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Insights into Chemistry of Biological Materials: Newly Discovered Silica-Aragonite-Chitin Biocomposites in Demosponges

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Biological materials are a rewarding area of modern materials science, yielding both evolutionary insights and inspiration for biomimetic research. In particular, biocomposite structures are valuable sources of novel structures with unusual chemical properties, and they are very informative for the mechanisms of biomineralization. Here we describe a unique biocomposite of amorphous silica, crystalline aragonite, and chitin from species of the order Verongida, a group of marine sponges. The structures have been analyzed with a diverse suite of techniques, revealing a chitinous template for siliceous overgrowth containing aragonite-based crystal aggregates. Sponge chitin is an example of a specific template where two minerals in amorphous and crystalline forms are formed together with an organic molecule.

1. Introduction

Biological structures are a source of inspiration for approaching a variety of technical challenges in materials science (reviewed in ref 1). Sponges (Porifera) are fascinating research subjects because of the hierarchical organization of their fibrous skeletons (Demospongiae) and mineralized spicules containing opaline silica (Demospongiae and Hexactinellida) or calcium carbonate (Calcarea). The skeletons of sponges are natural examples of rigid silica-based[2,3] or calcium carbonate-based[4] composites. As a result, the biomimetic potential of marine sponges for chemists and material scientists is very rich.[5] Composites form structural interfaces resulting in fatigue resistance and resiliency. Furthermore the presence of water has notable effects on the mechanical and materials properties.[6,7] For example the skeleton in the hexactinellid *Euplectella* comprises an elaborate cylindrical lattice with at least six hierarchical levels[8] spanning the length scale from nanometres to centimeters. The basic building blocks are laminated spicules that consist of a central proteinaceous axial filament surrounded by alternating concentric domains of consolidated silica nanoparticles and organic interlayers.[9]

Although many aspects of the chemical and materials properties of biocomposites can be modeled for biomimetic engineering, the design of novel biomaterials relies on an understanding of the organic matrices and templating structures in nature. Such studies are also of great interest in high-level phylogenetic studies, aiding an understanding of the evolutionary origins of biomineral structures recorded in the fossil record. Research in this area has benefited from the recent discovery of collagen

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within spicules of two representatives of Hexactinellida, *Hyalonema sieboldi* and *Monorhaphis chuni*,[10,11] and the occurrence of chitin within the framework skeleton of *Farrea occa*[12] and separate spicules of *Euplectella aspergillum*[5] and *Rossella fibulata*,[13] revealed by desilicification in alkali.

According to morphological and chemical analyses, the Verongida form a coherent order of keratosan demosponges.[14] Verongid genera are mainly distinguished by the structure and composition of their fibres,[15] and the group is distributed worldwide. They are traditionally thought to lack a mineral skeleton, possessing instead a collagenous mesohyl supported by spongin fibres that exhibit a granulated "pith" interior and a laminated "bark" exterior.[15] Recent re-examination showed that chitin, rather than spongin, forms the main organic component of verongid skeletal fibres, and that aragonite is also present.[16,17] Because sponges are often regarded as the most ancient metazoans (630−542 My),[18] the finding of chitin within their skeleton is of major significance. It is well established that chitin functions directly or indirectly as a template for nucleation of mineral phases in other invertebrates.[19,20] In this case, it was suggested that chitin serves as a template for calcium carbonate deposition in sponges.[17]

The formation and templating of silica (SiO_2) has also been studied in spicules of hexactinellids and demosponges.[3] However, despite the recognition of Si in the outer compact layers of fibres of verongid sponge *Aplysina aerophoba*, determined using X-ray microanalysis,[21] the synthesis of siliceous structures in Verongida has not previously been described. In other keratose demosponges, incorporated sediment and other foreign particles are the main source of inorganic skeletal material in spongin fibres.[22,23] Bavestrello et al.[24] hypothesized that the ability of several types of sponge to incorporate foreign silica particles could be a part of a more general process involving cell reactivity to silica from prokaryotes to vertebrates. However, *Verongula* and *Aplysina* species possess a skeleton without spicules or foreign detritus[25] (see Figure S1, Supporting Information (SI)). Moreover, one of the criteria that differentiate the orders Verongida and Dictyoceratida is the presence of foreign particles only in the latter.[26] That means, silica present in verongid fibres must be synthesized by the sponge.

The organic chemistry of sponges is also the focus of intense research for pharmaceutical and other applications. Sponges rely heavily on bioactive organic molecules for their defense,[27] including many halogenated compounds. Bromine-containing compounds related to tyrosine constitute by far the commonest class of secondary metabolites in Verongida.[28] Skeletal fibres are thought to play a role in sequestering and accumulating brominated compounds, perhaps as inactive residues.[21,29] Bromine is often concentrated within keratosan fibres, and up to 12% dry weight of their secondary metabolites contains the element.[29] The concentration of bromine within spongin fibres,[21] where chitin is also densely distributed,[17] leads to further questions regarding the possible role of bromotyrosine-like compounds in regulation of sponge biomineralization, which are discussed below.

The structure and formation of the fibres were investigated in detail to answer several key issues. We intended to resolve the role of chitin as a potential mineral template, and to clarify the role of any distinctive secondary metabolites in biomineralization. We also wanted to investigate whether true mineral−chitin biocomposites are present in demosponges as well as in hexactinellids, and to establish rigorously whether carbonate is the only authigenic mineral phase present in verongid fibres, by studying the structures at the highest possible resolution and with a wide array of techniques.

2. Experimental Section

2.1. Sample Preparation. We examined the fiber skeleton of three demosponges of the order Verongida: *Verongula gigantea* (Hyatt, 1875), collected from Trindade Island off Brazil (Figure 1A), *Aplysina cavernicola* collected from Mediterranean Sea, and *Aplysina cauliformis* collected from the Caribbean Sea. Trindade Island (20°30′S−29°20′W) is located 1,140 km E off Vitória, SE Brazil. It has an area of approximately 8 km^2, with sandy beaches, rocky coasts and tide pools. *V. gigantea* was collected through SCUBA diving in Ponta dos Farilhões, a vertical rocky wall with small caves near the bottom, 30 m depth, and high wave impact. Specimens of *A. cavernicola* were

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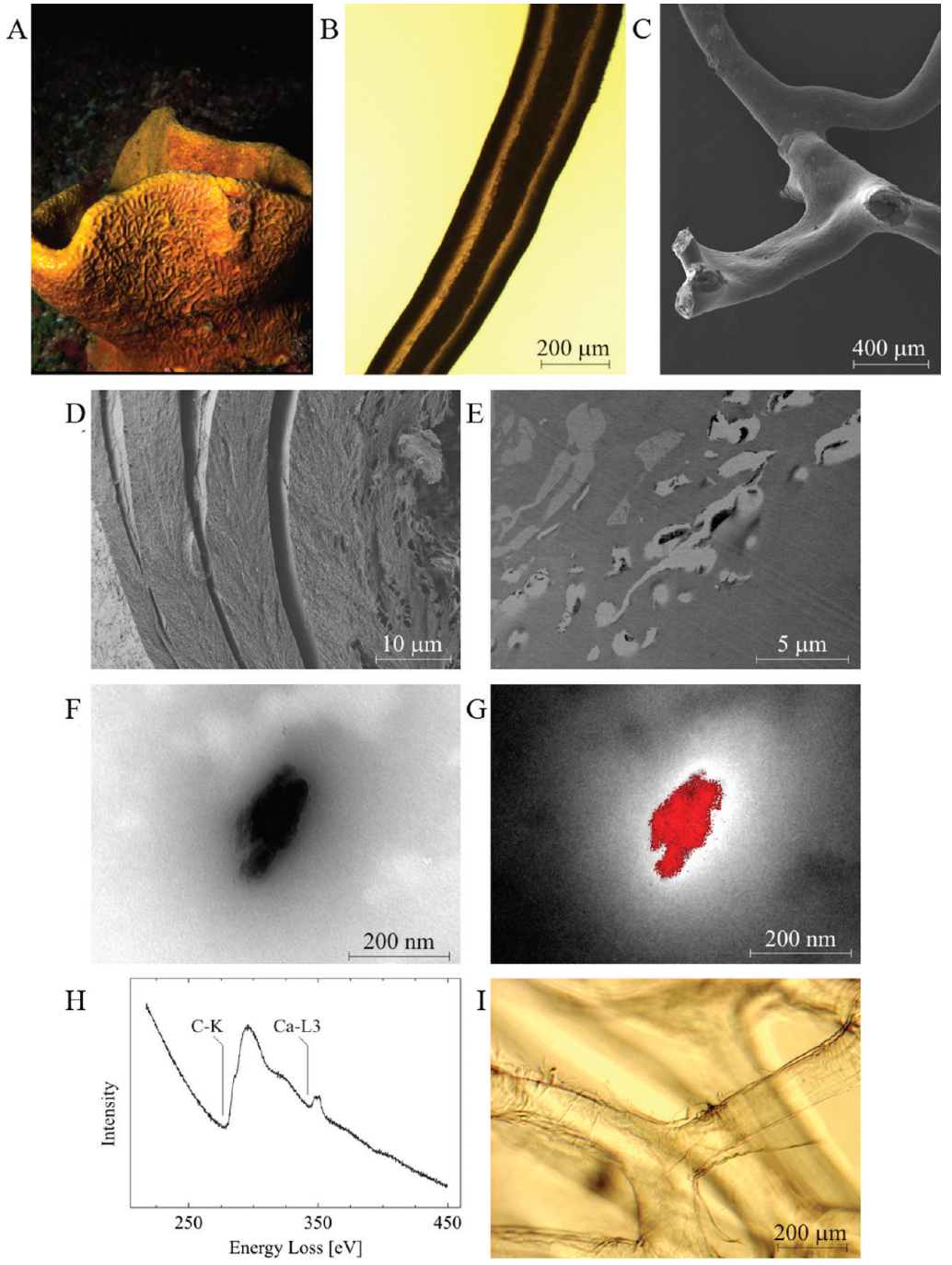

Figure 1. Multilayered skeletal fibres of verongid sponges with chitin as the main organic material. (A) *Verongula gigantea* from Trindade Island, Brazil, 10 cm tall. (B) Skeletal fibres of *V. gigantea* showing pith (dark colored) and bark (yellow and dark-brown colored). (C) reticulated fiber skeleton of *V. gigantea* seen in SEM. (D) Cross-section of a skeletal fiber showing a multilayered structure, which resembles silica spicules as described for hexactinellids and demosponges.[2,3] (E) STEM image of the cross-section of the mineralized dense outermost layer of the fiber showing the presence of electron-dense inclusions (dark). (F) TEM image which also shows the electron-dense formations. (G) Map of the distribution of calcium overlaid on an inverted high contrast image taken at 250 eV. This composite image indicates that electron-dense formations (F) contain calcium as a main element (indicated in red). (H), Electron energy loss spectrum taken from calcium accumulation seen in (F) and (G); calcium represented by a distinct peak at an energy loss of $\Delta E = 358$ eV. (I), Cylindrical translucent structures obtained by demineralization of skeletal fibres using 2.5 N NaOH at 37 °C. These structures were identified previously[17] as α-chitin (SI Figure S3).

collected, by SCUBA diving, from a population located between 14 and 16 m depth, on a vertical, calcareous rocky wall substrate in Maire Island, Marseille. Specimens of *A. cauliformis* were collected from a well illuminated coral reef located in the eastern part of Grand Bahama Island (Sweetings Cay), at 4−8 m depth. Sponge samples were put in ziplock bags underwater, brought back to the laboratory, and frozen less than an hour after collection. Prior to further treatment, the sponges were lyophilized. In cleaning and preparing the skeletons, we avoided the use of chemicals.

2.2. Alkaline Extraction of Sponge Fibres. To elucidate the nature of the fiber components, we demineralized the sponge fibres by alkali treatment. Fiber skeletons were washed three times in distilled water, cut into 2.0×2.5 cm^2 pieces, and placed in a 10 mL plastic vessel containing 8 mL of 2.5 M NaOH solution. The vessel was covered to restrict evaporation and

placed under thermostatic conditions (37 °C) with gentle agitation for 7 days. Immersion in NaOH led to an immediate loss of bromotyrosines and related brown pigments from the fibres. The effects of the alkali etching on the fibres were also examined using optical and scanning electron microscopy. The fibrous colorless material obtained after alkali treatment of the sponge samples was washed with distilled water five times and finally dialyzed against deionized water on Roth (Germany) membranes with a MWCO of 14 kDa. Dialysis was performed for 48 h at 4 °C. The dialyzed material was dried at room temperature and used for staining and analytical investigations. For appropriate comparisons during this and subsequent experiments involved in the process of sponge chitin characterization, we used alpha-chitin (Fluka) as a control. The material was purified with aqueous 1 M HCl for 2 h at 25 °C and then refluxed in 2 M NaOH for 48 h at 25 °C. The resulting α-chitin was washed in deionized water and centrifuged several times until neutrality was reached. This kind of treatment does not result in deacetylation or other modifications of the chitin,[30] as can be seen from solid-state NMR data (SI Figure 5). The NMR data further show that the described alkali-treatment removes other organic components such as proteins from the NaOH-resistant chitin.

2.3. Acidic Treatment of Sponge Skeletons. To elucidate the nature of the stable fiber components under acid dissolution, we demineralized fibres of investigated sponges by treatment with HCl. Fiber skeletons were washed three times in distilled water, cut into 2 × 2.5 cm^2 pieces, and placed in a 10 mL glass vessel containing 8 mL of 3 M HCl solution. The vessel was covered to restrict evaporation and placed under thermostatic conditions (37 °C) with gentle agitation for 7 days. Immersion in HCl did not lead to an immediate loss of brown pigments from the fibres. The fibres became white and fragile only after 5 days of incubation in HCl. The effects of the acidic treatment on the fibres were also examined using optical and scanning electron microscopy. The white material obtained after acidic treatment of the sponge samples was washed with distilled water five times and finally dialyzed against deionized water on Roth (Germany) membranes with a MWCO of 14 kDa. Dialysis was performed for 48 h at 4 °C. The dialyzed material was dried at room temperature and used for staining and analytical investigations.

Silicon concentrations in this acid resistant material were determined by the silicomolybdate method[31] according to U.S. Standard Methods 4500-Si E using Silicat-Test (Merck).

2.4. Analytical Methods. used in this work (FTIR, Raman, solid-state NMR, photoemission and X-ray absorption spectroscopy, electron spectroscopic images (ESI) and electron energy loss spectra (EELS), as well as SEM, STEM, TEM, HR-TEM, FIB TEM, are detailed and described in the SI.

3. Results and Discussion

3.1. Mineralized Chitin within Sponge Skeletal Fibres. The skeleton of *Verongula gigantea* (Figure 1A) is a three-dimensional construction with anastomosing fibres (Figure 1B and C) producing polygonal reticulation. Each fiber consists of concentric cylinders (Figure 1D). Transmission electron microscopy (TEM) studies of chemically untreated fibres corroborated not only their microstructural organization, but also the presence of electron-dense micro- and nanoformations located in the outermost chitinous layer with respect to the axial channel (Figure 1E).

We detected the presence of calcium in electron-dense nanostructures observed within ultrathin sections of *V. gigantea* fibres (Figure 1E) by means of energy filtering transmission electron microscopy (EFTEM). Electron spectroscopic imaging (ESI) (Figure 1F and G) and electron energy loss spectra (EELS) (Figure 1H) revealed discrete calcium localization in these structures.

The chemistry of verongid fibres can be studied by selective dissolution in alkaline or acidic solutions.[32] Alkalis can dissolve the siliceous material, leaving the carbonaceous intact. In contrast, acidic solutions can dissolve the carbonate to leave the siliceous phase. In order to isolate the mineral-free organic matrix we used alkali treatment at 37 °C as described previously,[17] yielding translucent tubular structures (Figure 1I) after five days. The results of physico-chemical analyses performed using NMR and Raman spectroscopy (see SI Figures S2 and S3) show strong evidence for the presence of α-chitin of poriferan origin.[17] Weighing of dried natural fibres and the same fibres after alkali-mediated demineralization showed that chitin makes up ca. 60% of the dry weight of the native sponge skeleton. No measurable traces of Ca or Si were found in alkali-treated chitinous fibres after their dialysis. However, calcium carbonate was identified in preliminarily dialyzed and lyophilized alkali extracts, as in previous results.[12,16]

A preliminary elemental analysis of the sponge fibres cut using FIB technique (Figure 2, also see SI Figures S5 and S6) showed the additional presence of calcium and silicon as well as carbon, nitrogen, oxygen, bromine, and other halogens. These results agree with those reported previously for *Aplysina aerophoba*,[21] although the nature of the phases containing these elements was unknown. Figure 2A and B definitively show the absence of foreign particles or spicule debris typical for skeletons of keratose sponges of the order Dictyoceratida.[24,26]

The treatment of native sponge fibres with 3 M HCL solutions resulted in dissolution of the acid-soluble mineral component. This yielded a perforated fiber surface (Figure 3A). The structure of the films is visible in light microscopy (Figure 3B) or scanning electron microscopy (SEM) (Figures 3C and D). Silicon concentrations were determined after alkali dissolution of isolated layers (Figure 3B) by the silicomolybdate method[31] to be about 100−150 μg of Si per mg of dried skeletal fiber.

Because autofluorescence is atypical for pure silica, but well-known for chitin and naturally occurring silica-chitin composites,[5,12] we used fluorescence microscopy and Calcofluor White staining for preliminary identification of chitin within these acid-resistant structures. The characteristic blue fluorescence of both unstained and stained samples indicated the presence of chitin (see SI

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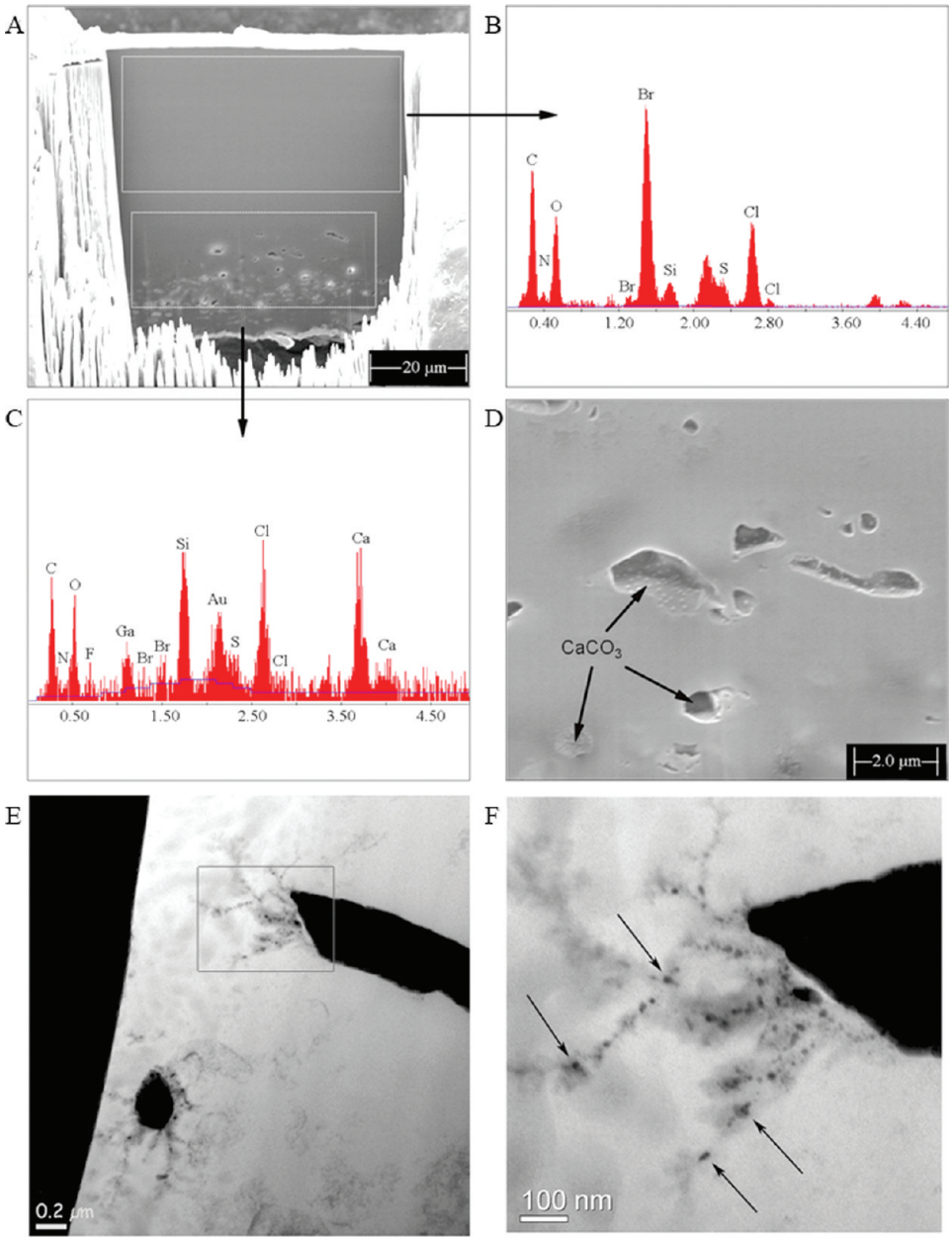

Figure 2. Microscale distribution of bromine, silica and calcium within a sponge skeletal fiber. (A) The longitudinal FIB (focused ion beam) cut of a *V. gigantea* sponge fiber shows the presence of two main regions of different structure. The EDX analysis of the outermost region (B) indicates the presence of bromine and silica. However, silica and calcium are present in the inner region which is in contact with the axial channel of the fiber (C). The SEM image of this region (D) shows micro- and nanoformations of calcium which are similar to those represented in Figure 1E and F. These formations were subsequently identified as calcium carbonate (Figures 4 and 5). (E) TEM image of the FIB cut of the fiber region represented in (D) showing that calcium carbonate grows on the chitin nanofibres. (F) Both, micro- and nanoparticles (arrows) of the mineral are tightly embedded into the organic matrix.

Figure S7). We suggest that the nanofibrillar network in this silica-based matrix (Figure 3E) is of chitinous nature. Samples of the matrix shown in Figure 3C and D were subsequently submitted to HR-TEM to examine the nature of this material, and locate additional occurrences of chitin. HR-TEM studies of the silicified matrix residues obtained after acid mediated demineralization of *V. gigantea* fibres revealed the presence of nanocrystallites of diameter 2 nm (Figure 3F). These structures were nearly identical in appearance and size to the chitin crystallites observed when examining an α-chitin standard (see SI Figure S8). They were also extremely similar to those previously reported by TEM observations of chitinous skeletal structures in insects, crustaceans and arachnid species.[33−35]

3.2. Identification of Silica and Aragonite. The chemical nature of the mineral phases within sponge fibres was then investigated by means of X-ray photoemission spectroscopy (XPS) and X-ray absorption spectroscopy (XAS) (Figure 4A and B), performed on the untreated fibres of *V. gigantea* (Figure 1B−D), *Aplysina cauliformis*

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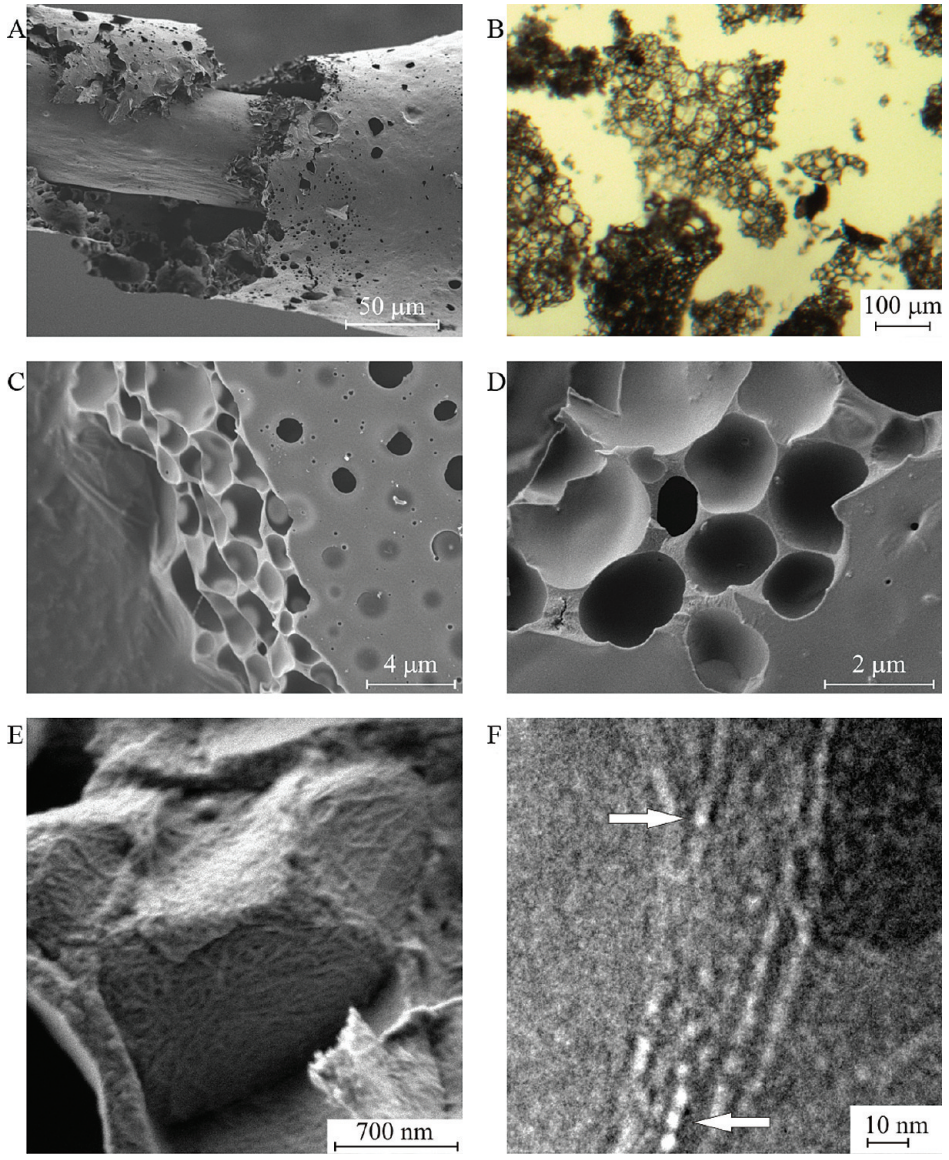

Figure 3. Silica in the outermost layers of the skeletal fibres of verongid sponges. (A) SEM image of a skeletal fiber after treatment with 3 M HCl at 37 °C for 3 weeks. The image shows perforated layers covering the pith region. (B) Light microscopic image of isolated layers showing their perforated structure. (C) SEM image showing the three-dimensional organization of the layers. (D) Nano- and microscale apertures visible using SEM within these formations corresponding to the mineral compounds that were dissolved by HCl. (E) The SEM image of the same fragment at higher magnification shows the presence of a nanofibrillar network. (F) Nanofibres in the silica matrix containing nanoformations of 2 nm diameter(arrows) typical for α-chitin crystallites as described previously.[33−35]

and *A. cavernicola*. The appearance of Si 2*p* photoemission demonstrates the presence of silicon in all fibres, and the exact position of the peak on the binding energy scale grants insight into its chemical bonding due to the well-known "chemical shift" of core-level binding energies.[36] For instance, pure silicon is observed at ∼99.5 eV binding energy, whereas SiO_2 is found at ∼104 eV.[37] The observed Si 2*p* binding energy of ca. 103 eV is similar to that reported before for silica-supported chitosan.[38] Therefore, we hypothesize that silicon oxide is present in skeletal structures of verongids, bound as a silica−chitin composite. This stoichiometry additionally renders foreign particles as a source of the silica signal unlikely. XAS spectra obtained for each of the sponge samples (Figure 4B) show a strong signal at the Ca 2*p* absorption edge, at which the fine structure strongly resembles that of the $CaCO_3$ reference sample.

Further analysis of the mineral structures was achieved by infrared (IR) and Raman spectroscopy. IR spectra were previously shown to be useful in differentiating between different polymorphic forms of calcium carbonate,[39] between different polymorphic forms of chitin,[17] and for silica identification including silica−chitin composites.[40] The obtained FTIR spectra (Figure 4C) as well as Raman spectra (see SI Figure S4) unambiguously

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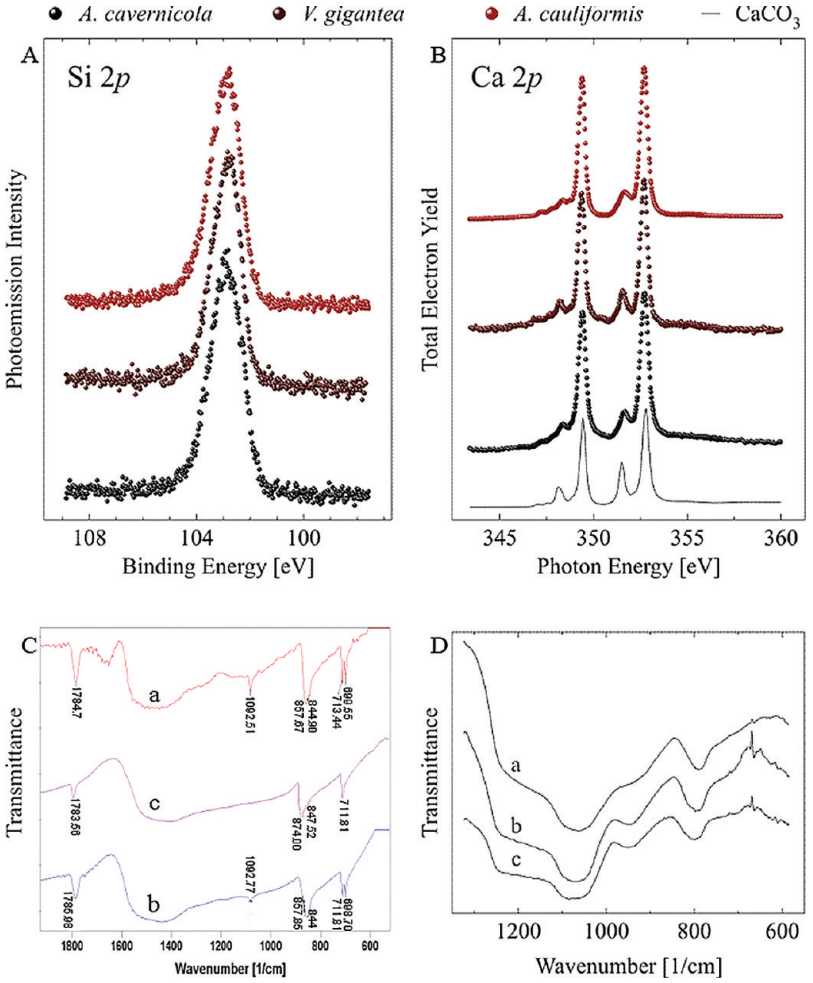

Figure 4. Calcium carbonate in the form of aragonite identified within the silica-chitin-based matrix of verongid skeletal fibres. (A) Photoemission spectra of natural sponge fibres suggesting the presence of silicon dioxide bound to an organic matrix. (B) X-ray absorption spectra showing that calcium carbonate is the second mineral component present within three Verongida species of different geographical origin. (C) Comparison of the FTIR spectra of crystalline mineral components isolated from the outermost layers of sponge skeletal fiber (a), aragonite standard (b), and calcite standard (c). Characteristic bands for the identification of the different compounds are marked. (D) FTIR spectra of natural opal (a),compared with the acid resistant layers isolated from *A. cavernicola* (b), and from *V. gigantea* (c).

confirm the presence of aragonite (Figure 4C) and silica (Figure 4D) within the chitin-based skeletal fibres of the investigated sponges.

The origin of the microscale apertures within the acid-treated matrix visible in SEM images (Figure 5A) could be explained by the presence of aggregated calcium carbonate particles of similar diameter to others observed in extracts obtained from fibres after agate mortar disruption and alkali treatment (Figure 5B). Closer examination using HR-TEM and electron diffraction measurements suggests that the textured core of these aggregates consists of tightly packed chitin (Figure 5C and D) scaffolding aragonite (Figures 5E and F, also see SI Figure S9). Consideration of the polarity of the —N—C(O)— bond in chitin molecules, in which the negative charge is shifted toward the oxygen atom, suggests that the formation of calcium carbonate may be initiated through the interaction of Ca^{2+} ions with the oxygen of the C═O.[41] Combining this data, we propose a model for the nanostructure of the silica−chitin−aragonite-based biocomposite in the form of the structural unit of the Verongid sponge skeleton (Figure 6). It is theoretically possible that Si binds to chitin after the macromolecular structure has been formed. It has been suggested[41] that silicate ions and silica oligomers preferentially interact with glycopyranose rings exposed at the alpha-chitin surface, presumably through polar and H-bonding interactions. Alternatively, and more plausibly from stereochemical considerations,[42] mono- or disaccharide Si derivatives may have been incorporated during polysaccharide chain synthesis.

3.3. Silica−Aragonite−Chitin-Based Biocomposite. The formation mechanisms of this unique biocomposite are still unclear, although certain possibilities can be considered. It is difficult to differentiate between organically mediated and inorganic precipitation; hence, a combination of different mechanisms in the same species cannot be ruled out. The observed three-dimensional morphology of this complex biocomposite can be understood as the result of the crystallization of aragonite

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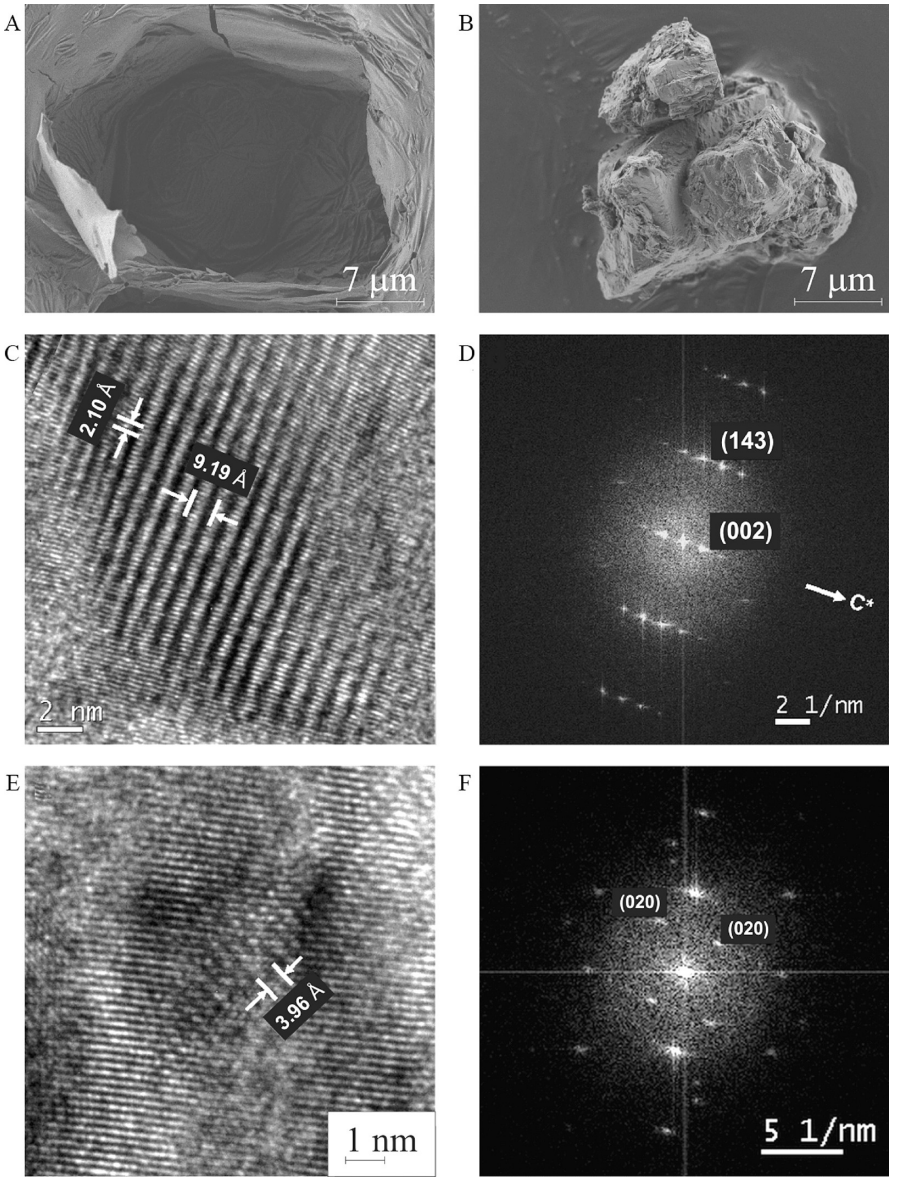

Figure 5. Chitin acting as a template for aragonite formation in skeletal fibres of verongid sponges. (A) SEM image showing the typical location of a calcium carbonate agglomerate (B) within a microscale aperture in silica-chitin-based layers of the skeletal fibres. (C) High resolution TEM image of the crystalline phase within this agglomerate, displaying spacings of 9.19, 4.45, and 2.10 Å corresponding to (002), (120), and (143)/(136) lattice planes typical for α-chitin. However, the 4.45 and 2.10 Å reflections could also belong to the aragonite crystal structure corresponding to (110) and (220) lattice planes (D). (E) HR-TEM image showing the presence of crystalline aragonite within the same mineral agglomerate. (F) Fast Fourier transform of (E) displaying orthorhombic structure of aragonite with denoted spacings of 3.96, 3.00, 2.78, and 2.11 Å corresponding to (020), (002), (121), and (220) reflections.

crystals in the presence of polymeric silica, a well-known trap for organic compounds.[31] As it is thought that some components of the organic matrix may be essential to the controlled precipitation of calcium carbonate,[43] the role of bromotyrosine-related compounds in regulation of calcium input into fibres may be significant. The bromotyrosine tetramer bastadin-5 from the verongid sponge *Ianthella basta* is an ionophore and a potent modulator of Ca^{2+} release from the sarcoplasmic reticulum.[44] This compound stimulates the release of Ca^{2+} from the sarcoplasmic reticulum by binding to the tetrameric heterodimeric channel protein, although the mechanism is not fully understood. Figure 2 shows that the bromine in the chitinous skeletal fiber of the sponge is mostly distributed in calcium-free regions. These results are similar to those recently reported by Schofield et al.[45] for calcium−bromine ratios in the chitinous cuticle of the crab claws. They suggested that one possible function of bromine is to harden and stiffen by increasing cross-links in chitin. The bromine-rich and correspondingly calcium-free regions were less hard but more fracture-resistant.

Sponges are an ideal subject for biomineralization and biomaterials studies due to their complex range of mineralogy, their phylogenetic position at the base of metazoans[24] and their ability to build hierarchically structured

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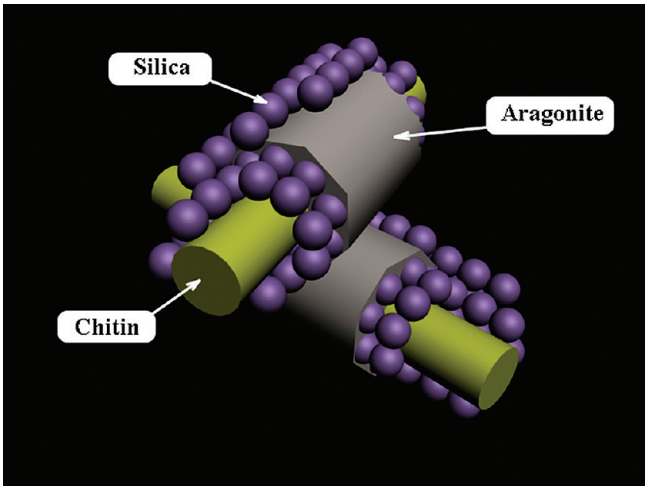

Figure 6. Proposed model of the nanostructural organization of skeletal fiber of verongid sponges showing its multiphase biocomposite nature.

skeletons.[1,8] Although in the phylum Porifera the skeleton may be composed of a large variety of minerals, calcium carbonate and siliceous structures very rarely coexist in the same sponge. The only previously known examples are (i) Coralline, or calcified sponges, which possess a solid calcareous skeleton and siliceous spicules; (ii) *Hemimycale columella* (Demospongiae, Poecilosclerida), in which calcium carbonate spherules occur alongside siliceous spicules;[46] (iii) *Cinachyrella alloclada* (Demospongiae, Spirophorida), in which the siliceous spicules are complemented by calcareous granules.[47] None of these constitute biocomposites.

The early fossil record is more complicated, with a bilayered structure suggestive of a silica−carbonate biocomposite present in the heteractinid sponge *Eiffelia globosa*,[48] which also shows morphological characters of both Calcarea and Hexactinellida. It has been argued that the different secretion mechanisms of spicules of these two phases in modern sponges preclude homologous biomineralization between the sponge classes.[4] However, the recognition of a chitin template for the verongid biocomposite described here shows that the process is potentially flexible. We regard homology of biomineralization as reflecting a continuous lineage of the same templating pathway inducing or controlling mineral crystallization. The common ancestors of the sponge classes could potentially secrete both carbonate and silica as a result of the shared collagen/chitin/bromotyrosine templates. Bromotyrosine derivatives used to be considered exclusive for the order Verongida, but recent records from the orders Poecilosclerida, Agelasida, Haplosclerida, Astrophorida, and Dicyoceratida[49] demonstrate their wide phylogenetic distribution and early origin within the Porifera.

Different lineages may then have utilized these basic processes in different ways through different cytological secretion arrangements, giving the appearance of independent biomineralization, but sharing a fundamental origin. In particular, the contrast between intracellular and extracellular spicule secretion appears now to be less significant.

It has been suggested that structured polysaccharide moieties of glycoproteins[50] and chitin itself[51] are important in controlling aspects of mineral crystal growth in vivo. Chitin is one of the key components in the complex pool of extracellular biopolymers. It contributes the framework for implementing biological hierarchy into calcified biominerals, producing an alignment between the orientation of chitin fibres and brachiopod and mollusc shells, and also for in vitro experiments.[19,20,52]

The chitin in different invertebrates could be regarded as the substrate to which other macromolecules are bound that in turn induces nucleation of the mineral phase.[52] The chitin molecule has C═O, O—H, and N—H groups and oxygen atoms, which have an affinity to the calcium, phosphate, carbonate, and hydroxyl ions of corresponding calcium phases. However, the same functional groups possess affinity to silicate ions. Because there is a possibility that such an oriented organic matrix acts as a template, or an ordered structural framework, we hypothesized the existence of naturally occurring silica−chitin composites. Moreover, silicon was found associated with glycosaminoglycans bound as an ether or ester-like silicate with C—O—Si or C—O—Si—O—Si—O—C bonds, in amounts of one Si atom/130−280 repeating units of the organic.[42]

The nanofibrous architecture may serve as a superior scaffolding architecture for promoting biomineralization.[53] Mineralized chitin offers the nanofibrous framework for mechanical support imparted by the skeleton of invertebrates, and also reservoirs for ions and small molecules. The self-assembling properties of chitin, and its templating activity with respect to silicification, are in accordance with modern views on the development of hierarchical silica-based architectures reported by Pouget et al.[54] In the proposed growth mechanism, macroscopic bundles of silica nanotubes result from the kinetic cross-coupling of two molecular processes: a dynamical supramolecular self-assembly and a stabilizing silica mineralization. The feedback actions between the template growth and the inorganic deposition are driven

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nonenzymatically by a mutual electrostatic neutralization. We suggest that this dynamical template concept could also be accepted as a fundamental mechanism for growth processes in chitin-based biological systems with respect to biomineralization phenomena. Chitin could also play a crucial role in biosilicification of evolutionarily earlier organisms like fungi, as seen in the accumulation and deposition of silica by microcolonial fungi associated with desert varnish.[55] We hypothesized previously[13] that chitin molecules are perhaps part of a very old organic template system involved in the biosilicification mechanism, which was established a long time before the origin of sponges, and which has the potential to evolve biosilicification iteratively.

4. Conclusions

The discovery of nanostructured silica−chitin-aragonite biocomposites as structural scaffolds in verongid sponge skeletons offers many opportunities for biomimetic approaches for constructing uniquely complex biomaterials. Chitin possesses (directly or via other molecules) templating capabilities with respect to calcification as well as to silicification. Within the present paper we show that it can act as a template for biomineralization even where two minerals—amorphous silica and crystalline aragonite—are embedding chitin thus forming a unique material. A few similar examples of related, multiphase biocomposites have been previously identified: (i) silica-chitin-apatite composites in Brachiopoda;[56] (ii) silica-chitin-goethite composites in limpet teeth;[57] and (iii) silica−chitin−willenite composites in copepoda teeth.[58] The silica−chitin−aragonite composite structures described here represent an additional example of multiphase biomineralization. The phenomenon has apparently evolved independently in several groups of Metazoa. It appears that sponges were the pioneering group.

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Supporting Information Available: Description of analytical methods and additional data related to chemistry and structure of biocomposite obtained using NMR, Raman spectroscopy, LSM, HRTEM, and FIB (TEM). This material is available free of charge via the Internet at http://pubs.acs.org.

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