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Nanocharacterization of Bio-Silica Using Atomic Force and Ultrasonic Force Microscopy

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Abstract: Nanotechnology has become central to our research efforts to fabricate relatively smaller size devices, which are more versatile than their older and larger predecessors. Silica is a very important material in this regard. Recently, a new biomimetically inspired path to silica production has been demonstrated. This processing technique was inspired from biological organisms, such as marine diatoms, which produce silica at ambient conditions and almost neutral ph with beautiful control over location and structure. Recently, several researchers have demonstrated that positional control of silica formed could be achieved by application of an electric field to locate charged enzymes responsible for the bio catalytic condensation of silica from solution. Secondly, chemical and physical controls of silica structural morphology were achievable. Atomic Force Microscopy (AFM) and Ultrasonic Force Microscopy (UFM) techniques are employed for the first time to provide both substantially improved resolution of the morphology and relative measurement of the modulus of elasticity of the structures. In particular, these measurements reveal the positive impact of a shear flow field present during the silica formation on both the "ordering" of the structure and the mechanical properties.

1. INTRODUCTION

There has been a dramatic increase in research activities involving biomineralization and biomimetic synthesis of silica over the last few years. Biotechnology has started to reveal the proteins, genes and molecular mechanisms that control the biomimetic synthesis of silica in marine organisms and biologists like Nils Kroger have started to isolate the genes and proteins responsible for biosynthesis and nanofabrication [1]. The discovery of this interface between inorganic world of silicon and the biopolymers that control this biosynthesis includes dynamic catalysts related to well known enzymes and offers prospects for structural and positional control at nanoscales that were previously unimagined.

Among the most interesting materials are those with functionalized biomolecular interfaces, which include receptor proteins. These could form the basis for developing advanced materials that serve as chemical and biological sensors [2]. Other areas of interest where these materials can be used include technological areas as diverse as molecular electronics and optical switch applications [3] and the lithographic fabrication of nanometer scale patterns [4]. In many cases materials have uses in biomedical applications, for example, in tissue engineering [5-7] or in developing self assembled drug and gene delivery vehicles [8]. Another very important area where bio-silica can be used is that of nanocomposites.

Silica is a very important material for nanofabrication and structural and positional control over its morphology at nanoscales will go a long way to serve the various applications discussed above. Biosilicates are increasingly being explored as inexpensive precursors for silicon-containing materials. For example, ancient diatom shells in the form of diatomaceous earth are used as silicon sources for zeolites [9] as well as silicon nitride [10]. Similarly, many other nature inspired efforts have been made to produce silicon-containing materials at ambient conditions. The main impetus has been on identifying various proteins, peptides and polysaccharides that direct the growth of silica at nanoscales in nature and specifically in diatoms and other marine organisms. The major objective of the present research is to characterize the biomimetically-produced silica employing AFM/UFM techniques.

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2. EXPERIMENTAL METHODS

Many researchers studying biosilification using peptides have observed formation of silica nano-spheres using a 19 amino acid R5 unit peptide [SSKKSGSYSGSKGSKRRIL] [11]. We have shown in the present research that similar results are observed using other peptides such as lysine. The primary reaction in this form of in vitro process of biosilification is:

$$Si(OH)_4 \rightarrow SiO_2 + 2H_2O$$

This reaction is carried out in the presence of either R5 or lysine or any other peptide, which can direct biosilification process. Following experimental methods were employed to characterize the bio-silica.

2.1 Static Test

In a static test different constituents are simply mixed and no electric or physical field, like a shear flow field, is applied. We mixed in a vial, 50 µl of the phosphate base, 5 µl of the tetramethoxy silane (TMOS) solution, and 5 µl of 50 mM the R5 solution. This mixture was stirred slowly for two minutes. Silica precipitates out and it can be clearly seen as a white precipitate. This resulted in the formation of silica spheres of roughly 500nm diameter [12]. To analyze this biomimetically produced silica, we take a drop of this precipitate in a pipette and put the drop on a microscopic glass slide and let it dry. After it has dried up we can clearly see the white silica powder on the slide. The silica nano-spheres are observed using an atomic force microscope.

2.2 Hydrodynamic tests

Three methods were employed to observe the effects of shear flow fields on the structural morphology of the silica being produced biomimetically and the details are given in Reference 13.

Method 1: The biomimetic process was carried out in a capillary tube where the reaction solution was moved back and forth inside the capillary using controlled amount of air to produce bubbles. The bubbles create micro-channels amongst themselves and cause the reaction solution to experience shear flow fields as the bubbles move relative to one another. This method, even though demonstrated the importance of shear flow fields, is very crude as bubble formation is not well-controllable.

Method 2: This method was employed to subject the silica processing to shear fields while it was being processed. The set-up consisted of a round Teflon disc with a circular groove cut into it. The groove was about 50 microns wide and 50 microns deep. The dimensions of the groove are important, as they are a controlling factor for the shear stress field being generated when the disc is rotated. The disc was rotated at varying rpms using the variable dc supply so as to subject the reaction solution to varying shear stresses. The silica produced was very carefully collected from the groove using a pipette. The silica collected from the groove was analyzed using first an optical and then the atomic force microscope. This method was not very effective as much of the reaction solution was lost at higher rpms due to centrifugal forces.

Method 3: The third method involved the use of a polyphenylene shaft rotating inside a stationary annular casing made of similar material with a clearance of 15 microns between the shaft and the casing. This approach is a much better method than the previous ones as it gives us more control over the flow fields, in other words the shear flow fields generated in this case are much more uniform and much easier to quantify. The shear stress on the liquid reaction film can be approximated as

$$\tau = \mu \, r_{shaft} \, \omega / w$$

Where 'w' is the thickness of the liquid film, which is equivalent to the clearance between shaft and casing (15 microns) in our case and ω is the angular velocity. Except at the ends, the shear rate is nearly uniform. By controlling the rpm of the shaft, the shear rate can be controlled with good accuracy.

2.3 Electro-static and Electro-hydrodynamic tests:

Electric fields were applied to achieve control, particularly the positional control that is lacking in other techniques, of the silica. For this purpose a cylindrical gold plated copper electrode with a diameter of 1mm was employed. The R5 enzyme is a cationic peptide and it can be moved under the effect of an electric field. R5 is approximately 71% by-mass polar and cationic in nature. The presence of an electrostatic field will cause the R5 to migrate towards the lower potential. Hence by applying electric field to the electrode, R5 can be made to move towards and then adsorb on the surface of the anode. Then this adsorbed surface is brought in contact with silane solution causing the nanosilica to form on the electrode surface. The electrode was lowered into a hemispherical bowl containing R5 solution. The bowl is at negative potential as compared to the electrode. By keeping the electrode dipped in the solution, the R5 gets adsorbed on the surface of the electrode. Then this electrode is lowered into silane solution. For electrohrdrodynamic tests the electrode is rotated inside the silane solution after R5 has been adsorbed on the surface. The same procedure was used for studying various other biocatalysts or peptides like lysine and proved to be very effective. This is a very efficient and effective method of examining the impact of shear field on biomimetically produced silica.
The shear stress that acts on the tip of the electrode is:

$$\tau_w \sim \rho r \omega^2 (\mu / \rho \omega)^{1/2}$$

Where ρTHE DENSITY OF REACTION SOLUTION IS, ω is the radial velocity and μ is the coefficient of friction [14]. The shear stress is uniform on the electrode sides, as the radius remains constant for the sides.

2.4 Characterization:

One of the most important goals of this study is to characterize the biomimetically-produced silica. The main instrument of choice to do this characterization study has been AFM/UFM. The most interesting samples were in the form of silica growing on the edges of the copper electrodes with gold plating. These samples provided for some of the most interesting and important results as will be discussed later. The electrodes were half an inch in length, but the silica was grown only on the lower quarter inch of these electrodes. Hence these electrodes were cut at quarter inch length from the tip to run ultrasonic force microscopy on them. After running UFM on the edges, these electrodes were cut once again at about 1 mm from the tip. This was done so as to stand the cut surfaces up on the transducer with the tip facing upwards. This is necessary since both electric and shear fields are different on the tip and the sides of the electrode, so different results are expected on the tip and the sides. The main coupling material, between the transducer and the sample, used in all of our samples was honey. It provides a coupling medium to transfer vibration from the transducer to the sample and also it holds the sample in place and does not let it move. The sample is left to settle for some time, the typical time is 5-10 minutes. The sample coupled with the transducer is set on the sample stage of the AFM and the transducer is hooked to the signal generator and lock in amplifier.

3. RESULTS AND DISCUSSION

The Scanning Probe Microscope used for all our experimentation was a *Dimension 3000 Large Sample Scanning Probe Microscope* manufactured by Digital Instruments, California.

3.1 Static Test Results:

For a static test where the silica condensation reaction occurred in a quiescent environment, the silica produced is a colloidal dispersion of spheres with a typical size in the range of 500nm. Figure 1 shows such dispersion. The size of the image is 10 microns x 10 microns. Note that some spheres are isolated, while others appear to be fused. The image acquired by employing tapping mode AFM is seen in figure 2. Again it clearly shows the spherical nature of silica precipitated out during a static test.

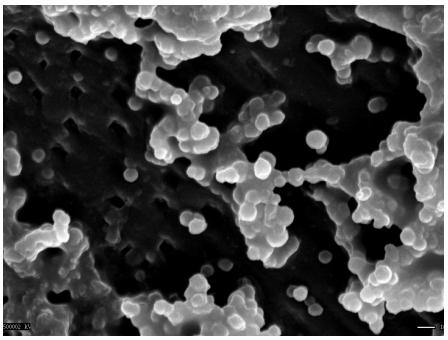

Fig 1 Image of Silica precipitated

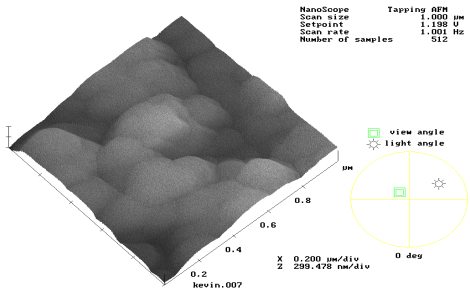

Fig 2 Tapping mode AFM image of Silica

3.2 Hydrodynamic Test Results

The sample preparation was similar to static test samples. The same microscopic slides were used to collect the precipitate. This silica on the slide was then imaged with the tapping mode AFM. The results obtained clearly indicated that shear flow field affects the structure of the silica being obtained tremendously. The normally spherical silica, obtained during static tests, is now elongated and fiber like. This is a very important result as this shows that the morphology of the biomimetic silica can be changed through application of a shear field. The results for method 2 are shown in figure 3.

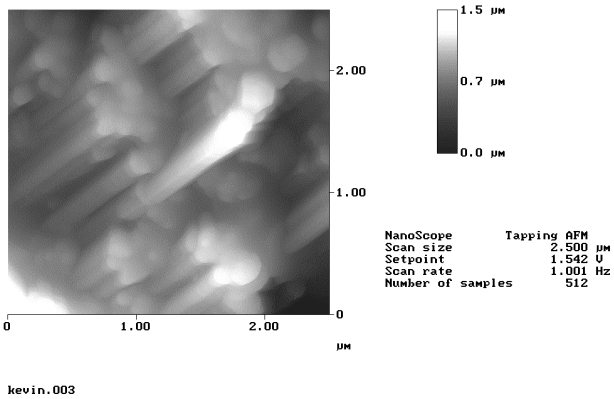

Fig 3 Tapping mode AFM image of silica obtained using method 2

Although the contrast is not as good as UFM scan, it's still clearly visible that we have obtained bundles of silica fiber like objects. The typical radius of these silica cylinders is of the order of 200 nm. Hence both size and structure were affected by the use of shear flow field.

Method 3 was adopted as an improvement over the method 2 because of the improved control. After the test was run, the precipitated silica was drawn from the annular test section onto a microscopic slide and allowed to dry up. The silica concentration in this method was much higher than what was obtained in method 2. Tapping mode AFM studies were conducted on these samples. As is clearly visible in figure 4, we get elongated structures, again with a typical diameter of 200 nm.

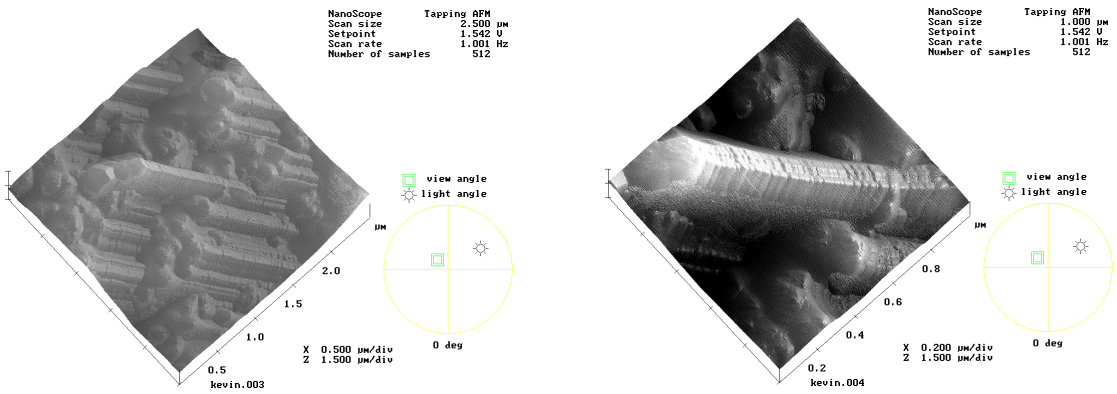

Fig 4 Tapping mode AFM images of silica obtained using method 3

This method provided very interesting and important results. It was found out that the structure and size of silica can be altered when subjected to shear flow fields. As was the case in this method the characteristic shape changed from spherical to cylindrical and the size decreased from 500 nm to 200nm. The main disadvantage of this method is that it gives us no control over the positioning of the silica.

3.3 Electro-Hydrodynamic Test Results:

A gold plated copper electrode was used to localize the enzyme, which acted as the catalyst in silica biomineralization. This helped in the positional control over the silica on the electrode surface. The silica grew on the electrode as a uniform layer. In these samples the bond between silica and the electrodes was strong we were able to run contact mode AFM.Silica grows in a uniform layer over the sides of the electrode, which can be attributed to a constant shear flow field. Figure 5 shows the AFM and UFM image of the bare gold surface, which can be compared to later images of silica on this gold surface. After running the test this gold surface is covered by a layer of silica. Figure 6 shows the AFM/UFM scan over such silica growth. This scan was done on the cylindrical side of electrode, where shear flow field is constant.

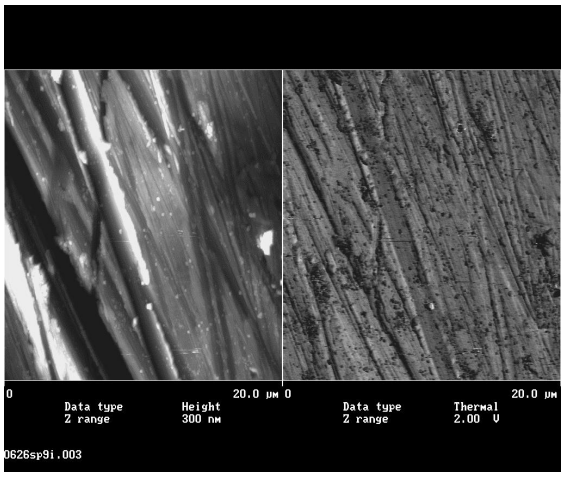

Fig 5 AFM/UFM scan of the bare gold plated electrode surface

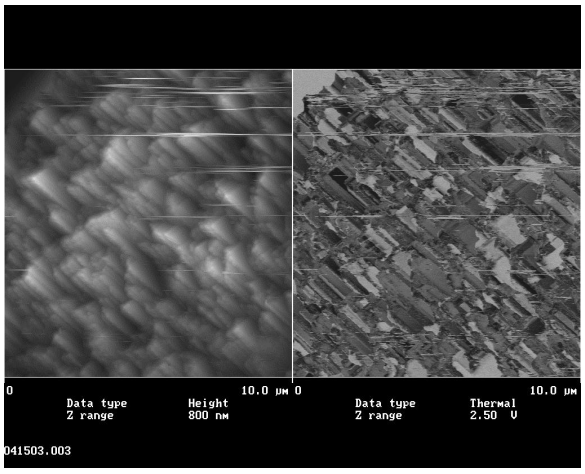

Fig 6 Scanning Probe scan of the electrode side showing plate like structure of Silica

As is evident from figure 6 the structure of silica is plate like as compared to spherical and cylindrical obtained during static and hydrodynamic tests respectively. Also the orientation of the plate like structures is

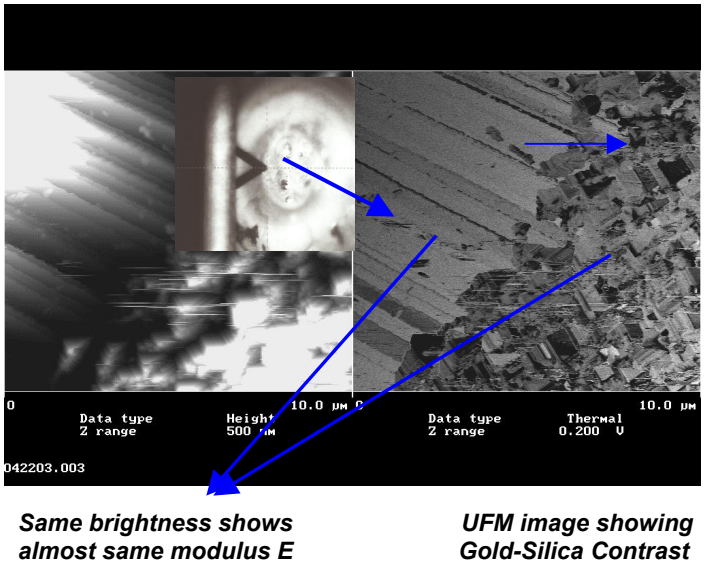

Fig 7 AFM/UFM scan of the center of electrode tip

in the same direction as that of shear flow. Another feature of this scan is that the contrast of the image in the UFM image is almost same over the scan area in the direction of its growth. This goes to prove that the Elastic Modulus E of silica growing over the scan area is constant. This can again be attributed to the constant shear flow field over the electrode sides.

The tip of the electrode was further scanned using Tapping mode AFM. The main advantage of the tip is that we obtain a bare spot in the middle where no Silica grows. This allows for the scanning of gold and silica in the same scan which can be used to compare the contrast between these two different materials and we can infer mechanical properties of Bio-Silica being grown by above processes as shown in Figure 7. Young's modulus for thin plated gold is reported to be 78 ± 10 GPa [12]. Based on the interpretation that same brightness of gold tip and bio-silica in UFM suggest similar stiffness of the two materials. Thus, the modulus of elasticity E for bio-silica , to a good approximation, is 78 GPa, which is in agreement with the range of values for various types of silica, 70-92 GPa with an error of 20%.

4. CONCLUSIONS

1. Structure of bio-silica is spherical with a characteristic size of 500nm for quiescent in vitro reactions.
2. Structure of bio-silica changes from spherical to cylindrical when subjected to shear flow fields.
3. The characteristic size decreased from 500 nm for static tests to 200 nm to silica produced under the effect of shear flow fields.
4. Applying electric fields can control the peptide adsorption on the surface of an electrode.
5. These electric fields, in synergy with shear flow fields can be used to control both structure and location of silica growth.
6. The structure of silica changes from cylindrical to plate like under the synergetic effect of electric and shear flow fields.
7. The young's modulus for silica produced by electro-hydro dynamic technique can be approximated equal to that of the gold film on the electrode, which is approximately 78-80 GPa.

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