higher proportions of sterols and sterol esters in skeleton extracts as compared to a much higher abundance of waxes and triglycerides in previously studied extracts from scleractinian soft tissues suggested a role in the mineralization process. Most probably they are involved in the stabilization of amorphous calcium carbonate and/or the formation of $CaCO_3$ vesicles [49,113]. However, the relevance this family of molecules, if any significant, in the deposition of the skeleton of corals has to be almost completely discovered. The recent studies on other calcifying organisms denote a growing involvement in the biomineralization processes [49,113].

4. Influence of intra-skeletal organic matrix in calcium carbonate precipitation

The influence of OM macromolecules on the precipitation of $CaCO_3$ has been mainly investigated in *in vivo* using coral larvae and by means diverse *in vitro* experimental assays.

The *in vivo* skeletogenesis in settled coral larvae has been investigated in few studies [23,26,31,78,116,117]. The skeletal formation in *S. pistillata* was studied by a coral nubbin attached to a glass coverslip [78]. Four main stages of skeletogenesis were observed: (i) a thin layer of coral tissue deposited; (ii) primary fusiform crystals deposited forming a discontinuous skeletal front; (iii) needle-like crystals appeared, covering the primary crystals; (iv) a lengthening of the needle-like crystals that resulted in occlusion of the spaces between adjacent crystals. Recently, Gilis et al. [117] carried out in aquarium conditions a microscopic and spectroscopic investigation at diverse scales of all skeletal elements deposited by *P. damicornis* recruits, from 12 h to 22 days after settlement on a substrate surface. Aragonite was the major phase, with the exception of tiny rod-shaped crystals of calcite, observed only in the initial stages of basal plate formation [117]. Aragonite showed a wide range of randomly distributed complex morphologies, on substrate areas not yet completely mineralized. This mineralogical and morphological diversity in the early stages of larval biomineralization were associated to the involvement of different mechanisms of precipitation [117].

A common feature of these *in vivo* studies is the formation of a layer of tissue on which the mineral deposition starts. Indeed, the initial formation of an OM acting as template for the mineral deposition is a common feature of biologically controlled biomineralization processes [1–3].

In vitro experiments using the primary tissue culture of the coral *M. digitata* showed the formation of an OM at which extracellular mineralized particles were associated [95]. In an innovative *in vitro* approach the precipitation of $CaCO_3$ in coral was investigated by means of coral tissue cultures that aggregate to form "proto-polyps" [118]. This experimental system facilitates calcification at the cellular level and simultaneously allows *in vitro* manipulations of the calcifying fluid. Viable cell cultures of *S. pistillata* were maintained for 6 to 8 weeks in enriched seawater medium with aragonite saturation state similar to open ocean surface waters. The primary cell cultures assembled into "proto-polyps", which formed an OM on which aragonite crystals precipitated. The precipitation of aragonite was independent of photosynthesis by the zooxanthellae, and did not occur in control experiments lacking coral cells.

In *in vitro* cell free experiments the capability of OM molecules to influence the precipitation of $CaCO_3$ was studied by overgrowth [119] experiments carried out on a surface skeleton section of *L. pertusa*, *Montipora caliculata*, *Acropora digitifera*, *Balanophyllia europaea*, *Leptopsammia pruvoti*, *Cladocora caespitosa* and *Astroides calycularis*. Aragonite formed on the surface of all coral skeletons (Table 1). This overgrowth process, which could be due to secondary nucleation events, made crystals having species specific size and texture, and thus reflecting an interaction with OM molecules released from the skeletal substrate [120,121].

The *in vitro* cell free precipitation of $CaCO_3$ was also carried from solutions in the presence of OM soluble and insoluble components [28,120–123]. The first study using this assay was carried out using the OM from *B. europaea* [28]. This OM was shown to favor the precipitation of aragonite from solution where only the precipitation of calcite is observed. The OM another Mediterranean coral, *C. caespitosa*, was investigated as well [122]. Both studies commonly showed the significant role of the SOM proteins in controlling not only the polymorphism of $CaCO_3$, but the assembly, morphology and shape of the precipitated particles. It was also shown that the general strategy for the morphogenesis of fibrous aragonite lies in the nanoscale aggregation and subsequent coalescence processes.

Analogous *in vitro* cell free $CaCO_3$precipitation experiments were carried out using the OM extracted from other tropical corals [120] and Mediterranean corals [121]: *L. pertusa*, *M. caliculata*, *A. digitifera*, *C. caespitosa* and *A. calycularis*. The results showed that the control on morphology and polymorphism of $CaCO_3$ is specie specific (Table 1, Fig. 3). The coral biomineralization process was also investigated in a viscous agarose sol with dissolved The SOM from *B. europaea* or *L. pruvoti* was also investigated using a counter-diffusion-system. These experiments showed that the SOM concentration determines the $CaCO_3$ polymorphic distribution [123].

Table 1
Summary of the *in vitro* cells free $CaCO_3$ precipitation experiments in the presence of organic matrix (OM) from the Mediterranean coral *L. pruvoti*, *B. europaea*, *A. calycularis* and *C. caespitosa* (data from [121]) and the tropical corals *M. caliculata*, *A. digitifera* and *L. pertusa* (data from [120]. The precipitation of $CaCO_3$ was carried out from a 10 mM $CaCl_2$ solution containing and optimized concentration of SOM or IOM by the $(NH_4)_2CO_3$ vapor diffusion assay. The main features of the OM fractions are also reported.

Species[£]	OM composition		$CaCO_3$ overgrowth[%]		$CaCO_3$ precipitation[%]		
		SOM/IOM[$]	C	A	SOM (C)	IOM (C)	*e*OM (C)[*]
L. pruvoti sol. azo.	2.5 ± 0.1[a]	*p* (=)	s. cryst.	needle	db., s. ag.	cr. ag.	s. ag.
	0.3[b]	*s* (−)	{10.4}	bundles		{10.4} {10.8}	(ag. nano p.)
	−[c]	*l* (=)	(7–29)[&]	(1–3)[&]	(5–57)[&]	(12–72)[&]	(5–32)[&]
B. europaea sol. zoo.	2.9 ± 0.1[a]	*p* (=)	s. cryst.	prisms	db., s. ag.	cr. ag.	s. ag.[*]
	1.5[b]	*s* (−)	{10.4}{hk.0}		(add. part.)	{10.4}{10.8}	(ag. nano p.)
	+[c]	*l* (=)	(6–33)[&]	(0.3–1)[&]	(2–20)[&]	(16–53)[&]	(3–10)[&]
A. calycularis col. azo.	2.7 ± 0.1[a]	*p* (=)	s. cryst.	prisms of	db., s. ag.	s. cr.	s. ag.
	0.2[b]	*s* (−)	{10.4}	needles	(add. part.)	{10.4} {10.8}	(ag. nano p.)
	+[c]	*l* (−)	(10–50)[&]	(2–5)[&]	(4–40)[&]	(4–21; 113–139)[&]	(37–68)[&]
C. caespitosa col. zoo.	2.5 ± 0.1[a]	*p* (=)	s. cryst.	prisms of	db., s. ag.	cr. ag.	s. ag.
	0.3[b]	*s* (−)	{10.4} {hk.0}	needles	(ag. nano p.)	{10.4}{10.8}	(ag. nano p.)
	−[c]	*l* (−)	(4–28)[&]	(1–4)[&]	(7–10)[&]	(26–85)[&]	(3–16)[&]
M. caliculata col. zoo.	3.4 ± 0.1[a]	*p* (=)	s. cryst.	prisms of	db., s. ag.	cr. ag.	s. ag.
	1.4[b]	*s* (−)	{10.4} {hk.0}	needles	(ag. nano p.)	{10.4}	(ag. nano p.)
	+[c]	*l* (−)	(4–28)[&]	(2–5)[&]	(5–20)[&]	(5–30)[&]	(3–15)[&]
A. digitifera col. zoo.	2.7 ± 0.1[a]	*p* (=)	–	needle bundles	db., s. ag.	cr. ag.	s. ag.
	5.0[b]	*s* (+)			(ag. nano p.)	{10.4}	(ag. nano p.)
	+[c]	*l* (=)		(0.5–1)[&]	(5–40)[&]	(20–80)[&]	(3–15)[&]
L. pertusa col. zoo.	3.9 ± 0.1[a]	*p* (=)	s. cryst.	prisms of	db., s. ag.	cr. ag.	s. ag.
	1.5[b]	*s* (−)	{10.4}	needles	(ag. nano p.)	{10.4}	(ag. nano p.)
	−[c]	*l* (=)	(10–50)[&]	(2–5)[&]	(5–40)[&]	(20–100)[&]	(3–10)[&]

The main crystalline calcium carbonate crystalline phase, aragonite (A) and calcite (C) are reported. The crystallization experiments were carried out in a 10 mM $CaCl_2$ solution by the method of the ammonium carbonate gasses diffusion.

[a] Mass percentage of OM entrapped in the skeleton.
[b] Mass ratio between SOM and IOM. This data showed a great variability from one experiment to another and the median value is reported.
[c] Relative content of acidic amino acid between SOM and IOM: (+) indicates higher and (−) indicates lower.
[£] sol. = solitary coral; col. = colonial coral; zoo. = zooxanthellate coral; azo. = zooxanthellate coral.
[%] The precipitated $CaCO_3$ particles showed a great variety of shapes and morphologies. s. cryst. = rombohedral single crystal; db = dumbbells; s. ag. = aggregate made of spherulites and spherical particles; cr. ag. = aggregate made of single crystals; ag. nano p. = the aggregate is formed of nanoparticles; add. part. = additional morphologies were observed.
[$] p, s and l indicate protein, sugars and lipids, respectively and their relative content between SOM and IOM is evaluated as higher (+), equal (=) or lower (−).
[&] Indicates the range of dimension of the particles (in μm).
[*] Only in the presence of *B. europaea* the precipitation of aragonite was observed. When indicated the errors are reported as standard deviations.

In a very important research the ability to precipitate $CaCO_3$ given by four highly acidic proteins expressed from the coral *S. pistillata*, and not of the whole OM [120–123], was tested [111]. The results showed that each acidic protein was able not only bind Ca^{2+} stoichiometrically, but also precipitate aragonite *in vitro* in seawater in conditions where the precipitation does not occur spontaneously [111]. On the other side the use of a single component of the OM could hide the cooperative effect of the OM molecules in the overall control over calcification, as proposed [124].

An important, although not always common, outcome from the cell free *in vitro* precipitation of $CaCO_3$ in the presence SOM was the detection of amorphous $CaCO_3$ (ACC) as precursor of the crystalline phases [28,121], mainly when the precipitation was carried out from artificial seawater. Two forms of ACC having a different content of water and a diverse thermal stability were detected. Only the ACC obtained in the presence of SOM from *B. europaea* thermally converted in aragonite, while the other ACCs obtained in the presence of SOM from *L. pruvoti*, *C. caespitosa* or *A. calycularis* thermally converted in magnesium calcite. This observation suggests the formation of ACC as transient form during the coral skeleton formation. This, despite the fact that ACC has not been observed in *in vivo* studies into adult coral skeletons [116].

5. Intra-skeletal organic matrix and effects of ocean acidification

Nowadays, there is great concern on corals survival under envisaged scenarios of increased pCO_2 in the atmosphere due to anthropic activities, and the associate ocean acidification (OA) with reduction of $CaCO_3$ saturation state in seawater [125–128].

This review reports that the precipitation of aragonite in corals is controlled mainly by the OM molecules. Among them the acidic proteins have an important role in controlling the nucleation of aragonite. Their activity takes place in the calcification media where the pH is controlled by several chemical and biological processes [*e.g.* 91–94]. According to Mass et al. [111] the nucleation activity of acidic proteins takes place *in vitro* on the base of thermodynamic considerations in seawater either at pH 8.2 and 7.6, *via* an electrostatic interaction with protons on bicarbonate anions.

The following experimental results are in line with this observation [111], suggesting that the calcification process is minimally affected by the predicted change in surface ocean pH in the next decades [125–128].

New recruits of coral *Favia fragum* were kept in seawater with aragonite saturation states ranging from ambient to undersaturated [26]. Aragonite was deposited by all corals, but those in undersaturated seawater showed a delay in initiation of calcification. In addition changes in texture and composition of the aragonite crystals were detected. These results suggest that the coral maintains the saturation state of media within calcifying compartment above that of the external seawater.

Similar conclusions were obtained analyzing the seawater acidification impacts on intracellular pH in calcifying cells and extracellular pH in the media at the tissue–skeleton interface in the coral *S. pistillata*. The seawater acidification decreased the pH of the media at tissue–skeleton interface, but this decrease was gradual relative to the surrounding seawater, leading to an

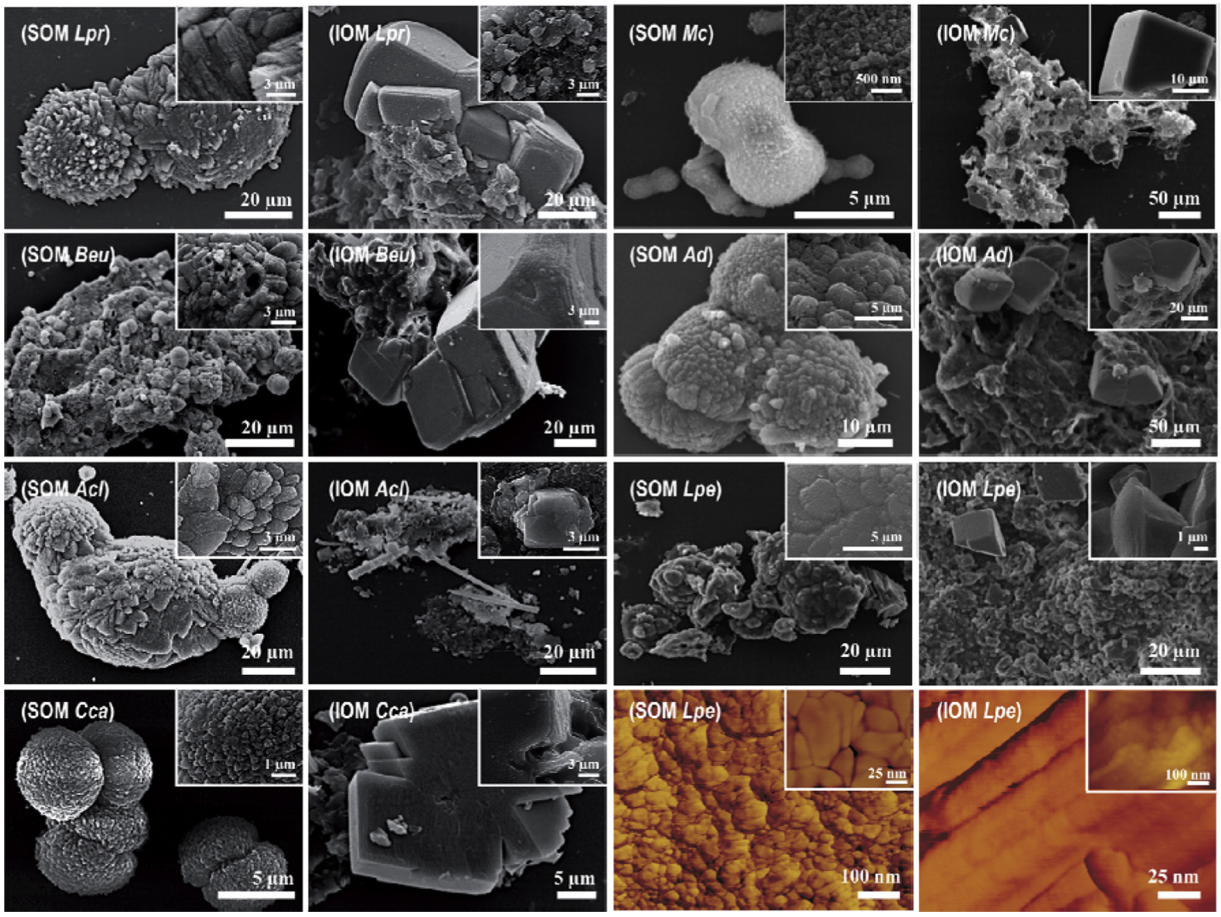

Fig. 3. Scanning electron microscopy images of $CaCO_3$ precipitates obtained *in vitro* from 10 mM $CaCl_2$ solution by the $(NH_4)_2CO_3$ vapor diffusion assay in the presence of SOM and IOM from obtained by decalcification of the skeleton of *L. pruvoti* (*Lpr*), *M. caliculata* (*Mc*), *B. europaea* (*Beu*), *A. digitifera* (*Ad*), *A. calycularis* (*Acl*), *L. pertusa* (*Lpr*) and *C. caespitosa* (*Cca*). The images show a great variability of morphologies, shapes and aggregation of diverse $CaCO_3$ building units that in all the cases were made of calcite, unless for the presence *B. europaea* SOM that co-precipitate aragonite. The morphology, shape and aggregation of calcite are more affected by SOM than IOM, pure calcite form perfect rhombohedra in the used precipitation conditions [28,120,121]. In the right bottom two representative atomic force microscopy images are shown, these are similar for all samples.

increasing pH difference between media at tissue–skeleton interface and seawater [29].

Skeletal parameters analyses of samples obtained by *in vivo* experiments on corals grew in different condition of acidity showed that only the ultra-structural features of aragonite fibers were influenced by the seawater acidity [129]. It also important to note that the density of population of corals grew in a natural environment mimicking future scenarios of OA decreased [130], suggesting that other physiological/ecological parameters, beside the calcification, are affected by the OA.

6. Conclusions

All the studies on the deposition of aragonite in scleractinian coral skeletons converge on the concept that it is a biological controlled process, although this process can influenced by environmental parameters.

This even if coral skeleton does not show an apparent elegant and accurate architectural assembly of the aragonitic building units as it is in echinoderm and mollusk skeletons. Nevertheless, the OM controls synthesis and microarchitectural organization of aragonitic fibers and the EMCs, producing skeletons having a final morphology which is species specific. This, despite the fact that scleractinian corals have a simple biological structure, when compared with echinoderm and mollusk. Thus, scleractinian corals represent almost an ideal gym for the study of the biomineralization processes. Moreover, there are many clues that multidisciplinary approaches, exploitation of several new experimental techniques and the actual growing interest of the response of coral calcification to OA, will increase even more the interest of all the biomineralization community on these organisms.

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