Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Review
Volume 12, Issue 3, 2023, 64
https://doi.org/10.33263/LIANBS123.064

Iron-sponges Interrelations: from Biocorrosion to Nanostructured Biocomposites[◊]

Anita Kubiak [1,2,*], Martyna Kotula [1,2], Bartosz Leśniewski [1,2], Martyna Pajewska-Szmyt [2,*]

[1] Faculty of Chemistry, Adam Mickiewicz University, Uniwersytetu Poznańskiego 8, 61-614 Poznan, Poland; anikub@amu.edu.pl (A.K.); markot6@amu.edu.pl (M.K.); barles5@amu.edu.pl (B.L.);
[2] Center of Advanced Technology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 10, 61-614 Poznan, Poland; mpszmyt@amu.edu.pl (M. P-Sz.);
* Correspondence: anikub@amu.edu.pl (A.K.); mpszmyt@amu.edu.pl (M. P-Sz.);

[◊] Paper dedicated to Professor Hermann Ehrlich on the Occasion of His 65th Birthday.

Scopus Author ID 57223308578
Received: 11.02.2022; Accepted: 17.03.2022; Published: 15.04.2022

Abstract: Iron (Fe) is the 4th most widespread element on Earth that constitutes a crucial player in geochemistry, biological processes, and industry as metal. The study of iron biomineralization is a well-recognized interdisciplinary area that has been growing over the years. This paper presents an overview of iron-sponges interrelation, particularly from the view of nanostructured biocomposites. This review highlights the potential mechanism of iron-sponge interrelations, including biocorrosion as well as the possible role of selected amino acids and structural proteins in the mineralization process concerning the development of novel nanoorganized hybrid materials. This is the first review that describes the interrelations between iron and structural biopolymers of marine sponge skeletons to the best of our knowledge. We believe that this review will highlight new perspectives in bioinspired materials science and modern biomimetics.

Keywords: marine sponges; iron; spongin; biocorrosion; bio-fouling; biocomposites

1. Introduction

Iron is the fourth most abundant element in the Earth's crust [1] and plays a crucial role in diverse industrial directions. The annual iron and steel production is over 3,000 million tons [2]. Iron also plays an essential role in many biological processes, including biomagnetism, cellular respiration, oxygen transport, and the synthesis of deoxyribonucleic acid (DNA) [3–5]. In human metabolism, iron homeostasis is an important reaction because iron overload can harm the liver, heart, pancreas, thyroid gland, and central nervous system [6–8]. In nature, iron-based minerals and biominerals are broadly distributed. Their formation enables organisms to store iron for future metabolic needs and avoid high intracellular concentrations of ferrous iron due to forced biomineralization [9]. Other potentially useful iron biominerals characteristics are magnetism, hardness, and density [10].

The process by which inorganic ions accumulate in coordinated formations together with organic protein molecules is known as biomineralization [11]. Various forms of iron-containing biominerals, such as secondary iron sulfides and iron oxides like γ-FeOOH (lepidocrocite), α-FeOOH (goethite), Fe_2O_3 (hematite), Fe_3O_4 (magnetite), green rust (mixed-valence hydroxide), $FeCO_3$ (siderite) and $Fe_3(PO4)_2$ (vivianite) have been discovered in both pro and eukaryotic organisms, including humans [9]. These biominerals can be synthesized intracellularly [12–16] and extracellularly [17–20]. Research on iron biomineralization is a growing and interdisciplinary area of modern applied science, which encompasses biotechnology [21,22], nanotechnology [23,24], biomaterials, and biomedicine [25–27].

Seawater contains several different physicochemical fractions of dissolved iron, including Fe^{3+}, inorganic, colloidal Fe, and organically complexed iron [28–30]. It is a crucial nutrient for marine organisms [31,32]. Iron affects the nitrogen cycle [33], and the biological carbon pump [34,35] also undergoes internal transformations such as abiotic and biotic retention, recycling, and remineralization [35–38]. Marine photosynthesis is responsible for about 50% of the global CO_2 uptake [39]. Iron is supplied to the waters mainly from atmospheric sediments [40], hydrothermal vents [38,41], margin sediments [42], artificial fertilization [43], and groundwater discharges [44]. An important source of iron for marine organisms is the biocorrosion of metal constructs and shipwrecks [45,46]. Marine underwater-located metal structures are colonized by microorganisms and deposited by macroorganisms such as sponges (Figure 1) [47,48,49].

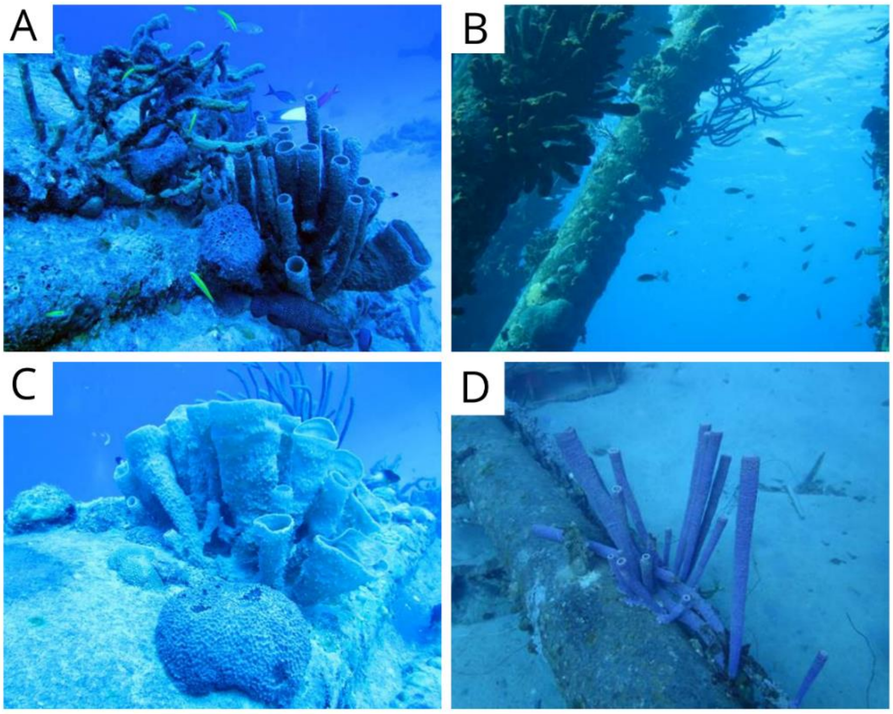

Figure 1. Diverse marine sponges growing on iron-based constructions. (**A**),(**C**): Front Porch (wreck), N 12°09.904' W 68°17.194', (**B**): Hilma Hooker (wreck), N 12°06.271' W 68°17.291', (**D**): Salt Pier N 12°05.006' W 68°16.912'. The underwater photographs were made by V.N. Ivanenko (Lomonosov Moscow State University) during The Bonaire Marine Biodiversity Expedition (2019) organized by Naturalis Biodiversity Center in Leiden, ANEMOON Foundation, and STINAPA Bonaire National Parks Foundation.

Key information on the origins of animal biomineralization came from marine sponges, which were among the first animals to build mineralized skeletons through biologically controlled processes [50]. They are mostly sessile invertebrates belonging to the phylum Porifera and one of the oldest phylogenetically multicellular organisms [51]. Based on morphological data and molecular and genetic analysis, these aquatic animals are divided into four classes: Demospongiae, Hexactinellida, Homoscleromorpha, and Calcarea. The largest class of sponges is Demospongiae and comprises about 80% of all living sponges [52]. Marine sponges present a specific skeletal structure and chemical composition, consisting of inorganic and organic parts. The mineral part is mainly calcium carbonate or amorphous noncrystalline silica [53]. The organic part consists of chitin, or spongin fibers, a collagen-like protein synthesized by spongocytes. These fibers impart flexibility to the skeletons of these organisms [54].

The structure of the sponge body is the aquifer used for pumping and filtering water for nutrition and oxygen absorption [55,56]. The filtration volume may range from 15,000 to 24,000 liters per day per kg of sponge [57,58]. They feed on organic particles, bacteria, and even viruses, trapping particles as small as 0.2 microns [57]. Trace metals, including iron, dissolved in seawater can also be selectively accumulated by sponges [59,60]. It was found that marine sponges only grow in the presence of iron ions [61], and crystalline iron mineralization among them was first noted in 1968 [62].

The relationship between biominerals and marine sponges remains an inspiration for scientists to create new nanostructured biomaterials. For the first time, this review describes the interrelations between iron and marine sponges and their use in developing new nanostructured composites.

2. The Presence of Iron in Marine Sponges

One of the subclasses of demosponges is keratosan sponges. Their mineral-depleted skeletons consist of "keratose" or "horn" fibers, which are made of proteinaceous spongin [63]. The taxonomic name Keratosa is ascribed to the group consisting of the orders Dictyoceratida and Dendroceratida [64,65]. Since ancient times, Dictyoceratida sponges have been considered "bath sponges". They are sponges with well-developed skeletons of spongy fibers hierarchically organized into primary, secondary, and sometimes tertiary fibers and constitute a significant part of the body volume [66]. A typical example is the Mediterranean bath sponge *Spongia officinalis* [67].

The first existence of crystalline iron mineralization in the phylum Porifera and the first mark of hard tissue formation among representatives of Keratosa were discovered in 1968 [62]. In the studied species (*S.officinalis, S. graminiea,* and *Ircinia fasciculata*) the endosome was brick red or reddish red. The color varied from sample to sample and was caused by highly light-refracting particles attached to the sponge skeleton fibers. The granules were spherical and varied in size from 0.5 to 2.5 μm. These particles were tightly bound and completely embedded in the spongin fibers. It has been proved that the reddish-brown granules are formed of very fine crystallites of a poorly organized lepidocrocite (γ-FeOOH) [62].

Vacelet *et al.* [68] found that lepidocrocite present in *S. officinalis* usually contains calcite and a small amount of goethite (α-FeOOH). The amount of iron varies greatly depending on the habitat and age of the spongin fibers. From the distribution of iron biominerals, conclusions were drawn about the growth and structure of the sponge skeleton: (a) mineralization is a slow process compared to fiber formation; (b) the secondary connective fibers may have discontinuous growth; (c) the collagen microfibers show a specific pattern in successive layers of sponges; (d) although the sponge is a collagen highly resistant to *in vitro* enzymatic digestion, the sponge is able to remodel its skeleton by self-digesting the fibers [68].

Meldrum and co-workers [69] identified that the organic matrix of the sponge *I. oros*, has an iron-containing precipitate as a crystalline lepidocrocite (γ-FeOOH) by electron diffraction and ^{57}Fe Mossbauer spectroscopy. The crystals were inserted in spongin fibers, indicating that the binding and subsequent aggregation of iron by macromolecules present in spongin is responsible for mineralization. It has been found that iron biomineralization does not take part in any particular structural function but could be implicated in several biological pathways such as detoxification [69,70].

Sponges filter large amounts of seawater and potentially accumulate heavy metals, including iron and other environmental pollutants. They have the characteristics of good bioindicators and are convenient tools for characterizing the state of the marine ecosystem. In the seawater, concentrations of dissolved Fe typically range from <0.2 to ∼1nmol×L^{-1} and are generally lowest at the surface [71–76]. However, in minimum oxygen zones away from coastal seas, dissolved Fe concentrations of 1-1.7 nmol×L^{-1} have been observed [77–79]. It was shown that some marine sponges from the Red Sea, Gulf of Aqaba could accumulate trace elements, including iron, in concentrations higher than in seawater [80]. Iron hyperaccumulation has also been observed in sponges from the South Brittany Coast, France [81]. Moreover, high iron concentrations have been reported in several species of sponges habituating near the island of Qeshm and Lark [82] and in the coastal waters of the Gulf of Mannar [83]. According to Bowen and Sutton [84], trace metals found in marine sponges can come from many sources: (a) microbial symbiotes, (b) sedimentation to which sponges are constantly exposed, (c) particles and bacteria filtered out by the sponge as a food source, (d) inclusion sediment and larger substrate particles through the sponge.

Iron is believed to be a crucial element in the proliferative and morphogenetic processes of perimorphs, which is the formation of multicellular aggregates from dissociated single cells of marine sponges. Based on septin gene expression and incorporation studies, it has been shown that Fe^{+3} ions are required for cell proliferation in *Suberites domuncula* perimorphs [85]. Fe^{+3} also induced ferritin expression and promoted the synthesis of a liceous skeleton structure known as spicule [86]. The finding supports this outcome that the enzymatic activity of silicate, which turns organosilicon into silicic acid, also depends on Fe^{3+}. Furthermore, the presence of Fe^{3+} is important for the expression of the scavenger receptor molecule, which is probably involved in spicule morphology [61,87]. It was also discovered that Fe^{3+} promotes the formation of osculum, which is an excretory structure of marine sponges [88].

For a better understanding of iron-related bio functions in marine organisms, Guan and co-workers [89] isolated. The iron-binding natural compounds haliclonamide A and haliclonamide B from the marine sponge *Haliclona sp*. They found that the structure of haliclonamide A consisted of novel cyclic peptides containing oxazole and methyloxazoline rings and that these two compounds formed a stable complex with Fe^{3+} but not with Fe^{2+}. Furthermore, the Fe^{3+} concentration in this sponge tissue was 10 times higher than in other tissues and 100 times higher than in seawater! This suggests that iron uptake activity by marine organisms may occur via nonsiderophore metal-binding peptides in the ocean [89].

3. Possible Mechanisms of Iron-Sponge Interrelation

Since the biomineralization of iron in marine sponges is mainly localized in spongin fibers (see above), the association of iron with proteins should be considered first. As a transition metal, iron has extremely useful ligand binding and redox properties [90]. It has the ability to accept and donate electrons, switching between ferrous readily (Fe^{3+}) and ferric (Fe^{2+}) forms [91]. These properties make iron a useful component of many crucial in nearly all physiological and cellular. Therefore, this element is essential for all living organisms.

Nevertheless, iron poses a significant challenge to the cells: the extremely low solubility of Fe^{3+} at neutral pH limits its bioavailability, and the reactivity of Fe^{2+} as a catalyst for the Fenton reaction results in the conversion of harmless H_2O_2 into the toxic hydroxyl radical ($HO^•$) [90,92]. Iron storage proteins are an integral part of iron metabolism, which is finely tuned to maintain homeostasis. In mammals, complex regulatory mechanisms have been developed to ensure adequate intestinal absorption, transport, utilization, and free toxic iron ions [7,92–95].

Literature reports have shown that spongin fibers contain amino acid sequences, including cysteine, tyrosine, histidine, or lysine [52,96]. Their presence makes the spongin surface endowed with structurally distinct functional groups (e.g., -SH, -OH, $-NH_2$, -COOH) [97]. These functional groups containing electron donor elements (O, N, S) are capable of forming complexes with ferric ions (Figure 2) [98]. There is a large group of iron-sulfur cluster proteins in which the composition of Fe-S clusters and the oxidation state of iron can vary [99–102]. In most cases, the terminal ligands attached to iron are derived from thiol groups from cysteinyl residues. Therefore, it is believed that functional groups derived from amino acids, especially cystine/cysteine sulfur, may play a key role in the iron biomineralization process [62,68]. For example, Tsurkan *et al.* [97] reduced hyperfine coupling in the EPR spectra of the obtained Spongin-Atacamite biocomposite attributed to covalent Cu-S bonds. This confirmed the stabilizing role of amino acid side chains (cysteine) between transition metal ions and spongin.

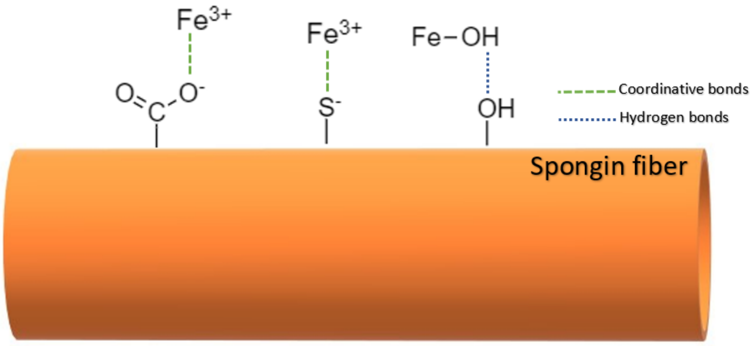

Figure 2. Proposed mechanism of iron-spongin interaction.

At a seawater pH of 8.1, Fe^{2+} is rapidly oxidized to Fe^{3+} [103]. The inorganic form of Fe^{3+} in seawater is determined by its hydrolyzing behavior and readiness to nucleate into molecular Fe^{3+} hydroxides. In general, seawater iron is present mainly in the form of iron oxyhydroxide, which has very low solubility and a thermodynamically stable $^{3+}$ oxidation state [104–106]. Under alkaline seawater conditions, the thiol groups (SH-) of cysteine are converted to thiolate anions (RS-) [107]. Then, the interaction of cysteine with noncrystalline iron(III) hydroxide involves the oxidative dehydration of cysteine, which can form disulfide bonds (S-S) to produce cysteine along with the simultaneous reduction of some of the interfacial ferric sites, thus transforming the solid phase into a mixed-valence Fe^{2+}/Fe^{3+} compound that dissolves more readily than the starting material [108,109]. The dissolution/precipitation mechanism then leads to the formation of more thermodynamically stable iron mineral phases like lepidocrocite [110–112]. Cornell and Schneider [113] have shown that with cysteine at pH 8.0, there is a rapid conversion of noncrystalline iron(III) hydroxide into mainly crystalline lepidocrocite with a small amount of goethite. These biominerals are found in the spongin-based skeletal structures of marine sponges.

4. Biocorrosion of Marine Metal Constructions as a Source of Iron

Metal corrosion is a spontaneous process that transfers electrons from a zero-valent metal to the final acceptor. This process occurs when electron transfer from an electron donation site, known as the "anode site", is directed to another "cathode site" site. We can also consider these sites as reduction and oxidation reactions, respectively, the gain or loss of electrons, in a reaction called redox. Both reactions are interdependent, and the transfer between the two sites is always from the most negative to the most positive potential. This transfer of electrons from the negative pole to the positive pole will dissolve the anode site or cause corrosion [114,115].

Corrosion in the marine environment is complicated because of different corrosion processes. Seawater is home to many marine organisms, including microorganisms, plants, and animals. A lot of material corrosions in the marine environment are related to the interactions between the materials and the marine organisms. Scientists found two main mechanisms, including microbiological corrosion and marine bio-fouling [116]. Biofouling is a complex process that first involves colonization by microorganisms such as bacteria, viruses, microalgae, protozoa, diatoms, and fungi. Its next phase is deposition by calcareous hard growth organisms such as barnacles, tubes, clams, and soft growth organisms such as sponges, algae, anemones, seaweeds, and tunicates [48].

Corrosion of metallic materials accelerated directly by the activities of microorganisms or indirectly by their metabolites is called microbiologically influenced corrosion (MIC). [117,118]. This phenomenon can be divided into two main categories. One of them concerns mainly passive metals and alloys and is associated with an increase in the metal potential under aerobic conditions and may induce pitting corrosion processes. Another relates to non-passivating materials such as copper and carbon steel and takes place under anaerobic conditions and concerns sulfide-producing microorganisms [119]. The MIC on iron-containing constructions is often caused by iron-oxidizing bacteria (IOB) and sulfate-reducing bacteria (SRB). In marine conditions, these two microorganisms accelerate the corrosion of materials through a synergistic effect. The IOB uses oxygen in the medium to create a suitable growth environment for anaerobic SRBs and then promotes matrix corrosion by the SRB. During this process, the SRB and IOB work together to form biofilms on the iron-based surface, typically consisting of corrosion products of the two bacteria, extracellular polymeric matter, and sessile cells. The entire process includes (a) an adsorbed film is formed on the iron-based surface; (b) planktonic microorganisms migrate to the surface of the metal drawn by the adsorbed film; (c) planktonic microorganisms adhere to active sites on the surface of the material and turn into sessile microorganisms; (d) sessile microorganisms grow and produce metabolites to form biofilms; (e) as the metabolites and corrosion products increase, mature and stable biofilms are formed which begin to corrode; (f) over time, the stability of the biofilms diminishes, and then some of them will fall off to form heterogeneous biofilms [116,120].

Marine bio-fouling has a huge negative impact on the infrastructure and equipment operated in the marine industry. It increases the mass and roughness of ships' hulls, which increases frictional resistance and then causes additional fuel consumption. This phenomenon also causes or accelerates corrosion of metal structures, increasing the risk of failure of ship equipment and structures [121]. Studies have provided general considerations on the formation and growth of marine biofouling that is associated with biofilm, including the following steps (Figure 3): (a) the absorbed film is rapidly formed on the submerged surface due to the adsorption of proteins, glycoproteins, and polysaccharides; (b) marine microorganisms adhere to the absorbing film and gradually transform into a biofilm, secreting extracellular polymeric substances consisting of proteins and polysaccharides into the capsule, and they become fixed; (c) microorganisms, such as diatoms, larvae and microalgae spores, accumulate on the surface of materials because the biofilm can provide them with nutrients; (d) the larvae of marine macroorganisms such as sponges settle and develop on the surface of the materials as macrofoulers [48,116].

This common formation process for fouling organisms illustrates the relationship between microorganisms and macrofouling, such as sponges. Microorganisms are a source of biofouling due to the appropriate sites and conditions they create and the nutrients they provide to attract new organisms. The actions of microorganisms on metal constructions can regulate the formation of sponges, while their accumulation can provide some protection against the elimination of microorganisms and biofilms.

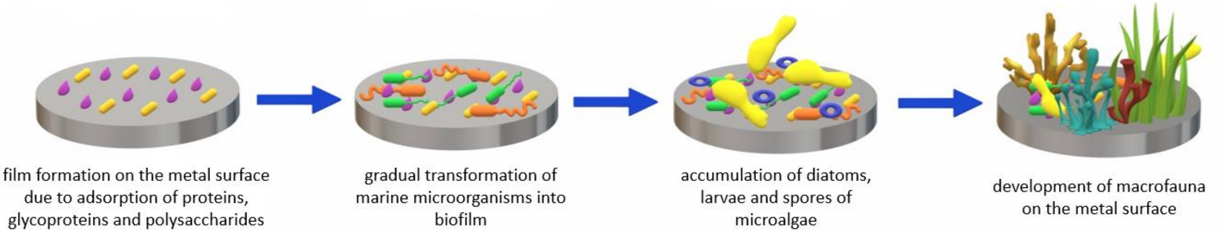

Figure 3. Process of marine bio-fouling.

It is believed [122] that the inner layer of the steel corrosion product is $Fe^{II}_4Fe^{III}_2(OH)12SO_4 \cdot 8H_2O$, mixed compound Fe (II-III) being the initial sediment formed as a result of the abiotic corrosion phase on the steel surface in the marine environment. Then this compound can be oxidized to γ-FeOOH lepidocrocite, α-FeOOH goethite, and Fe_3O_4 magnetite. The loose and porous layer of γ-FeOOH of the lepidocrocite, a mineral found in spongin, is the main corrosion product in the seawater environment, which is mainly influenced by the flow of oxygen and the moderate temperature of the environment [122–124].

5. Iron-Sponge Biocomposites

Marine sponges are recognized as one of the richest sources of new marine natural compounds and biomaterials [125–129]. One of them is naturally 3D prefabricated biomaterial spongin. The exact composition of the spongin is unknown. It is mainly composed of protein-derived collagen and contains a significant amount of cysteine and halogenated (I, Br, Cl) amino acid residues. It was also found to contain calcium, silica, and xylose [52,96,130]. This composition is responsible for mechanical strength and exceptional resistance to a wide range of proteolytic enzymes and acids [96].

Moreover, spongin is thermally stable up to 300°C, which inspired scientists to use it as an organic matrix in various hydrothermal synthesis reactions using Extreme Biomimetics [130]. This approach is based on functionalizing thermostable biomaterials with inorganic compounds under conditions simulating natural processes occurring at extremely low or extremely high temperatures, under high pressure, salinity and pH levels [130–137]. This strategy enabled the designing of the first nanostructured 3D spongin – atacamite composite [97] and obtaining new hybrid materials based on spongin fibers and metal oxides, such as MnO_2 [138] and TiO_2 [139].

Szatkowski and co-workers [140] used the three-dimensional skeleton of the fibrous bath sponge of the Mediterranean sponge *H. communis* for *in vitro* hydrothermal synthesis of hematite using $FeCl_3$ under difficult conditions (90 ^{o}C, pH 2) according to the Extreme Biomimetics principles for the synthesis of the α-Fe_2O_3-spongin composite. The α-Fe_2O_3 sponge-based composite has been tested for potential use as an anode material in a condenser. The results show that the components constructed with this novel composite material positively affect the capacity of energy storage devices. Based on the obtained results, a probable mechanism for forming interactions between α-Fe_2O_3 nanoparticles and spongin fibers was also proposed (Figure 4). The mechanism of the composite formation would involve the adsorption of nucleation sites on the surface of the fibers, followed by the formation of bonds between Fe^{+3} and oxygen atoms through hydrogen and covalent bonds and the probable chelation effect. The hematite particles may also be bound to cysteine residues' sulfur moiety [140].

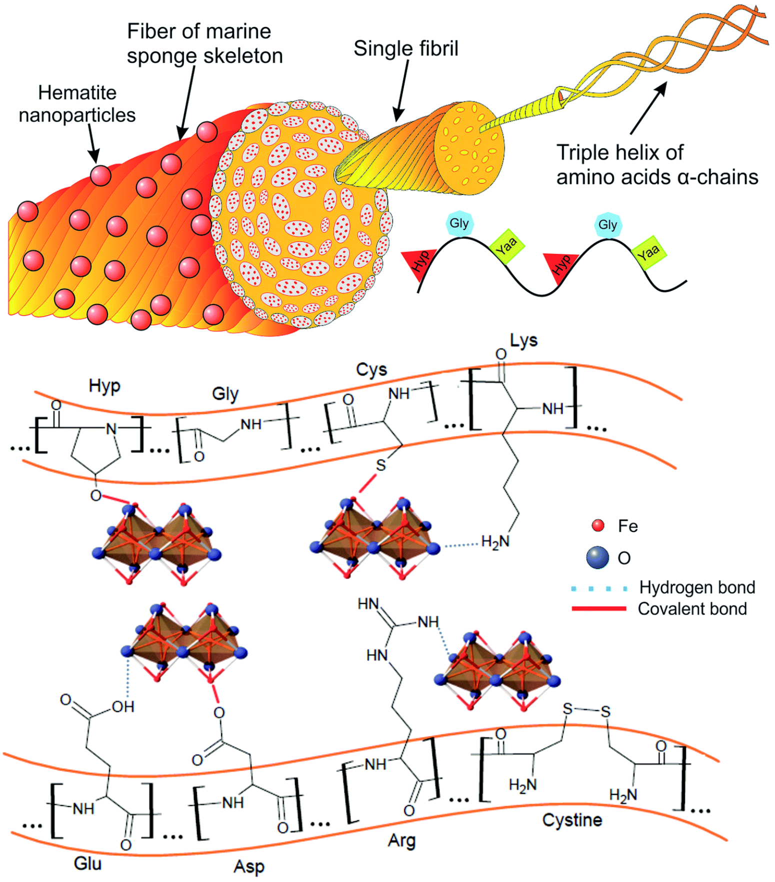

Figure 4. The probable interaction mechanism between α-Fe_2O_3 and spongin from *H. communis* after hydrothermal synthesis at 90°C. Reprinted from [140] (licensed under CC BY-NC 3.0).

Another composite material is a magnetic three-dimensional spongin scaffold with Fe_3O_4 nanocore developed by Akbari and co-workers [141]. The creation of well-dispersed spherical nanoparticles strictly associated with the spongin scaffold was confirmed by scanning field emission electron microscopy (FE-SEM). Composite showed high efficiency in removing two cationic dyes of crystal violet and methylene blue. Magnetic spongin exhibits absorption capacity. Consequently, it can be used as an eco-friendly and simple adsorbent for the elimination of various organic impurities and as a carrier in drug delivery [141].

Several species of Demospongiae contain structural amino polysaccharide chitin and not spongin as the main skeletal component [142–148]. Chitin from marine sponges occurs as ordered crystal nanofibrils that form the structural components in the sponge skeleton of representatives of the Verongiida order [149–152]. It is renewable, bioactive, biodegradable as well as thermostable up to 400°C [153–156]. According to the Extreme Biomimetic approach, these properties can be used to create new, biocompatible organic-inorganic hybrid materials. Thus, for the first time, Wysokowski and co-workers [157] obtained three-dimensional chitin-Fe_2O_3 biocomposites by hydrothermal synthesis. For this purpose, chitin scaffolds extracted from the skeleton of the marine sponge *Aplysina aerophoba* served as a framework for *in vitro* creating iron oxide from a saturated solution of iron (III) chloride under hydrothermal conditions (pH~1.5, 90°C). The obtained nanostructured composite was characterized by light, fluorescence and scanning electron microscopy, electron diffraction, Raman spectroscopy, and HR-TEM. The results show that the deposition of the mineral phase occurs both on the surface of chitin fibers and inside chitin microtubules, which allows for efficient mineralization of chitin with respect to iron oxide.

6. Conclusions

The chemistry and structural biology of skeletal constructs in marine sponges remain to be very complex and not yet fully discovered, including diverse aspects of iron-sponges interrelations. The higher concentration of iron ions in the water, caused by the biocorrosion of metal-based constructs, can significantly affect the living marine sponge and the formation of unique iron-based mineral phases. As it turns out, iron ions take part in biomineralization, through which the lepidocrocite is formed on the organic part of the sponges' three-dimensional skeletons - the spongin. The mechanism of this process is not fully understood yet. This combination inspired scientists to create nanostructured iron-spongin biocomposites that could potentially be used as pollutant adsorbents, drug carriers, and anode material. Nevertheless, it is a very new topic, and it is worth taking a closer look at it due to the unique properties of spongin and iron. It would also be necessary to solve the mystery of the enigmatic nature of spongin, which has not been solved for 300 years.

Funding

This research was funded by the National Science Centre within the framework of the project Maestro 12 No. 2020/38/A/ST5/00151.

Acknowledgments

We cordially thank Dr. Viatcheslav Ivanenko from Lomonosov State University in Moscow (RF) for unique underwater images of selected sponges.

Conflicts of Interest

The authors declare no conflict of interest.

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