Characterization of Spicule Structure

Fariborz Tavangarian, Jennifer L. Gray, Trevor Clark, and Chao Gao

Abstract Nature has been a great source of inspiration for engineers and scientists for centuries. It provides unique ideas to overcome the unmet needs of human beings. Spicules are structural elements of *Euplectella Aspergillum* sponges that reside in the deep ocean. They have an exceptional microstructure that provides excellent mechanical properties. Although spicules are composed of a brittle material, silica (SiO_2), they behave differently under load compared to other ceramics. This behavior is due to their concentric cylindrical structure. To produce a similar structure with potential engineering and biomedical applications, one needs to investigate its microstructure in depth. In this study, we examined the microstructure of spicules to understand their architecture as a foundation to better design biomedical implants for tissue engineering applications.

Keywords Spicules · Mechanical properties · Tissue engineering · Bio-inspiration · Implants

Introduction

Many of the improvements and innovations in technology that help to improve our way of life come from observing and mimicking nature. Processes, functions, and designs in nature have stood the test of time and continue to perform optimally in trying scenarios, making them ideal for implementing into our everyday lives.

F. Tavangarian (✉)
Mechanical Engineering Program, School of Science, Engineering and Technology, Pennsylvania State University, Harrisburg, Middletown, PA 17057, USA
e-mail: fut16@psu.edu; f.tavangarian@gmail.com

J. L. Gray · T. Clark
Materials Research Institute, The Pennsylvania State University, University Park, PA 16802, USA

C. Gao
Department of Mechanical and Industrial Engineering, Norwegian University of Science and Technology, 7034, Trondheim, Norway

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One particularly intriguing design is found in marine sponges. Studies have shown siliceous sponges are incredibly strong and durable under load, and have credited this to their unique structure on the micron and nanoscale. The building blocks of the sponge skeleton are comprised of small silica-based scaffolding called spicules, and collagen [1]. The siliceous sponges are not the only sponge family which contains spicules, rather there is also a classification of sponges that contain calcareous spicules. However, these spicules are more glasslike in nature and do not have the unique structural attributes of the former. The location of spicule formation in the sponge skeleton is largely attributed to genetic influence, giving different sponge classifications their own unique shape [2].

Two different types of sponges produce spicules derived from silica, the *Demospongiae* and the *Hexactinellida.* The main differences between the two are the orientation and pattern of the spicules as well as the cellular makeup [3]. The traits of both types of spicules produce a strong foundation that enables the sponge to survive the oceanic pressure and maintain an upward direction when growing. This is critical as it lets the sponge successfully move more water through the body, a process essential for survival. Spicules are tested very favorably using the Brillouin Scattering method, and the same test is used to evasively test the strength of spider webs [4]. The unique capacity for stress these spicules possess is credited to their structure. The anatomy of a spicule is organic at the origin but the majority of the structure is an inorganic material. The siliceous spicule is protected by a collagen sheath, forming a sort of net around the outermost part of the silica-based layers. The purpose of this collagen is to facilitate the formation of the lamellae layers and keep the overall shape of the spicule cylindrical [5]. The following segment of the spicule is a hard, rigid section comprised of many circular layers stacked on top of each other with the axial canal at the center [1]. These layers are strong and brittle, and make up the majority of the spicule's diameter. The axial cylinder is the next component and it is made of a dense mass of silica [1]. This is the part of the spicule that the significant elasticity originates from. The silica here is somewhat flexible, allowing the spicule to absorb impact if necessary. This softer cylinder is complemented by the rigid outer section resulting in a structural element that is both strong and durable. The axial filament is the final region of the siliceous spicule, located at the center of the spicule in the axial canal, and it is made of proteins called silicateins. These silica-based proteins are the brains of the spicule, responsible for using the silicate in the water around them. This organic core then converts it into silica to deposit into a spicule layer [5].

The combination of a flexible core and a rigid strong outer layer makes these spicules structurally intriguing. A better understanding of the microstructure of the spicules will help us to improve our knowledge and apply it toward new structures from brittle materials with improved performance under load-bearing applications. In this paper, we study the structure of spicules using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to investigate the cross section of spicules and inspect how organic/inorganic layers come in contact with each other. Epoxy was used to fix the sponges in place and to generate cross sections. The samples were sanded and polished to generate a clean surface for microscopic studies. To prepare very thin layers for TEM, focused ion beam (FIB) was used. The results can be used to produce similar structures from brittle materials with unexpected mechanical properties similar to spicules.

Materials and Methods

Sample Preparation

Euplectella Aspergillum sponges have been cut into small sections and then put into circular silicone molds with a height and diameter of 25 mm. Two-part epoxy (Allied High Tech Products 145-20,005 as resin and 145-20,010 as hardener) was poured into the molds and put aside until solid samples were obtained. Then the samples with a height of 10–15 mm were prepared using a slicing saw (TechCut 5, Allied High Tech Products, USA). The surface of each sample was sanded using SiC grits from 120 down to 1200 and then polished with 0.05 μm colloidal silica (Allied High Tech Products, Lot: 017,543/CR, USA) to make sure a smooth sample is prepared for microscopic evaluation. Some specimens were etched using ammonium hydroxide and hydrogen peroxide and deionized (DI) water (1:1:5) followed by a quick hydrofluoric acid (HF): DI water (1:50) for 1 min. To prepare a sample for TEM evaluation, first, a scaffold of the sponge was cut and then place fixed on top of a stub using a hot glue. Then one strand of the sponge was selected and milled using focused ion beam (FIB).

Sample Characterization

Scanning electron microscopy (SEM) was utilized to investigate the microstructure as well as the morphology of the samples. For this purpose, FEI Helios Nanolab 660 (Hillsboro, OR, USA) and Apreo S (ThermoFisher Scientific) were used with an acceleration voltage of up to 20 kV. To prepare a thin slice of the cross section of spicules, focused ion beam (FIB, ThermoFisher Scientific, Scios 2, USA) was used. A strand of spicule was selected and then milled by FIB to generate the required specimen for TEM analysis. Transmission electron microscopy (TEM, ThermoFisher Scientific, Talos F200c, USA) was utilized to investigate the nanostructure and elemental analysis of the samples.

Results and Discussion

Figure 1 shows a *Euplectella Aspergillum* sponge with the microstructure of spicules in the base and body of the sponge. As seen, the strands (fiber) on the body of the sponge are composed of concentric cylindrical structure (Fig. 1b). However, in the base, they are composed of several spiculic structures covered with a shield (Fig. 1c). The high strength of spicules reported in the literature can be ascribed to their layered structure [6, 7]. When a crack is initiated on the surface of the outer layer of spicule, it can only propagate to its interface with the next layer. When the crack reaches the interface, it stops as the next layer is discrete and separated from the outer layer. The interface between the two layers arrests the crack and prevents it from further propagation. To initiate a new crack, higher stress is required. Consequently, the layered structure provides higher strength compared to a solid rod. Also, crack deviation is another mechanism that may play a role in preventing crack development and hence increase the strength of the spicules. Similar mechanisms have been observed in other naturally occurring structures in nature such as what has been reported in abalone nacre [8, 9].

To study the layered structure, the cross sections of spicules were investigated by SEM before and after etching. Figure 2 shows the cross sections of the samples before and after the etching process. As can be seen, the samples are composed of concentric cylindrical structures. This layered structure can prevent the progress of the cracks from one layer to the next and prevent catastrophic failure in spicule structure. The concentric cylindrical structure is not very clear in those samples before etching (Fig. 2a). However, after the etching process, the concentric cylinders can be distinguished from each other due to the removal of the organic materials in between the layers and consequently the higher contrast between the layers and the interfaces.

To investigate the morphology and the existing elements in the samples, a thin layer of the cross section was milled by FIB and then EDS analysis was performed. Figure 3 shows the TEM images as well as the elemental analysis of the sample. The yellow arrows show the organic material in between the silica layers. As seen, the organic material is mostly composed of Na and K but the inorganic portion of the structures which are the cylindrical layers are composed of silica. Exposure of the samples to an electron beam for a longer period of time caused damage to the structure and

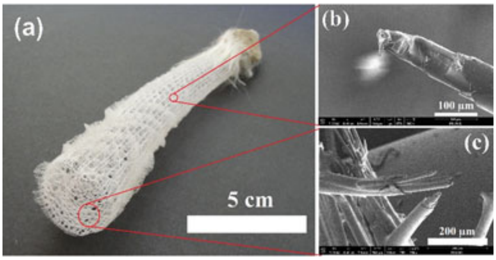

Fig. 1 **a** Image of *Euplectella Aspergillum* sponge, **b** the microstructure of a spicule, as seen, consisted of concentric cylindrical structure, and **c** several spicules are covered in a shield at the base

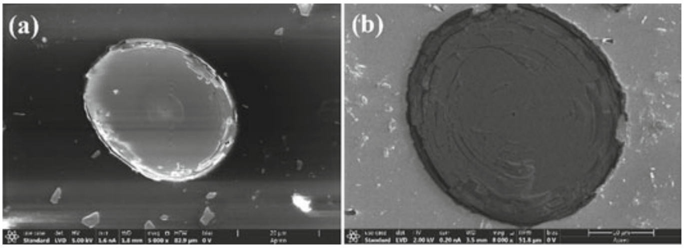

Fig. 2 The cross-sectional area of a typical spicule **a** before and **b** after etching

changed the morphology at the interface between the inorganic and organic materials (the pic is not shown here). A cryo microscope TEM with a direct electron detector should be used for imaging at much lower doses than in a conventional microscope. It is equipped with a K2 direct electron detector for very low-dose TEM imaging of beam-sensitive samples. In addition, it can also be equipped with a Gatan Quantum GIF with EELS/EFTEM (Electron Energy Loss Spectroscopy/Energy Filtered TEM) capabilities. Using this GIF allows the scientists to collect elemental maps on the K2 direct electron detector in EFTEM mode or collect EELS spectra on the K2 using the GIF which will allow elemental mapping in STEM mode. The ability to use the K2 for both imaging as well as elemental mapping will allow characterization of the sample structure as well as elemental distributions, at lower electron doses than would otherwise be possible, therefore minimizing beam damage. In future studies, cryo microscope should be used to better study and analyze the spicule samples.

Conclusions

Recently, spicules have received much interest due to their unique architecture. They show excellent mechanical properties although they are mainly composed of silica (SiO_2) which is a brittle material. Studies have proved that spicule-inspired structures with a concentric cylindrical architecture do not break catastrophically. This behavior is due to the unique structure of spicules. As shown in this study, spicules are composed of concentric cylinders with organic materials in between the layers. The layers are mainly composed of SiO_2 and the organic layers in between them consist of Na and K. Exposure of the samples to an electron beam for a long time damaged the samples and changed the morphology of the structure. To better analyze the specimens, one should use cryo TEM to decrease the exposure dose and prevent structural changes. Further studies are required to better understand the interface of organic/inorganic materials and the morphology of the interface between silica and organic materials. Inspiring by this structure can open up a new venue for designing novel structures with potential applications in biomedical implants, automotive, building, and aerospace industries.

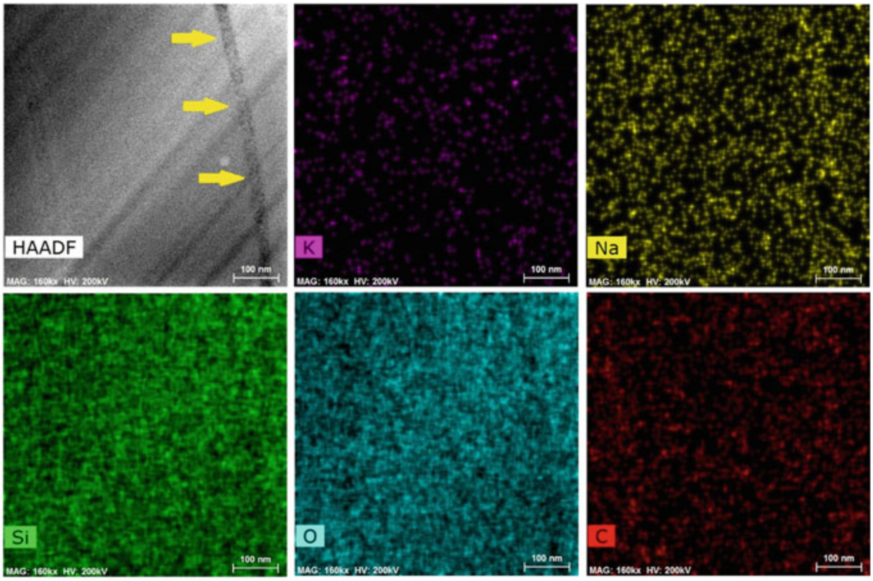

Fig. 3 High-angle annular dark field (HAADF) image of spicule along with the elemental analysis of the sample

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