

Research Article

Nanostructural Organization of Naturally Occurring Composites—Part II: Silica-Chitin-Based Biocomposites

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Investigations of the micro- and nanostructures and chemical composition of the sponge skeletons as examples for natural structural biocomposites are of fundamental scientific relevance. Recently, we show that some demosponges (*Verongula gigantea*, *Aplysina* sp.) and glass sponges (*Farrea occa*, *Euplectella aspergillum*) possess chitin as a component of their skeletons. The main practical approach we used for chitin isolation was based on alkali treatment of corresponding external layers of spicules sponge material with the aim of obtaining alkali-resistant compounds for detailed analysis. Here, we present a detailed study of the structural and physicochemical properties of spicules of the glass sponge *Rossella fibulata*. The structural similarity of chitin derived from this sponge to invertebrate alpha chitin has been confirmed by us unambiguously using physicochemical and biochemical methods. This is the first report of a silica-chitin composite biomaterial found in *Rossella species*. Finally, the present work includes a discussion related to strategies for the practical application of silica-chitin-based composites as biomaterials.

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1. INTRODUCTION

This is the second paper on naturally occurring silica-based biocomposites of sponges origin. The initial paper [1] studied the presence of fibrillar collagen as a component of glassy anchoring spicule of *Monorhaphis* sp. glass sponge.

Biocomposites of marine origin including sponge skeletal formations are a constant source of inspiration for finding solutions to a variety of technical challenges in bionics, architecture, optics, engineering, as well as materials science and biomedicine (reviewed in [2, 3]). The biomimetic potential of marine sponges seems to be a goldmine to material scientists. Several main aspects relating to sponges as biomaterials and biocomposites are recently described as follows [3]:

(i) hexactinellid spicules as natural glass-based composites with specific mechanical properties [4, 5];

(ii) skeleton of *Euplectella* sp. (Hexactinellida) as a hierarchical natural structural material of remarkable design [6, 7];

(iii) basal spicules of Hexactinellida as biological glass fibers with specific optical properties [8–10];

(iv) silicatein-based biocatalytic formation of nanocomposite materials (reviewed in [11]);

(v) biomimetically inspired hybrid materials based on sili-cified sponge collagen [12–14].

In case of hexactinellid spicules, it is reported that they are highly flexible and tough, possibly not only because of their layered structure and the hydrated nature of the silica as suggested earlier [15], but of the presence of collagen [16] or chitin [17]. According to paleontological and molecular data, the sponge class Hexactinellida may be the oldest

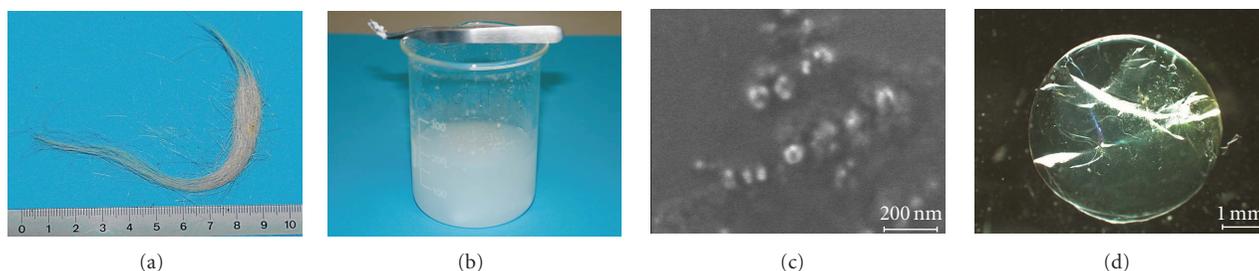


FIGURE 1: Highly flexible spicules of marine glass sponge *Rossella fibulata* (a). Pieces of clean spicules were placed in deionized water and disrupted using magnet stirrer. Visual observations show that the water solution became of a milky color typical for colloidal suspensions of silica (b). Silica nanoparticles are associated around oriented organic matrix of nanofibrillar nature (c). Transparent hard glassy spheres were obtained using this fraction of colloidal suspension after dropping into 99% TMOS solution at room temperature (d).

metazoan taxon in earth's history [18, 19]. Recently, we suggested that silica-chitin scaffolds may be key templates for skeleton formation also in ancestral unicellular organisms, rather than silica-protein composites [17]. From this point of view, we hypothesized that chitin molecules are probably part of very old organic template system involved in a biosilicification phenomenon, which was established a long time before the origin of glass sponges and collagen as structural protein with respect to high templating activity for biomineralization.

The objective of the current study was to test the hypothesis that chitin is an essential component of the silica spicules of Antarctic glass sponge *Rossella fibulata* (Figure 1(a)) as well, and if so, to unravel its involvement in the mechanical behavior of these spicules. Nanomechanical properties, nanohardness and elastic modulus, of a closely related sponge *Rossella racovitzea* were determined previously by using a vertical indentation system attached to an atomic force microscope [15]. The *Rossella* spicules, known to have optical wave conduction properties, are 10–20 cm long with a circular cross-section of diameter 200–600 μm . The spicules are composed of 2–10 μm -thick layers of siliceous material that has no detectable crystallinity. Measurements through the thickness of the spicules indicated uniform properties regardless of layering. Both the elastic modulus and nanohardness values of the spicules are about half of that of either fused silica or commercial glass optical fibers. The fracture strength and fracture energy of the spicules, determined by 3-point bend tests, are several times those of silica rods of similar diameter. The spicules offer bioinspired lessons for potential biomimetic design of optical fibers with long-term durability that could potentially be fabricated at room temperature in aqueous solutions [8]. Unfortunately, the nature and origin of organic matrix were not investigated in these pioneering studies.

We decided also to re-examine the results of some previously reported studies concerning the presence of polysaccharides within silica-containing spicules of another hexactinellid sponge. For example, Travis et al. [20] reported the presence of parallel-oriented cellulose-like filaments with an average width of 1.9 nm observed in organic matrix material after HF-based desilicification of the spicules of hexactinellid

Euplectella sp. These matrices also contain considerable amounts of hexosamine.

In this study, we performed structural, spectroscopic, and biochemical analysis of organic matrix isolated from spicules of *R. fibulata*. Finally, the present work includes a discussion relating to strategies for the practical application of silica-chitin- and silica- N-acetyl glucosamine (NAG)-based composites as biomaterials.

2. EXPERIMENTAL

2.1. Chemical etching of glass sponge skeletons

The object of our study was *Rossella fibulata* Schulze & Kirkpatrick, 1910 (Hexactinellida: Porifera), collected in 2005 in the Scotia Sea, Antarctic, at a depth of 200 m.

Spicules of *R. fibulata* were treated according to the following procedure. Sponge material of *R. fibulata* was stored for several days in fresh sea-water. The sponge was dried afterwards for 4 days at 45°C. Finally, the sponge skeleton was cleaned in 10% H_2O_2 and dried again at 45°C. Tissue-free dried sponge material was washed three times in distilled water, cut into 3 cm long pieces and placed in a solution containing purified *Clostridium histolyticum* collagenase (Sigma Aldrich, Saint Louis, USA) to digest any possible collagen contamination of exogenous nature. After incubation for 24 hours at 15°C, the pieces of glass sponge skeleton were again washed three times in distilled water, dried, and placed in a 15 mL vessel containing chitinase solution (as described below) to digest any possible exogenous chitin contaminations. After incubation for 48 hours at 25°C, fragments of skeleton were again washed, dried, and placed in 10 mL plastic vessel containing 8 mL 2.5 M NaOH solution. The vessel was covered and placed under thermostatic conditions at 37°C without shaking.

The effectiveness of the alkali etching was also monitored using optical and scanning electron microscopy (SEM) at different locations along the length of the spicular material and within the cross-sectional area. The colourless alkali-insoluble material obtained after alkali treatment of the glass sponge samples was washed with distilled water five times and finally dialysed against deionized water on Roth

(Germany) membranes with a MWCO of 14 kDa. Dialysis was performed for 5 days at 4°C. The dialyzed material was dried at room temperature and used for structural and analytical investigations.

2.2. Development of the silica nanoparticulate fraction using mechanical disruption of cleaned *R. fibulata* spicules.

The spicules of *R. fibulata* were treated using enzymes as described above, cleaned, dialyzed, and cut in 3 cm long pieces. These fragments of clean spicules were placed in deionized water and disrupted mechanically using glass covered stirring bars (25 × 6 mm) and magnet stirrer during 24 hours at 25°C. After centrifugation (1500 × g) for 5 minutes the debris of mechanically disrupted spicules was collected and milky colour supernatant was carefully decanted. Obtained debris-free suspension (Figure 1(b)) was used for SEM and TEM investigations.

2.3. FTIR spectroscopy

IR spectra were recorded with a Perkin Elmer FTIR Spectrometer Spectrum 2000, equipped with an AutoImage Microscope using the FT-IRRAS technique (Fourier transform infrared reflection absorption spectroscopy) as described previously [21].

2.4. Transmission electron microscopy (TEM)

Conventional transmission electron microscopy [1] was performed with a Philips CM200 FEG\ST Lorentz electron microscope at an acceleration voltage of 200 kV. For electron microscopy, a drop of the water suspension containing the sample was placed on the electron microscopy grid. After one minute, the excess was removed using blotting paper and thereafter dried in air. The electron microscopy grids (Plano, Germany) were covered with a holey carbon film.

2.5. Scanning electron microscopy (SEM) analysis

The samples were fixed in a sample holder and covered with carbon, or with a gold layer for 1 minute using an Edwards S150B sputter coater. The samples were then placed in an ESEM XL 30 Philips or LEO DSM 982 Gemini scanning electron microscope.

2.6. Chitinase digestion and test

Dried 20 mg samples of purified sponge spicules, previously pulverized to a fine powder in an agat mortar, were suspended in 400 µL of 0.2 M phosphate buffer at pH 6.5. Positive control was prepared by solubilizing 0.3% colloidal chitin in the same buffer. Equal amounts of 1 mg/mL of three chitinases (EC 3.2.1.14 and EC 3.2.1.30): N-acetyl-β-glucosaminidase from *Trichoderma viride* (Sigma-Aldrich) number C-8241, and two poly (1,4-β-[2-acetamido-2-deoxy-D-glucoside]) glycanohydrolases from *Serratia marcescens* (Sigma-Aldrich) number. C-7809 and *Streptomyces*

griseus (Sigma-Aldrich) number. C-6137, respectively, were suspended in 100 mM sodium phosphate buffer at pH 6.0. Digestion was started by mixing 400 µL of the samples and 400 µL of the chitinase-mix. Incubation was performed at 37°C and stopped after 114 hours by adding 400 µL of 1% NaOH, followed by boiling for 5 minutes. The effectiveness of the enzymatic degradation was monitored using optical microscopy (Zeis, Axiovert). The Morgan-Elson assay was used to quantify the N-acetylglucosamin released after chitinase treatment as described previously [22]. The sample which contains chitinase solution without substrate was used as a control.

2.7. Preparation of α-chitin

Alpha-chitin was prepared from a commercially available crab shell chitin (Fluka). The material was purified with aqueous 1 M HCl for 2 hours at 25°C and then refluxed in 2 M NaOH for 48 hours at 25°C. The resulting α-chitin was washed in deionized water by several centrifugations until neutrality was reached. The whole procedure was repeated twice. α-chitin was also used as a standard for FTIR and for Fourier transform (HRTEM) studies.

2.8. Preparation of colloidal chitin

Ten grams of α-chitin (Fluka) was mixed with 500 mL of 85% phosphoric acid and stirred for 24 hours at 4°C. The suspension was poured into 5 L of distilled water (DW) and centrifuged (15000 × g for 15 minutes). The resulting precipitate was washed with DW until the pH reached 5.0 and then neutralized by addition of 6 N NaOH. The suspension was centrifuged (15000 × g for 15 minutes) and washed with 3 L of DW for desalting. The resulting precipitate was suspended in DW and dialyzed. The chitin content in the suspension was determined by drying a sample.

2.9. Silicification of colloidal chitin and NAG

In the first step, 0.93 g of colloidal chitin or NAG (Sigma-Aldrich, Miss, USA) previously suspended in 34.6 g of methanol was added to 51.8 mL of deionized water. The suspension pH was then raised above 10 with the addition of 100 µL of 1 N NaOH solution. Finally, 169 µL of tetramethylorthosilicate (TMOS, 99 wt%, ABCR GmbH, Germany) were added and the solution was stirred at room temperature. After 1 hour the suspension was filtered and the recovered precipitate rinsed with deionized water, then with methanol, and finally air-dried.

3. RESULTS AND DISCUSSION

Most of the glass sponges inhabit soft muddy substrates. One of the strategies of survival under such conditions is the formation of root structures that prevent the body of the animal from sinking into the ground [23]. Due to their preferred deep-sea habitat, the Hexactinellida have been poorly investigated with respect to their general biology [24, 25] and the nature of organic components which build their skeletal

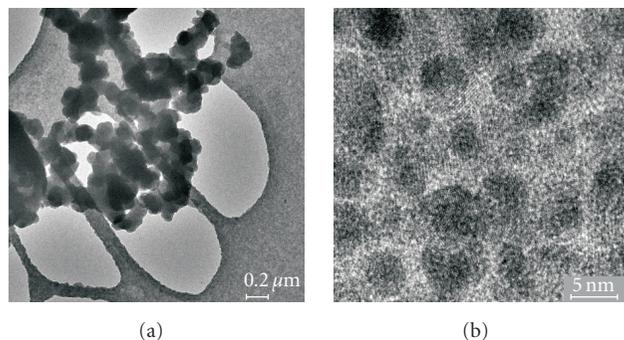


FIGURE 2: TEM images of silica-organic matrix-based suspension obtained after mechanical disruption of spicules in deionized water (a). Crystallites of organic nature are embedded in amorphous silica (b).

structures. It was generally accepted that their skeletons are composed of concentric layers of amorphous hydrated silica, containing varying amounts of organic material [26, 27] deposited around a proteinaceous axial filament [28, 29].

The finding of collagen within basal spicules of *H. sieboldi* [12, 16] and chitin in skeletons of *F. occa* [17] and in spicules of *E. aspergillum* [3], stimulated our attempts to find materials of organic nature in other species of glass sponges. In the case of *R. fibulata*, we have not observed any visible signs of demineralization of these materials using optical microscopy and SEM after 14 days and at the similar experimental conditions as in the study on *H. sieboldi* and *Monorhaphis sp.* On the contrary, spicules of *R. fibulata* show high resistance to alkali treatment even after 3 months of demineralization. This was similar the resistance observed for *E. aspergillum* [3]. This phenomenon led us to the assumption that siliceous skeletons of investigated sponges possess a material which protects amorphous silica from dissolution in alkali, and is highly resistant to alkali digestion. It is well known that chitin in alkali is stable with respect to degradation [30]. Correspondingly, in our experiments, chitin was the first candidate for a biomaterial with this property.

Initially, we performed experiments on mechanical disruption of cleaned *R. fibulata* spicules (Figure 1(a)) in deionized water as described above. This method of desintegration of spicules was very effective. Visual observations show that water solution became of a milky color typical for silica colloidal suspensions (Figure 1(b)) even after 6 hours. Debris-free suspensions obtained in this way were stable during 3-4 days. SEM of the suspensions confirmed their nanoparticulate structure (Figure 1(c)). Silica nanoparticles of diameter between 20 and 35 nm are associated around oriented organic matrix of nanofibrillar nature as shown in Figure 1(c). To verify whether this kind of silica-organic matrix obtained from the colloidal suspension mimic the biosilicification, we carried out in vitro experiments in which we exposed it to silicic acid solution derived from TMOS. We developed hard and transparent glassy spheres (Figure 1(d)) which were stable in water and in air during several months.

TEM investigation of these colloidal suspensions (Figure 2(a)) used for the development of spherical glassy

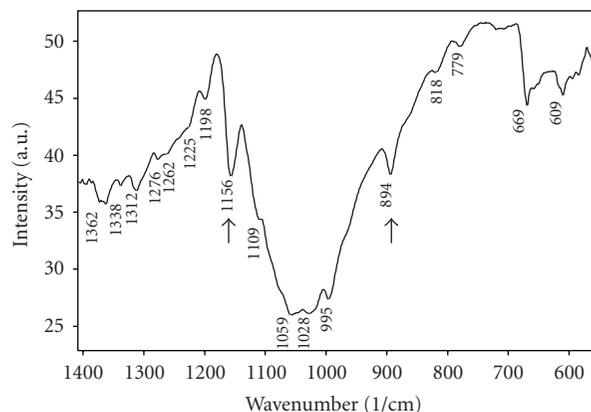


FIGURE 3: FTIR spectra of organic matrix isolated after desilicification of *R. fibulata* spicules show strong evidence for β -1, 4-glycosidic linkage at 890–896 cm^{-1} and for ether bond in pyranose ring at 1153–1157 cm^{-1} (arrows). There is no evidence for the presence of Si–O–Si bonds.

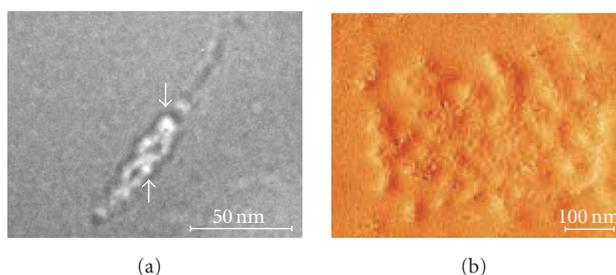


FIGURE 4: High-resolution transmission electron microscopy image of the fragment of isolated chitin nanofibril (a); the arrows indicate the presence of crystallite-like structures with a diameter which corresponds to that of chitin crystallites (2 nm). AFM micrograph of chitin nanofibrillar matrix (b).

materials clearly revealed that organic crystallites of approximately 3 nm in diameter are embedded in amorphous silica matrix. Observed HRTEM image (Figure 2(b)) is highly similar to previously reported HRTEM images of chitin nanocrystallites of the same diameter [31]. Therefore, in following experiments it was decided to isolate organic matrix from silica-containing spicules of *R. fibulata* using a desilicification procedure based on alkali treatment [16, 17].

To test our hypothesis that alkali-insoluble residues of *R. fibulata* spicules are of chitinous nature, we carried out different highly sensitive structural and biochemical analysis as described below.

FTIR observation of purified, dialysed, and dried samples of the alkali-insoluble organic matrix isolated after demineralization of *R. fibulata* spicules (Figure 3) confirmed occurrence of pyranose rings and β -1, 4 linkages, with peaks at 1156 cm^{-1} and at 894 cm^{-1} very similar to those of α -chitin. It was reported previously that the spectral feature between 1153 and 1157 cm^{-1} is mainly associated with an ether bond in a pyranose ring [32, 33]. The β -linkage was also indicated

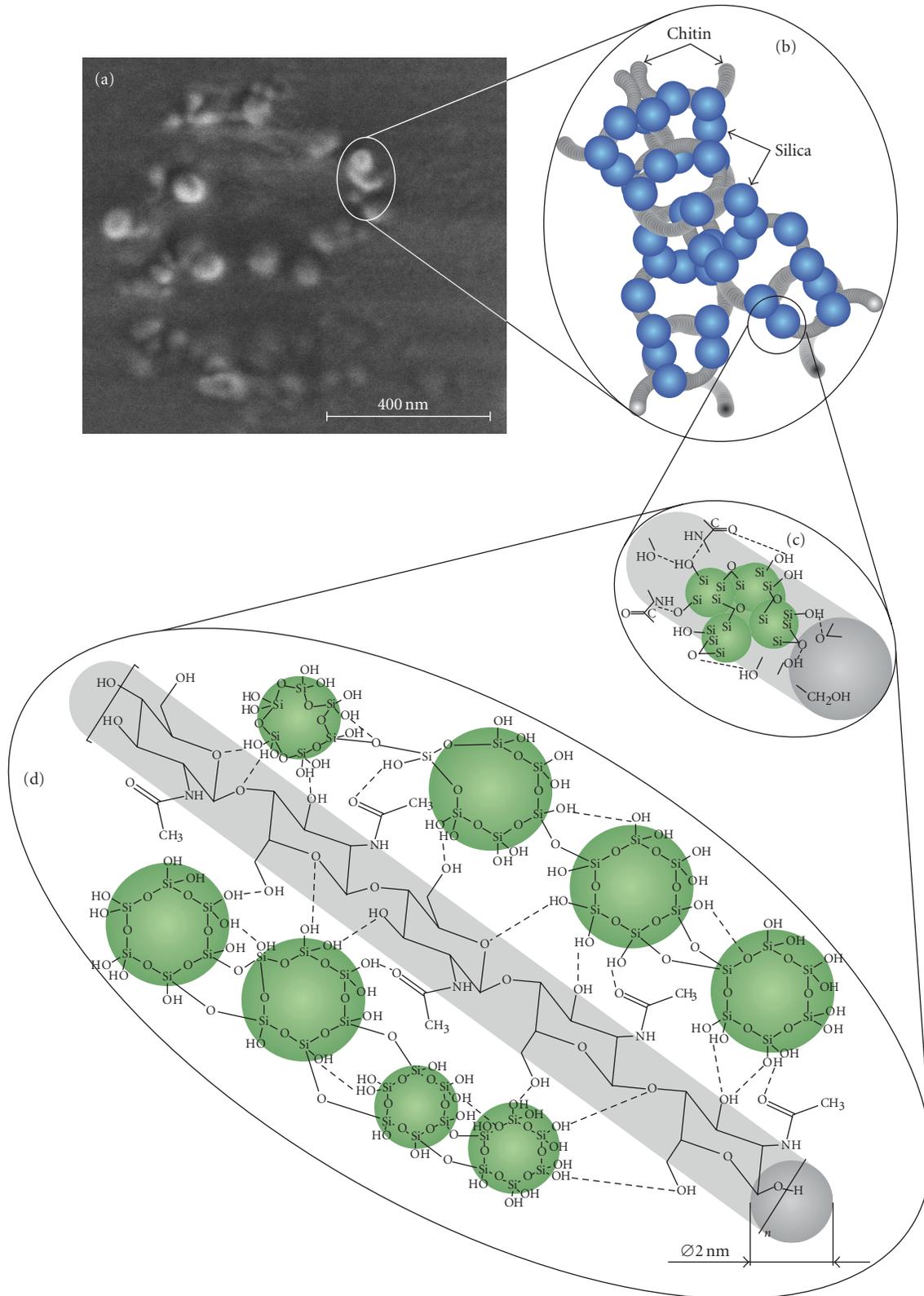


FIGURE 5: Proposed model of nanostructural organization of the naturally occurring silica-chitin composite unit (a) isolated from spicules of *R. fibulata*. Silica nanoparticles tightly surround chitinous nanofibrils (b). Schematic view (c) shows a possible nanodistribution of silica on the surface of chitinous nanofibril. Image (d) represents the hypothesized scheme of interaction between silica and poly-N-acetyl glucosamine-fragment of the chitin nanofibril and formation of the corresponding hydrogen bonds.

by the infrared absorption, in which a typical peak at $890\text{--}892\text{ cm}^{-1}$ for α -chitin was observed [34, 35].

In recent years, high-resolution electron microscopy has proved to be an important tool for analysis of the structure of fibrous crystalline polysaccharides, such as cellulose and chitin [36–39]. Therefore, the samples of organic matrix used for FTIR were subsequently submitted to HR-TEM analyses in order to examine the crystalline nature of this material and the plausible additional occurrence of chitin. HRTEM and AFM studies (Figures 4(a) and 4(b), resp.) of the organic matrix residue obtained after demineralization of *R. fibulata* spicules revealed the presence of nanocrystallites having a diameter of 2 nm. These structures were extremely similar to those previously reported by TEM observations of chitinous skeletal formations in insects, crustaceans, and arachnid species [40–42]. For further examination, high-resolution electron micrographs were taken from particular sample regions (data not shown). The Fourier transform of the high-resolution micrograph revealed a spacing of 4.79 \AA (*a*-axis), 10.2 \AA (fiber axis), 3.73 \AA , and 2.77 \AA . Such distances, corresponded to [(100) (040)], (001), [(130), (050)], and [(103), (043) (113)] reflections, proving the orthorhombic structure typical for α -chitin, as described in detail by Carlström [34] and Minke and Blackwell [42]. These measurements confirm our earlier observations [17, 21] that chitin in marine sponges appear to be consistently in the alpha modification.

To quantify chitin in our samples, we measured the amount of N-acetyl glucosamine released by chitinases using a Morgan-Elson colorimetric assay [22], which is the most reliable method for the identification of alkali-insoluble chitin because of its specificity [43]. We detected $19.2 \pm 1.5\text{ }\mu\text{g}$ N-acetyl-glucosamine per mg of spicule of *R. fibulata*.

The finding of silica-chitin natural composites as the component of the *R. fibulata* spicules is in good agreement with results of *in vitro* experiments on silicification of a β -chitin-containing cuttlebone-derived organic matrix as reported by Ogasawara et al. [44]. These authors suggest that silicate ions and silica oligomers preferentially interact with glycopyranose rings exposed at the β -chitin surface, presumably by polar and H-bonding interactions. We believe that chitin is acting as an organic template for silica mineralization in *Rossella* species in a very similar fashion as in *F. occa* [17] and *E. aspergillum* [3]. On the basis of the results presented in this work, we propose a model for the nanostructure of the naturally occurring silica-chitin composite unit, including interaction between poly-N-acetyl glucosamine-fragment of the chitin nanofibril and silica nanoparticles, which can be seen in Figure 5.

Because sponges are often regarded as the most ancient metazoans (630 to 542 My) [45, 46], the finding of chitin within skeletal formations of these organisms is of major scientific significance, since it gives important indications to the basic pattern of the Metazoa. As chitin also serves as a template for calcium carbonate deposition in sponges [21], this suggests that the evolution of mineralized skeletons in early metazoans share a common origin with respect to chitin as a unified template for biomineralization, similar to collagen as common structural protein in nature [12]. This feature

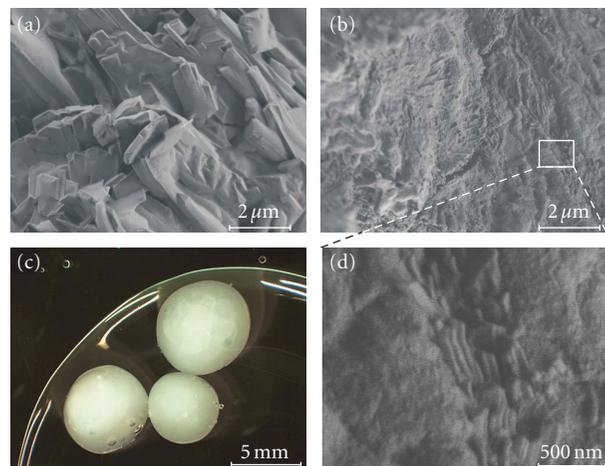


FIGURE 6: Crystals of N-acetyl glucosamine obtained from solution (a) could be also visualized using SEM even if being included into amorphous silica matrix (b). SEM image (d) revealed strong evidence that oriented crystals of NAG are observed in form of nanocrystals compactly embedded within this matrix. Light micrograph (c) of the silica-NAG spherical composites, which are highly stable in water-containing solutions.

may be considered a basic metazoan character and thus also has implications for the question of establishing the monophyletic status of the taxon Metazoa.

A comprehensive understanding of silica-chitin-based sponge skeletons with respect to chemical composition and structure may prove to be a novel model for the biomimetic synthesis also of N-acetyl glucosamine (NAG) and poly-NAG-based composites analogous to well established chitosan-silica hybrid materials [47, 48] with very attractive bioactive properties for applications in biomedicine. It was reported [49] that silicon was found to be a constituent of certain glycosaminoglycans. It was concluded that Si is present as silanolate, that is, an ether (or ester-like) derivative of orthosilicic acid, and that $R_1\text{--O--Si--O--}R_2$ bridges play a role in the structural organisation of glycosaminoglycans. Thus Si may function as a biological cross-linking agent and contribute to architecture and resilience of connective tissue [49].

To test our hypothesis that also NAG as monomer unit of poly-NAG and chitin could be used as substrate for silicification, we obtained silica-NAG-based materials in the form of rods or spheres (Figure 6) using TMOS and sol-gel techniques *in vitro* as described in Section 2. The diameter of these spheres could be varied between 2 and 10 μm . SEM investigations on micro- and nanostructural organization of silica-NAG composites revealed strong evidence that oriented nanocrystals of NAG (Figure 6(a)) could be also observed in form of nanocrystals compactly embedded within amorphous silica matrix (Figures 6(b) and 6(d)). Probably this kind of NAG nanodistribution is responsible for observed high mechanical stability and resistance of these composite materials to swelling and following dissolution in water containing solutions (Figure 6(c)). These properties could be probably of interest for technical purposes similar to

intercalated chitosan/layered silicate nanocomposites prepared to develop robust and stable sensors useful for anionic detection in aqueous media as reported on [50, 51].

We suggest that silica-chitin and silica-NAG (-poly NAG) composites could be highly optimized biocompatible structures that would support and organize functional tissues if applied in tissue engineering of bone and cartilage replacements similar to silica-chitosan-based biomaterials [52]. Experiments on biocompatibility of silica-chitin and silica-NAG composites derived in vitro are currently in progress.

4. CONCLUSION

Chitin and poly-N-acetyl glucosamine are well investigated materials of biological origin with wide fields of application in biomedicine because of their unique multifunctional engineering mechanical properties and biocompatibility [53–56]. With respect to polysaccharides, including sponge chitin, it is theoretically possible that inorganic Si binds after the macromolecular structure has been formed. An alternative, more plausible from stereochemical considerations [49], would consist in the incorporation of preformed mono- or disaccharide Si derivatives during the synthesis of the polysaccharide chain. The finding of nanostructured silica-chitin biocomposites as structural scaffolds of glass sponge skeletons introduces a new aspect into the discussion surrounding the chemistry, diversity, and nanolocalization of these materials. Chitin as a template for biomineralization probably belongs to the basic pattern of the Metazoa.

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REFERENCES

- [1] H. Ehrlich, S. Heinemann, C. Heinemann, et al., “Nanostructural organization of naturally occurring composites. Part I. Silica-collagen-based biocomposites,” *Journal of Nanomaterials*. In press.
- [2] P. Fratzl, “Biomimetic materials research: what can we really learn from nature’s structural materials?” *Journal of the Royal Society, Interface*, vol. 4, no. 15, pp. 637–642, 2007.
- [3] H. Ehrlich and H. Worch, “Sponges as natural composites: from biomimetic potential to development of new biomaterials,” in *Porifera Research-Biodiversity, Innovation & Sustainability*, M. R. Custodio, G. Lobo-Hajdu, E. Hajdu, and G. Muricy, Eds., Museu Nacional, Rio de Janeiro, Brasil, 2007.
- [4] G. Mayer, “Rigid biological systems as models for synthetic composites,” *Science*, vol. 310, no. 5751, pp. 1144–1147, 2005.
- [5] S. L. Walter, B. D. Flinn, and G. Mayer, “Mechanisms of toughening of a natural rigid composite,” *Materials Science and Engineering C*, vol. 27, no. 3, pp. 570–574, 2007.
- [6] J. Aizenberg, J. C. Weaver, M. S. Thanawala, V. C. Sundar, D. E. Morse, and P. Fratzl, “Skeleton of *Euplectella* sp.: structural hierarchy from the nanoscale to the macroscale,” *Science*, vol. 309, no. 5732, pp. 275–278, 2005.
- [7] J. C. Weaver, J. Aizenberg, G. E. Fantner, et al., “Hierarchical assembly of the siliceous skeletal lattice of the hexactinellid sponge *Euplectella aspergillum*,” *Journal of Structural Biology*, vol. 158, no. 1, pp. 93–106, 2007.
- [8] R. Cuttaneo-Vietti, G. Bavestrello, C. Cerrano, et al., “Optical fibres in an Antarctic sponge,” *Nature*, vol. 383, no. 6599, pp. 397–398, 1996.
- [9] J. Aizenberg, V. C. Sundar, A. D. Yablon, J. C. Weaver, and G. Chen, “Biological glass fibers: correlation between optical and structural properties,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 10, pp. 3358–3363, 2004.
- [10] W. E. G. Müller, K. Wendt, C. Geppert, M. Wiens, A. Reiber, and H. C. Schröder, “Novel photoreception system in sponges? Unique transmission properties of the stalk spicules from the hexactinellid *Hyalonema sieboldi*,” *Biosensors and Bioelectronics*, vol. 21, no. 7, pp. 1149–1155, 2006.
- [11] H. C. Schröder, D. Brandt, U. Schloßmacher, et al., “Enzymatic production of biosilica glass using enzymes from sponges: basic aspects and application in nanobiotechnology (material sciences and medicine),” *Naturwissenschaften*, vol. 94, no. 5, pp. 339–359, 2007.
- [12] H. Ehrlich and H. Worch, “Collagen, a huge matrix in glass-sponge flexible spicules of the meter-long *Hyalonema sieboldi*,” in *Handbook of Biomineralization Vol.1. The Biology of Biominerals Structure Formation*, E. Bäuerlein, Ed., Wiley VCH, Weinheim, Germany, 2007.
- [13] S. Heinemann, H. Ehrlich, C. Knieb, and T. Hanke, “Biomimetically inspired hybrid materials based on silicified collagen,” *International Journal of Materials Research*, vol. 98, no. 7, pp. 603–608, 2007.
- [14] S. Heinemann, C. Knieb, H. Ehrlich, et al., “A novel biomimetic hybrid material made of silicified collagen: perspectives for bone replacement,” *Advanced Engineering Materials*, vol. 9, no. 12, pp. 1061–1068, 2007.
- [15] M. Sarikaya, H. Fong, N. Sunderland, et al., “Biomimetic model of a sponge-spicular optical fiber-mechanical properties and structure,” *Journal of Materials Research*, vol. 16, no. 5, pp. 1420–1428, 2001.
- [16] H. Ehrlich, A. Ereskovsky, A. L. Drozdov, et al., “A modern approach to demineralization of spicules in glass sponges (Porifera: Hexactinellida) for the purpose of extraction and examination of the protein matrix,” *Russian Journal of Marine Biology*, vol. 32, no. 3, pp. 186–193, 2006.
- [17] H. Ehrlich, M. Krautter, T. Hanke, et al., “First evidence of the presence of chitin in skeletons of marine sponges. Part II. Glass sponges (Hexactinellida: Porifera),” *Journal of Experimental Zoology Part B*, vol. 308B, no. 4, pp. 473–483, 2007.
- [18] J. Reitner and D. Mehl, “Early Paleozoic diversification of sponges: new data and evidences,” *Geologisch Paläontologische Mitteilungen Innsbruck*, vol. 20, pp. 335–347, 1995.
- [19] J. P. Botting and N. J. Butterfield, “Reconstructing early sponge relationships by using the Burgess Shale fossil *Eiffelia globosa*, Walcott,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 5, pp. 1554–1559, 2005.
- [20] D. F. Travis, C. J. Francois, L. C. Bonar, and M. J. Glimcher, “Comparative studies of the organic matrices of invertebrate mineralized tissues,” *Journal of Ultrastructure Research*, vol. 18, no. 5–6, pp. 519–550, 1967.

- [21] H. Ehrlich, M. Maldonado, K.-D. Spindler, et al., "First evidence of chitin as a component of the skeletal fibers of marine sponges. Part I. Verongidae (Demospongia: Porifera)," *Journal of Experimental Zoology Part B*, vol. 308B, no. 4, pp. 347–356, 2007.
- [22] N. Boden, U. Sommer, and K.-D. Spindler, "Demonstration and characterization of chitinases in the *Drosophila* K_c cell line," *Insect Biochemistry*, vol. 15, no. 1, pp. 19–23, 1985.
- [23] K. Tabachnik, "Adaptation of the hexactinellid sponges to deep-sea life," in *Fossil and Recent Sponges*, J. Reitner and H. Keupp, Eds., pp. 378–386, Springer, Berlin, Germany, 1991.
- [24] H. M. Reiswig and D. Mehl, "Tissue organization of *Favosites* (Porifera, Hexactinellida)," *Zoomorphology*, vol. 110, no. 6, pp. 301–311, 1991.
- [25] S. P. Leys, G. O. Mackie, and H. M. Reiswig, "The biology of glass sponges," *Advances in Marine Biology*, vol. 52, pp. 1–145, 2007.
- [26] F. E. Schulze, "Hexactinellida," in *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898-1899*, C. Chun, Ed., vol. 4, pp. 1–266, Gustav Fischer, Jena, Germany, 1904.
- [27] C. L. De La Rocha, "Silicon isotope fractionation by marine sponges and the reconstruction of the silicon isotope composition of ancient deep water," *Geology*, vol. 31, no. 5, pp. 423–426, 2003.
- [28] J. C. Weaver and D. E. Morse, "Molecular biology of demosponge axial filaments and their roles in biosilicification," *Microscopy Research and Technique*, vol. 62, no. 4, pp. 356–367, 2003.
- [29] M. J. Uriz, X. Turon, M. A. Becerro, and G. Agell, "Siliceous spicules and skeleton frameworks in sponges: origin, diversity, ultrastructural patterns and biological functions," *Microscopy Research and Technique*, vol. 62, no. 4, pp. 279–299, 2003.
- [30] A. Einbu, S. N. Naess, A. Elgsaeter, and K. M. Varum, "Solution properties of chitin in alkali," *Biomacromolecules*, vol. 5, no. 5, pp. 2048–2054, 2004.
- [31] E. Atkins, "Conformation in polysaccharides and complex carbohydrates," *Journal of Biosciences*, vol. 8, no. 1-2, pp. 375–387, 1985.
- [32] G. Cárdenas, G. Cabrera, E. Taboada, and S. P. Miranda, "Chitin characterization by SEM, FFTIR, XRD, and ¹³C cross polarization/mass angle spinning NMR," *Journal of Applied Polymer Science*, vol. 93, no. 4, pp. 1876–1885, 2004.
- [33] J. Maitan, K. Bilikova, O. Marcovic, et al., "Isolation and characterization of chitin from bumblebee (*Bombus terrestris*)," *International Journal of Biological Macromolecules*, vol. 40, no. 3, pp. 237–241, 2007.
- [34] D. Carlström, "The crystal structure of α -chitin (poly-*N*-acetyl-D-glucosamine)," *Journal of Biophysical and Biochemical Cytology*, vol. 3, no. 5, pp. 669–683, 1957.
- [35] K. Bachmed, F. Quilès, M. Wathier, et al., "Use of dancyl *N*-acetyl glucosamine as substrate for chitin synthetase activities," *Progress Biochemistry*, vol. 40, no. 7, pp. 2523–2529, 2005.
- [36] A. Gemperle, Z. Holan, and V. Pokorny, "The Glucan-chitin complex in *saccharomyces cerevisiae*. IV. The electron diffraction of crustacean and yeast cell wall chitin," *Biopolymers*, vol. 21, no. 1, pp. 1–16, 1982.
- [37] W. Helbert and J. Sagiya, "High-resolution electron microscopy on cellulose II and α -chitin single crystals," *Cellulose*, vol. 5, no. 2, pp. 113–122, 1998.
- [38] M.-M. Giraud-Guille, H. Chanzy, and R. Vuong, "Chitin crystals in arthropod cuticles revealed by diffraction contrast transmission electron microscopy," *Journal of Structural Biology*, vol. 103, no. 3, pp. 232–240, 1990.
- [39] A. C. Neville, D. A. D. Parry, and J. Woodhead-Galloway, "The chitin crystallite in arthropod cuticle," *Journal of Cell Science*, vol. 21, no. 1, pp. 73–82, 1976.
- [40] M.-M. Giraud-Guille, "Plywood structure in nature," *Current Opinion in Solid State & Materials Science*, vol. 3, no. 3, pp. 221–227, 1998.
- [41] J. D. Goodrich and W. T. Winter, " α -chitin nanocrystals prepared from shrimp shells and their specific surface area measurement," *Biomacromolecules*, vol. 8, no. 1, pp. 252–257, 2007.
- [42] R. Minke and J. Blackwell, "The structure of α -chitin," *Journal of Molecular Biology*, vol. 120, no. 2, pp. 167–181, 1969.
- [43] C. E. Bulawa, "Genetics and molecular biology of chitin synthesis in fungi," *Annual Review of Microbiology*, vol. 47, pp. 505–534, 1993.
- [44] W. Ogasawara, W. Shenton, S. A. Davis, and S. Mann, "Template mineralization of ordered macroporous chitin-silica composites using a cuttlebone-derived organic matrix," *Chemistry of Materials*, vol. 12, no. 10, pp. 2835–2837, 2000.
- [45] M. Brasier, O. Green, and G. Shields, "Edicarian sponge spicule clusters from southwestern Mongolia and the origins of the Cambrian fauna," *Geology*, vol. 25, no. 4, pp. 303–306, 1997.
- [46] J. Reitner and D. Mehl, "Monophyly of the taxon Porifera," *Verhandlungen des naturwissenschaftlichen Vereins Hamburg*, vol. 36, pp. 5–32, 1996.
- [47] Y. A. Shchipunov, T. Y. Karpenko, A. V. Krekoten, and I. V. Postnova, "Gelling of otherwise nongelable polysaccharides," *Journal of Colloid and Interface Science*, vol. 287, no. 2, pp. 373–378, 2005.
- [48] Y. Shirosaki, K. Tsuru, S. Hayakawa, et al., "In vitro cytocompatibility of MG63 cells on chitosan-organosiloxane hybrid membranes," *Biomaterials*, vol. 26, no. 5, pp. 485–493, 2005.
- [49] K. Schwarz, "A bound form of silicon in glycosaminoglycans and polyuronides," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 70, no. 5, pp. 1608–1612, 1973.
- [50] M. Darder, M. Colilla, and E. Ruiz-Hitzky, "Biopolymer-clay nanocomposites based on chitosan intercalated in montmorillonite," *Chemistry of Materials*, vol. 15, no. 20, pp. 3774–3780, 2003.
- [51] S. S. Ray and M. Bousmina, "Biodegradable polymers and their layered silicate nanocomposites: in greening the 21st century materials world," *Progress in Materials Science*, vol. 50, no. 8, pp. 962–1079, 2005.
- [52] S. F. Wang, L. Shen, Y. J. Tong, et al., "Biopolymer chitosan/montmorillonite nanocomposites: preparation and characterization," *Polymer Degradation and Stability*, vol. 90, no. 1, pp. 123–131, 2005.
- [53] M. Rinaudo, "Chitin and chitosan: properties and applications," *Progress in Polymer Science*, vol. 31, no. 7, pp. 603–632, 2006.
- [54] J. N. Vournakis, E. R. Pariser, and S. Finkielstein, "Poly-*N*-acetyl glucosamine," US patent no. 5,623,064, 1997.
- [55] J. Vournakis, E. R. Pariser, S. Finkielstein, and M. Helton, "Biocompatible poly-beta-1, 4-*N*-acetyl glucosamine," US patent no. 6,686,342, 2004.
- [56] Q. K. Kang, C. M. Hill, M. V. Demcheva, J. Vournakis, and Y. H. An, "Poly-*N*-acetyl glucosamine-SO₄ for repairing osteochondral defect in rabbits," *Key Engineering Materials*, vol. 288-289, pp. 83–86, 2005.



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