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# Preparation of monolithic silica–chitin composite under extreme biomimetic conditions

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ABSTRACT

Chitin is a widespread renewable biopolymer that is extensively distributed in the natural world. The high thermal stability of chitin provides an opportunity to develop novel inorganic–organic composites under hydrothermal synthesis conditions *in vitro*. For the first time, in this work we prepared monolithic silica–chitin composite under extreme biomimetic conditions (80 °C and pH 1.5) using three dimensional chitinous matrices isolated from the marine sponge *Aplysina cauliformis*. The resulting material was studied using light and fluorescence microscopy, scanning electron microscopy, Fourier transform infrared spectroscopy. A mechanism for the silica–chitin interaction after exposure to these hydrothermal conditions is proposed and discussed.



## 1. Introduction

The term “Extreme Biomimetics” was first proposed by Hermann Ehrlich in 2010 [1] as a new pathway for bioinspired materials science. In contrast to traditional aspects of biomimetic synthesis of biocomposites, Extreme Biomimetics is based on metallization and mineralization of specific biomolecules [2] under conditions which mimic extreme aquatic niches like hydrothermal vents, geothermal pipelines or hot springs (see for review [3–7]). Therefore, the basic principle of this concept is to use biopolymers, which are chemically and thermally stable under these specific conditions *in vitro*. Biomacromolecules of thermophilic microbial origin [5] as well as chitin are the best candidates for selection and application of such biopolymers as templates and scaffolds. They have already seen use in the hydrothermal synthesis of metal oxides [8–11] as well as silica [12].

Chitin is a renewable biopolymer, which was originally found within cell walls of yeast, fungi, and diatoms. It is the main structural component in skeletons of arthropods, worms, and some corals and sponges (for review see [1,13,14]). Moreover, chitin is the template for biomineralogical formation of biocomposites like chitin–silica [15] and aragonite–chitin–silica [16] structures in numerous invertebrates [14]. Additionally, chitin is stable at temperatures up to 400 °C [17–20]. This property is the key factor for the *in vitro* development of novel chitin-based composites at high temperatures according to the concepts laid out by Extreme Biomimetics.

Silica precipitation is a widespread and important process in many hot spring systems in which the flow of hydrothermal fluids reduce the formation of heterogeneously amorphous silica masses, and both mineralization and fossilization of different kind of organisms can occur [6]. Therefore, we decided to use chitin isolated from the marine sponge *Aplysina cauliformis* [13,16] as a model template for the *in vitro* silicification at conditions corresponding to extreme natural environments like those seen in hot springs (80 °C and pH 1.5) [1,21]. The tube-like structure and 3D framework of sponge

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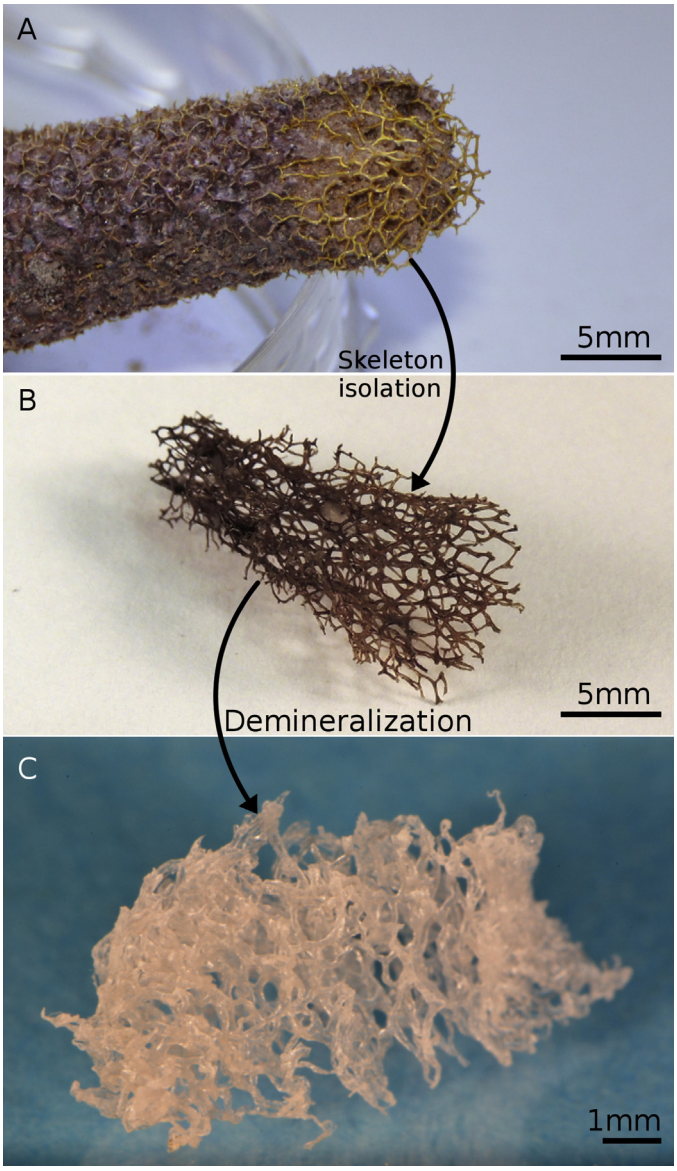


**Fig. 1.** Dried fragment of marine sponge *A. cauliformis* (A), is a prime source for obtaining the 3D skeletal structures (B), which, consequently, are source of colourless chitinous tube-like frameworks isolated after the alternating acid- and alkaline-based treatment (C).

chitin makes it particularly well-suited for the development of scaffolds with high potential for applications in tissue engineering (for review see [13,16,22]).

## 2. Materials and methods

### 2.1. Isolation of chitin-based scaffolds from A. cauliformis sponge

The sponge *A. cauliformis* (Verongida: Demospongiae: Porifera) was purchased from INTIB GmbH (Germany).

Chitin was isolated from dried sponges (Fig. 1A) using chemical treatment. To remove other compounds from the chitin, the sample underwent a series of extraction steps as described previously [16]. Briefly, the procedure includes step-by-step treatment as follows: an acidic extraction, an alkali-based extraction, an optional hydrogen peroxide treatment, and washing steps using distilled water before and after each treatment step.

Step 1: The samples were washed with distilled water at 37 °C for 24 h. This resulted in the extraction of all water-soluble substances including several pigments. Lysis of the sponge cells was also caused by this step of the treatment.

Step 2: Acidic extraction at 37 °C involved sample treatment with an acid solution in order to degrade possible calcium carbonate containing constituents and to remove acid-soluble proteins and pigments. The samples were treated in 20% acetic acid with stirring for 24 h. The remaining 3D fibrous sponge skeleton (Fig. 1B) was neutralized and subjected to further treatment steps.

Step 3: Alkali-based extraction at 37 °C involved sample treatment with a solution of 2.5 M NaOH in order to degrade and remove the sponge lipids and proteins as well as to eventually remove residual silica and pigments. Alkali treatment was performed for 24 h under stirring. The remaining three-dimensional scaffolds consisting of a fibrous skeletal material were neutralized. The procedure listed above was repeated until a colourless fibrous material remained (Fig. 1C). Hydrogen peroxide (35%) treatment can optionally be performed at room temperature with stirring for 15 min in order to degrade residual pigments. After $H_2O_2$ treatment, the residual three-dimensional fibrous sponge skeletal material was washed using distilled water and stored at 4 °C.

### 2.2. Hydrothermal silicification of the sponge chitin scaffold

In the first step, 0.93 g of *A. cauliformis* chitinous matrix samples was placed in 10 ml plastic test-tubes with 5 ml of deionized water. The pH was then set to 1.5 with the addition of 1 M HCl solution. Finally, 50 μl of tetramethylorthosilicate (TMOS, 99 wt%, ABCR GmbH, Germany) was added. The pH was corrected to 1.5 by adjusting the HCl concentration, and the solution was stirred at 80 °C in a thermostat for 7 days.

After silicification under these conditions the solution was removed and the biomineralized scaffold was rinsed five times with deionized water. To remove silica nanoparticles not attached to the surface of chitin, the samples were washed in an ultrasound bath (Elmasonic GmbH, Germany) at room temperature for 1 h, and finally air-dried. As a control, chitinous scaffolds were also prepared within the same reaction system without the presence of any silica precursors.

### 2.3. Staining of chitin

To elucidate the particular location of chitin in the silicified scaffolds, we used Calcofluor White (Fluorescent Brightener M2R, Sigma, Germany) which shows enhanced fluorescence when binding to polysaccharides, such as chitin [23,24]. Pieces of silicified scaffold were placed in 0.1 M Tris–HCl at pH 8.5 for 5 min, then stained using 0.1% Calcofluor White solution for 30 min in darkness, rinsed three times with distilled water, dried at room temperature, and finally observed using Digital Microscope Keyence BZ-9000 (Japan) with three filters. The exposure time used for sample analysis 1/250 s.

### 2.4. Scanning electron microscopy (SEM)

The selected samples were fixed in a sample holder and covered with carbon for 1 min using an Edwards S150B sputter coater. The samples were studied using an ESEM XL 30 Philips or LEO DSM 982 Gemini scanning electron microscope.

### 2.5. Fourier transform infrared (FTIR) spectroscopy

IR spectra were recorded with a Perkin Elmer FTIR spectrometer Spectrum 2000, equipped with an AutoImage Microscope using