International Journal of
Science Inventions Today
www.ijsit.com
ISSN 2319-5436
Research Article

BIOMINERALISED SILICA-NANOPARTICLES DETECTION FROM MARINE DIATOM CULTURE MEDIA

P.Gnanamoorthy*, S. Vasudevan and V. Ashok prabu

Centre of Advanced Study in Marine Biology,Faculty of Marine Sciences, Annamalai University,Parangipettai - 608 502.

ABSTRACT

Diatoms are unicellular algae the most spectacular among the microorganisms assemble into a micro-shell with a distinct 3-D shape and pattern of fine nanoscale features. In this investigation, we present results; Field Emission Scanning Electron Microscopy images show the presence of ordered arrays of silica nanoparticles. A number of diatoms with partially opened valves were observed on the surface of the diatom, which indicates that cell contents inside of diatoms could release the nanoparticles into the culture solution. We believe that the film forming silica nanoparticles are either released by the diatoms during reproduction or after cell death due to bacterial action. Further research will investigate whether the silica nanoparticles are produced intracellular and then released or whether synthesis occurs in cell culture medium. This approach provides an environmentally friendly means for fabricating silica nanoparticles for drug delivery, disease diagnostics, artificial opal films, decorative coatings and novel optical materials.

INTRODUCTION

Nanostructures generated by many living organisms, developed through evolution over a million of years, which can be a valuable creativeness for nanotechnology [1]. In nature large number of examples for microorganism, which assemble bio-minerals into intricate 3-Dimensional (3-D) nanostructures [2-3]. Processes for fabricating three-dimensional (3-D) nanostructured assemblies for use in advanced devices are under progress of development. Diatoms (unicellular algae) are the most spectacular among the microorganisms assemble into a micro-shell with a distinct 3-D shape and pattern of fine (nanoscale) features [4-5]. Diatom nanosilica have been extensively reported with number of applications such as photonics, molecular separation, immunoprecipitation, immunoisolation, microfluidics, sensing, biosensing, drug delivery and nanofabrication [5, 6-13].

Diatoms are microalgae, which are found in both freshwater and marine environments, as well as in moist soils, and on moist surfaces. They are either freely floating (planktonic forms) or attached to a substrate (benthic forms), and some species may form chains of cells of varying length. Individual diatoms range from 2 μm to several millimeters in size, although few species are larger than 200 μm in size. Diatoms as a group are very diverse with 12,000 – 60,000 species reported [14-15]. The diatom frustules have two halves consist of, the epitheca that is larger, and hypotheca the smaller. The hypotheca will grow out of the epitheca, and will be much easier than the epitheca to modify by metabolic consumption with some elements other than Si. Valves are the top and bottom parts of the frustules. The frustules of centric diatoms consist of a honeycomb of hexagonal chambers, called areolae. In general, each chamber has an outer surface, exposed to the external environment and an inner surface. One of the two surfaces is perforated by large, round holes called foramen, while the other surface contains one or two silica plates (cribellum and cribrum) perforated by a complex and highly symmetrical pore arrangement [16].

Silicic acid ($Si(OH)_4$) is the originator for silica formation from the aqueous environment is supplied via transmembrane transporter proteins called silicic acid transporters to diatom cell. The accumulated silicic acid is conveyed into particular intracellular vesicles called silica deposition vesicles (SDVs). The SDVs are subsequently motivated during cell multiplication, universally daughter cells build one valve and another one received from mother cell. These two models have been suggested for the rapid two dimensional precipitations of silica nanoparticles and valve morphogenesis [12]. Moreover, organic precipitation between aqueous and SDV encourages silicification into a honeycomb-like structure. Silica precipitation causes scattering effects generating new margins and sustained silica materialization [16]. In recent years the research on principles and mechanisms of biomimetic synthesis of silica nanostructure are concentrated [5].

In this study, we present results that demonstrate the formation of silica nanoparticles from diatom culture medium. We also propose that the silica nanoparticles are either released by the diatoms during reproduction or after cell death due to bacterial action. This approach will be provides an environmentally friendly means for fabricating silica nanoparticles for drug delivery, disease diagnostics, artificial opal films, decorative coatings and novel optical materials.

MATERIAL AND METHODS

Diatom collection:

Phytoplankton (Diatom) samples were collected from Vellar estuary, Tamil Nadu, India. The phytoplankton net is made up of bolting silk cloth no 30, mesh size 48 µm and mouth diameter of 0.35 m. During sampling the net was submerged in the water and towed horizontally from a mechanized boat with an outboard engine at a speed of 01 – 02 knots for half an hour. Collected samples was adopted for numerical analysis using the light microscope and identified by the keys were followed.

Laboratory Culture of Diatom:

The phytoplankton culture was carried out by following the standard methods of Anderson et al. [17] Totally six different diatom species were picked with the help of micropipette from the F/2 Guillard's medium[18]. Pure auxenic cultures of two diatom species i.e., planktonic two marine centric diatoms *Cosinodiscus* sp and *Odentella mobilensis*were produced under controlled conditions [temperature 25±0.50C/20±0.50C day/night cycles; photoperiods 12 hours light (fluorescent lamps) and 12 hours dark period] in the algal culture laboratory of Centre of Advanced Study in Marine Biology, Annamalai University of India were investigated.

Cleaning of Diatom Frustules:

In order to examine the diatom frustules under Field Emission scanning electron microscopy (FESEM), a cleaning procedure Butcher et al. [19] was followed to remove extracellular organic layers from the frustules. Auxenic cultures of diatoms in conical flask was shaken for 4 minutes to detach all the cells and 15 ml of each species was centrifuged at 6000 rpm for 10 min then the pellet was washed with deionized water four times to eliminate any additional fixatives. The pellets were treated with 10% H_2O_2 and kept in water bath for 15 min at 100 ºC and 10% aqueous HCl was added and then centrifuged at 2000 rpm for 10 minutes. The supernatant was pipetted out and the pellet was washed again with double distilled water for 3 times. Cleaned frustule valves were then stored in ethanol to avoid contamination.

Diatoms culture media filtration:

The live diatoms were harvested after 10-15 days of culturing and aliquots of growth media after the culturing period was filtered through the teflon filter (pore size 1 µm) and examined by light microscopy, scanning electron microscopy (SEM). The opalescence film on the filter paper is observed through filtration of culture media. In culture media without of diatoms is used as control.

Characterisation by field-emission scanning electron microscope (FESEM):

The structural characterisation of diatoms was performed by FESEM. The present investigations were performed using a Carls zeiss ultra 55 field-emission scanning electron microscope in combination with energy-dispersive spectroscopy (EDS) analysis. The one drop of diatom solution on carbon tap, after made into dry kept it in a desiccator for 72 hours. Then sputtered with gold around 10nm on the samples. Then different parts of the frustule from the central to the peripheral areas were scanned on the inner and outer

frustule surface.

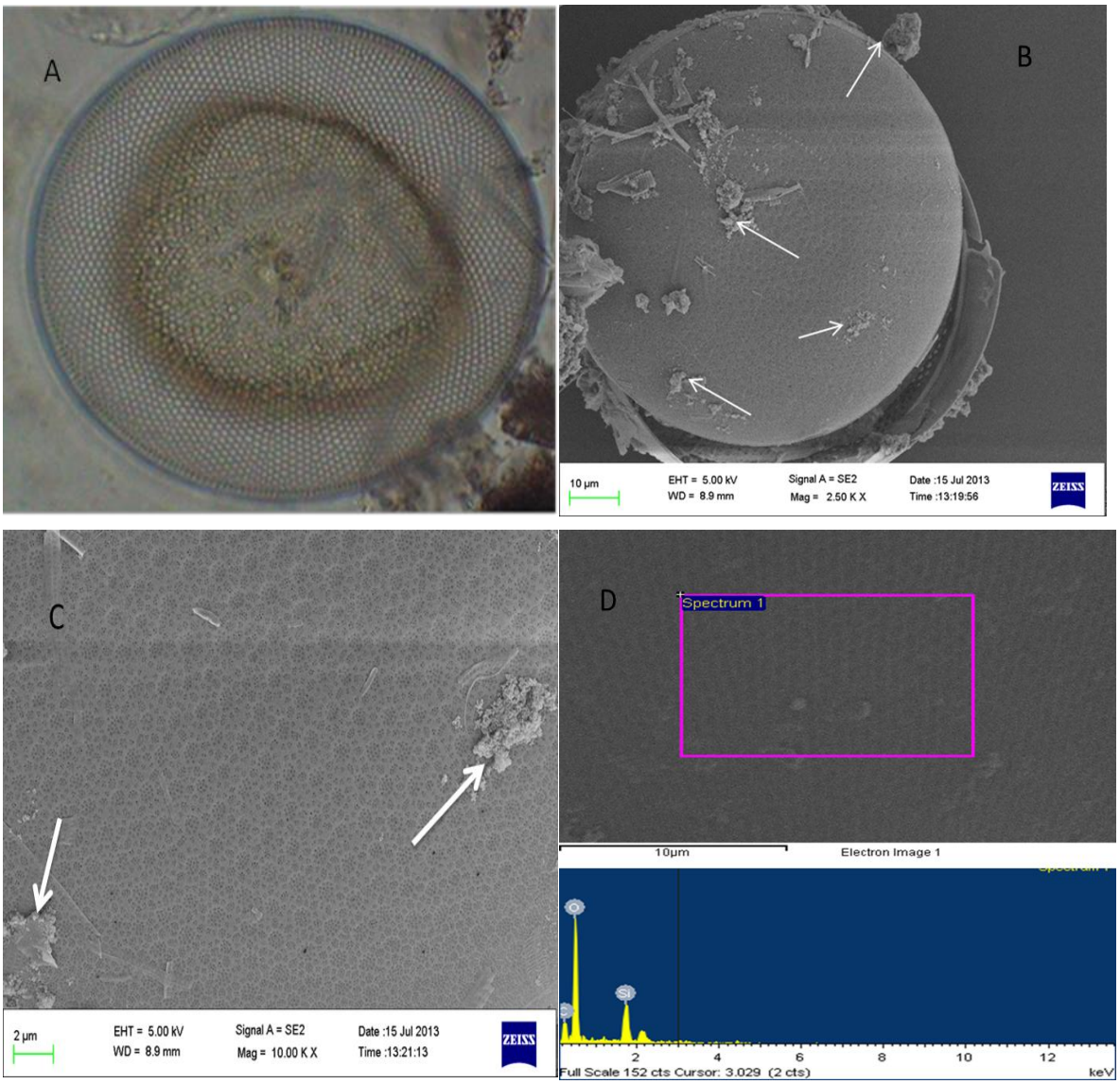

Figure 1: whole structure of *Coscinodiscus* sp from light microscope(A). FESEM image (10µm) of the diatom surface showing the silica nanoparticles on porous topography (B). (C) Well-arranged FESEM image of cribrum surface showing the silica nanoparticulate clusters. (D) Corresponding EDS graph shows the silica element presence.

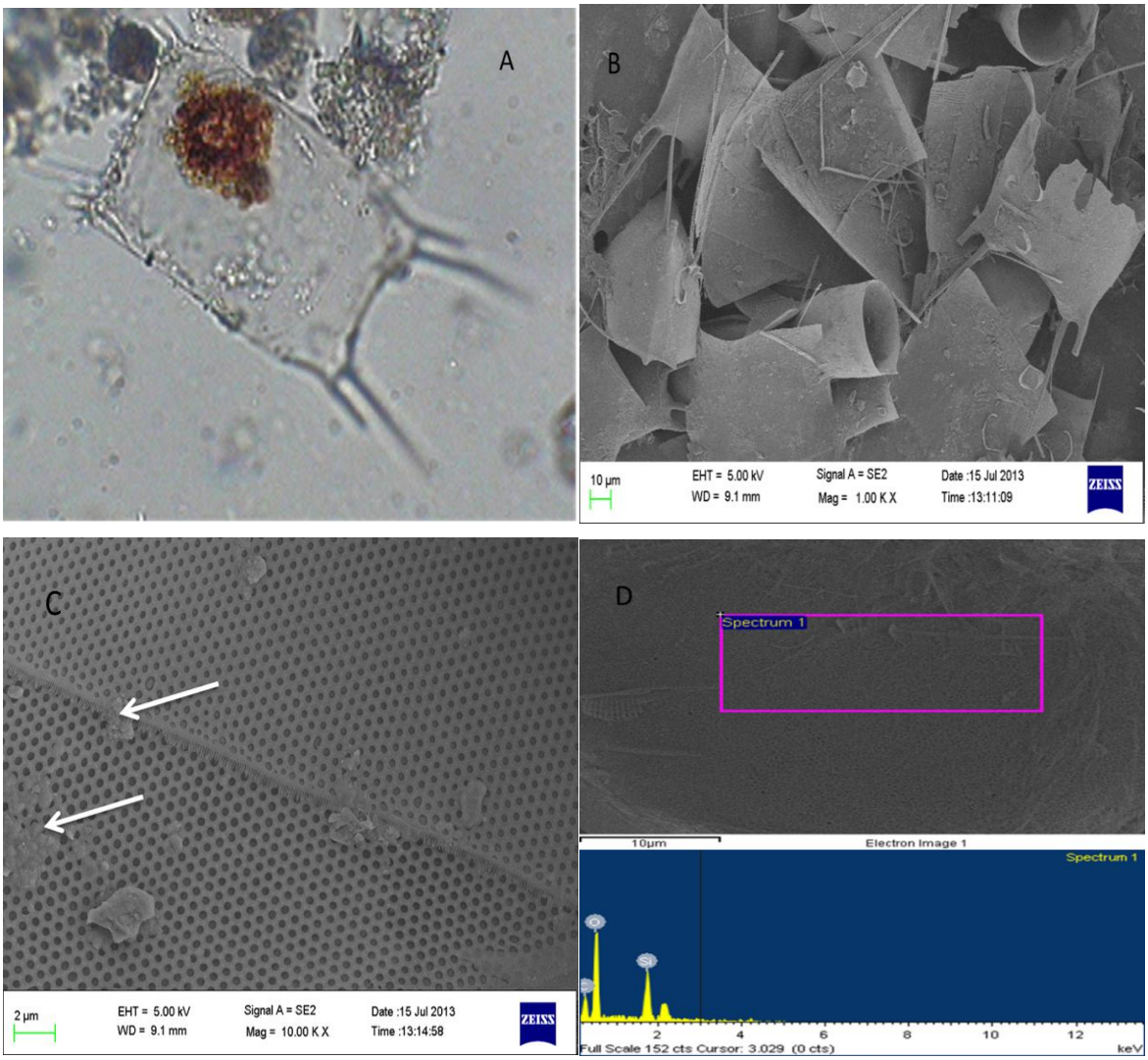

Figure2:whole structure of Odentella *mobilensis* from light microscope (A). FESEM image (10µm) of the frustules focusing of FESEM image shows of convex, with flat frustules (B). C outer surface of *Odentella mobilensis* image shows organisation of holes (foramen) are well-arranged foramen holes showing hexagonal organisation with silica nanoparticulate presented on diatom surface. (D) Corresponding EDS graph shows the silica element presence.

Element	Weight %	Atomic %
C K	13.65	19.92
O K	55.64	60.92
Si K	30.71	19.16
Totals	100.00	

Table1:EDS table shows the element compositions of *Coscinodiscus* sp

Element	Weight %	Atomic %
C K	17.18	25.16
O K	48.55	53.38
Si K	34.27	21.46
Totals	100.00	

Table 2:EDS table shows the element compositions ofOdentella mobilensis

RESULT AND DISCUSSION

In the present investigation two centric diatom species were cultured namely, *Coscinodiscus sp* and *Odentella mobilensis*. (Figure 1A and 2A). These species are frequently found in marine habitats and are ubiquitous components of diatom blooms. To gain a better understanding of about the precise mechanisms of frustule formation. In contrast to other biomineralised, this is largely attributable to silica itself, both from its chemistry, involving a complex inorganic polymerization process different from precipitation/dissolution reactions of carbonate or phosphate phases, and the limitations in suitable analytical techniques [20].

Light microscopy investigation shows their strong opalescent color from diatom body and this type of centric diatom has round valves and girdles that are 80 μm in diameter (Fig. 1A). the clean diatom frustules of *Coscinodiscus* sp, which are shown in Fig. 1B, the exterior of the valve has a convex shape, where sieve pores that are 132.1 nm in diameter can be seen [5]. Fig. 1C shows the concave internal surface of the valve, where a regular pattern of large pores (called cribrum) can be seen on surface of frustules showing the silica nanoparticulate clusters. We assumed this opalescent effect originated from these bonded silica nanoparticles. These exoskeletons (frustules) consist of SiO_2 nanoparticles assembled in a highly organized structure exhibiting porous networks at different scales. In the present investigation revealed that silica nanoparticles can be obtain from the diatom culture media. EDS analysis confirms the silica compositions are present (Fig, 1D).

Fig. 2A light microscope shows the diatom cell of *Odentella mobilensis* are centric diatom. A series of FESEM images of the outer layer of the frustules *Odentella mobilensis* were presented in fig 2B. Outer surface of *Odentella mobilensis* image shows well organisation of holes (foramen) showing hexagonal organisation and all the pores are circular with same size in well-arranged silica nanoparticles are indicated that cell material escaped from diatom shows the presence of spherical nanoparticles diatoms have been released during the death phase in culture media (Fig 2C). EDS graph of the centric marine diatom frustules (*Odentella mobilensis*) spot analysis, it was confirmed that the frustules contains from diatoms are contains mainly of oxygen and silicon in the form of amorphous silica (SiO_2) (Fig 2D and Table 2). In these studies, the valves (also called microshells) of the diatom *Odentella mobilensis* are regularly arranged with multi-level apertures of silica nanoparticles that are well organized in a flattened plane, exclusive for optical properties, large size,

and flat surface, which are make them informal to assemble and fix [21-22].

CONCLUSION

The formation of opalescent films by self-assembly of silica nanoparticles produced in the growth medium of marine algae (diatoms) was discovered. FESEM images show the presence of ordered arrays of silica nanoparticles. A number of diatoms with partially opened valves were observed on the surface of the diatom, which indicates that cell contents inside of diatoms could release into the culture solution. We believe that the film forming silica nanoparticles are either released by the diatoms during reproduction or after cell death due to bacterial action. Further research will investigate whether the silica nanoparticles are produced intracellular and then released or whether synthesis occurs in cell culture medium. This approach provides an environmentally friendly means for fabricating silica nanoparticles for drug delivery, disease diagnostics, artificial opal films, decorative coatings and novel optical materials.

REFERENCES

1. B. Bhushan, Y. C. Jung, and K. Koch, "Micro-, nano- and hierarchical structures for superhydrophobicity, self-cleaning and low adhesion", Philosophical Transactions of the Royal Society A, vol. 367, pp.1631–1672, 2009.
2. S.Mann, and G. A. Ozin, "Synthesis of inorganic materials with complex form", Nature, vol. 382, no.6589, pp. 313-318, 1996.
3. M. Sarikaya, "Biomimetics: Materials fabrication through biology", Proceedings of the National Academy of Sciences of the United States of America, vol. 96, no. 25, pp. 14183-14185, 1999.
4. A, Bozarth, U. G. Maier, and S. Zauner, "Diatoms in biotechnology: Modern tools and applications", Applied Microbiology and Biotechnology, vol. 82, pp. 195– 201, 2009.
5. D. Losic, G. Rosengarten, and J. G. Mitchell, "Pore architecture of diatom frustules: Potential nanostructured membranes for molecular and particle separations", Journal of Nanoscience and Nanotechnology, vol. 6, pp. 982–989, 2006.
6. T. Fuhrmann, S. Landwehr, M. El Rharbi-Kucki, and M. Sumper, "Diatoms as living photonic crystals", Applied Physics B: Lasers and Optics, vol. 78, pp. 257–260, 2004.
7. P. J. Lopez, J.Descles, A. E. Allen, and C. Bowler, "Prospects in diatom research",Current Opinion in Biotechnology, vol.16, pp.180–186, 2005.
8. D. Losic, J. G. Mitchell, R. Lal, and N. H. Voelcker, "Rapid fabrication of micro and nanoscale patterns by replica molding from diatom biosilica",Advanced Functional Materials, vol. 17, pp. 2439–2446, 2007.
9. D. Losic, J. G. Mitchell, and N. H. Voelcker, "Diatomaceous lessons in nanotechnology and advanced materials",Advanced Materials, vol. 21, pp. 2947–2958, 2009.
10. A. R. Parker, and H.E. Townley, "Biomimetics of photonic nanostructures",Nature Nanotechnology2, 347–353, 2007.

11. S. Lettieri, A. Setaro, L. De Stefano, M. De Stefano, and P. Maddalena, "The gas-detection properties of light-emitting diatoms", Advanced Functional Materials, vol. 18, pp. 1257–1264, 2008.
12. R. Gordon, D. Losic, M. A. Tiffany, S. S. Nagy, and F. A. S. Sterrenburg, "The glass menagerie: diatoms for novel applications in nanotechnology", Trends in Biotechnology, vol.27, pp. 116–127. 2009.
13. Y. Yu, J. Addai-Mensah, and D. Losic, "Synthesis of self-supporting gold microstructures with three-dimensional morphologies by direct replication of diatom templates", Langmuir, vol. 26, pp. 14068–14072, 2010.
14. D. Werner, "The Biology of Diatoms", University of California Press, 1977.
15. R. Gordon, and R.W. Drum, "The chemical basis for diatom morphogenesis", International Review of Cytology, vol. 150, pp. 243–372, 1994.
16. M. A. Sumper, "Phase separation model for the nanopatterning of diatom biosilica", Science, vol. 295, no.5564, pp. 2430-2433, 2002.
17. R. A. Andersen, S. L. Morton, and J.P. Sexton, CCMP - Provasoli-Guillard National Center for Culture of Marine Phytoplankton 1997 list of strains. Journal of Phycology, vol. 33, pp. 1, 1975.
18. R. R. L. Guillard, "Culture of phytoplankton for feeding marine invertebrates", In Smith, W. L. and Chanley, M. H. (eds), Culture of Marine Invertebrate Animals. Plenum, New York, pp. 29–60. 1975.
19. K. S. A. Butcher, J. M. Ferris, M. R. Phillips, M. Wintrebert-Fouquet, J. W. Jong Wah, N. Jovanovic, W. Vyvermann, and V. A. Chepurnov, "A luminescence study of porous diatoms", Materials Science and Engineering, vol. 25, pp. 658 – 663, 2004.
20. T. Coradin, P.J. Lopez, C. Gautier, and J. Livage, "From biogenic to biomimetic silica. ComptesRendusPalevol, vol. 3, pp. 43-452, 2004.
21. L. H. Wang, K. Y. Pu, J. Li, X. Y. Qi, H. Li, H. Zhang, C. H. Fan, B. Liu: "A Graphene/Conjugated Oligomer Hybrid Probe for Light-up Sensing of Lectin and Escherichia coli", Advanced Material,vol. 23, pp. 4386-4391, 2011.
22. W. Wang, T. Gutu, D. K. Gale, J. Jiao, G. L. Rorrer, and C. H. Chang, "Self-Assembly of Nanostructured Diatom Microshells into Patterned Arrays Assisted by Polyelectrolyte Multilayer Deposition and Inkjet Printing", Journal of American Chemistry Society, vol.131, no.12, pp. 4178-4179,2009.