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ORIGINAL ARTICLE

A lucrative chemical processing of bamboo leaf biomass to synthesize biocompatible amorphous silica nanoparticles of biomedical importance

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Abstract Synthesis of silica nanoparticles from natural resources/waste via cost effective route is presently one of the anticipating strategies for extensive applications. This study reports the low-cost indigenous production of silica nanoparticles from the leftover of bamboo (leaf biomass) through thermal combustion and alkaline extraction, and examination of physico-chemical properties and yield percentage using comprehensive characterization tools. The outcome of primed silica powder exhibits amorphous particles (average size: 25 nm) with high surface area (428 $m^2\ g^{-1}$) and spherical morphology. Despite the yield percentage of silica nanoparticles from bamboo leave ash is 50.2%, which is less than rice husk ask resources (62.1%), the bamboo waste is only an inexpensive resource yielding high purity (99%). Synthesis of silica nanoparticles from natural resources/waste with the help of lucrative route is at present times one of the anticipating strategies for extensive applications. In vitro study on animal cell lines (MG-63) shows non-toxic nature of silica nanoparticles up to 125 $\mu g\ mL^{-1}$. Hence, this study highlights the feasibility for the mass production of silica nanoparticles from bamboo leave waste rather using chemical precursor of silica for drug delivery and other medical applications.

Keywords Bamboo leaf biomass · Mass production · Silica nanoparticles · Biocompatibility · Drug delivery

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Introduction

Amorphous silica nanoparticles are commonly known to have a wide range of applications in many industries. The use of silica nanoparticles are extensive in biomedical field by synthesizing as a good biocompatible nanomaterial which can be used in drug delivery (Huang et al. 2014), enzyme encapsulation (Ab Wab et al. 2014), stabilising agent in therapeutics (Lu et al. 2010) and other therapeutic applications (Li et al. 2012). In addition, agricultural application of amorphous nanosilica has also been inspected in our previous study to overcome the shortage of silica in soil as well as bio-control action in plants and to enhance morphological and physiological parameters and diseases resistance (Suriyaprabha et al. 2012, 2014).

At present, the lucrative production of amorphous nanosilica is high on demand owing to its wide utility from medical to agriculture. Mass production of desirable and abundantly used biocompatible silica nanoparticles at low cost is essential to meet out the scarcity of using natural resource materials. While evaluating numerous chemical synthesis approaches, extraction of silica nanoparticles from biomass/biological resources is deemed to be one of the most economical production routes. As it is found in monocotyledonous plants, accumulation of silicon is found to be of greater extent (Epstein 1999; Savant et al. 1999). The production of silica nanoparticles is anticipated to be non-toxic and economic in nature. An investigation on forming amorphous silica nanoparticles from rice husk (RH) biomass is studied for different applications (Kalapathy et al. 2000; Carmona et al. 2013; Wong et al. 2014). However, finding the lucrative resources for large scale production of silica nanoparticles is still need to be refined as RH is also useful in animal feeds.

Bamboo is considered as one of the fast growing and highest yielding natural resources which is profusely available for construction from material to mankind. Countries that are rich in bamboo utilizes it for construction and pulp production (Villar-Cocina et al. 2014), thereby leaving the bamboo leaves as waste. Bamboo leave ash (BLA) is formerly studied for the production of pozzolanic material in cement composites (Amu and Adetuberu 2010). Indeed, the chemical analysis of the BLA showed that the concentration of combined Silica, Alumina, and Ferrous oxide exceeds 70% minimum standard required for pozzolanic materials (Utodio et al. 2015). Similarly, RH, bamboo also has more than 41% of SiO_2 in mature leaves (Motomura et al. 2002). However, to precisely evaluate the nanosilica productivity, both bamboo and RH biomass needs to be examined for pilot scale production so that lucrative synthesis of nanosilica with high purity for biological/industrial applications can be predicted.

One of the important aspects in the application of silica nanoparticles for biomedical applications is to assess the cytotoxicity levels in the living systems (Mondal et al. 2011; Dimkpa et al. 2012). Thus, it becomes an important issue to study the effect of size, shape, and surface functional groups on the bioavailability, uptake, subcellular distribution, metabolism, and degradation of the prepared nanoparticles.

To our knowledge, the extraction of high purity amorphous silica nanoparticles from BLA for industrial applications is scanty. Therefore, in this study, we synthesize silica nanoparticles from bamboo leaves using acid precipitation and characterize their structure, morphology, purity and yield with the help of varied characterization techniques. To substantiate the biomedical importance of the prepared bamboo leaf derived silica nanoparticles, the investigation on biocompatibility of the prepared particles is essential to screen the in vitro cytotoxic effect against animal cell lines.

Materials and methods

The leaves of bamboo species named *Dendrocalamus strictus* (Roxb.) Nees were collected in the surrounding areas of Yercaud hills, Salem district, Tamil Nadu, India. Mesophyll cells and parallel veins of the leaves of bamboo are used churn out bamboo leaf ash. All compounds which were used for the extraction were analytical grade and they were used as procured from Merck, India.

Extraction of nanosilica

The bamboo leaves were thoroughly cleansed so as to eradicate sandy and dust particles. Later, the leaves were dried and leaf powder was heated in the muffle furnace at 1023 K in an indigenous high temperature for 3 h. The obtained bamboo leaf ash (BLA) was treated with 6 N HCl under stirring at 343 K for 1.5 h to filter gathered impurities. Silica nanoparticles were extorted from BLA by means of acid precipitation process followed by alkali extraction (Palanivelu et al. 2014; Yuvakkumar et al. 2014). Then, the ash was rinsed for three times with distilled water to acquire the pH value 7.0. Later on, sodium hydroxide (2.5 N) was added under stirring for 2 h at 353 K to obtain silicate from ash of bamboo leaves. Supernatant consisting of the obtained sodium silicate (Na_2SiO_3) was added drop by drop with concentrated sulphuric acid until the pH level reaches 2.5 at which the solution became transparent white silica sol. The pH level was adjusted to acquire the precipitate of silica solution, and then it was carefully washed with double distilled water to shun the elements of sodium. The silica that was derived was set to dry in a hot air oven at 60 °C for 36 h. Finally, the pure silica powder was collected after calcination at 723 K for 2 h. All these processes are carried out in an indigenously designed high temperature tubular muffle furnace reactor set-up to produce silica nanoparticles at large scale.

Muffle furnace reactor set-up

The photographic image of the indigenous set-up is given in Fig. 1. High temperature tubular muffle furnace reactor set-up is indigenously designed to produce silica nanoparticles from bamboo leaf ash for mass production. High temperature tubular muffle furnace reactor set-up is designed with two major compartments namely, a) High Temperature Tubular Rotating Muffle Furnace and b) Glass Reactor. Tubular furnace set-up is designed in connection with a geared motor to facilitate slow circular rotation with uniform burning of the leaves. One of the salient features of this reactor set-up is the heavy weight rod that was fitted at the bottom of the furnace to tilt the furnace in a horizontal axis by dumble action hand. It is a cost effective way to harvest the burnt ash in collector vessel/glass reactor. The transparent cylindrical glass vessel acts as a collecting drum where silica nanoparticles are extracted from the bamboo leaf ash. Glass container is built-in with the acid-proof Teflon blades connected to a motor to achieve homogeneous stirring of the solution. The automated reactor set-up aids in the mass production of silica nanoparticles from bamboo wastes.

Characterization of nanosilica

Synthesized powders were subjected to various characterization studies for discovering their structure and

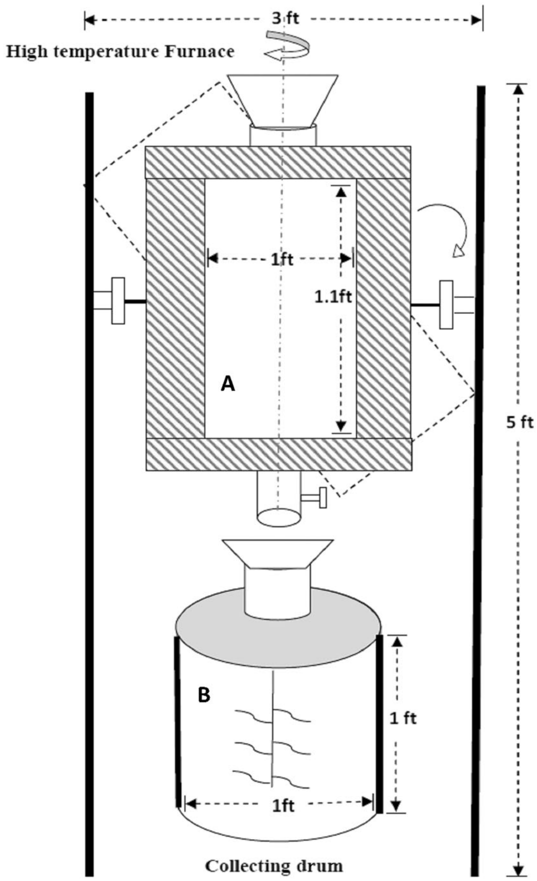

Fig. 1 Block diagram of the indigenous high temperature tubular muffle furnace reactor

morphology. X-ray diffractometer (XRD) (X' Pert Pro, PANalytical, the Netherlands) using Cu K_α ($\lambda = 1.5406$ Å) as a radiation source over the 2θ range of 10°–80° at 293 K was used to explore the crystalline nature of silica nanoparticles. The peaks of silica functional groups were attained from the Fourier transform infrared spectra (FTIR) in the wave number region of 4000–400 cm^{-1} with the help of FTIR spectrometer (Spectrum 100, PerkinElmer, USA). Particle size analysis (Nanophox, Sympatec, Germany) of dispersed silica nanoparticles was done to derive particle size distribution curve. The specific surface area of the prepared silica nanoparticles was investigated by means of Brunauer-Emmett-Teller (BET) surface area analyzer (Autosorb AS-1MP, Quantachrome, USA) through N_2 adsorption–desorption measurements. The specific surface area (SSA) was estimated from the relative pressure and volume of an adsorbate gas founded on BET equation (Brunauer et al. 1938). Silica nanoparticles were observed for its morphology and size using transmission electron microscopy (TEM) (CM 200, Philips, USA). The morphology and elemental composition of nanosilica was calculated employing scanning electron microscopy coupled with energy dispersive X-ray examination (SEM-EDAX) (JEOL JSM-6390LV, Japan).

Dry weight percentage

A quantity of the bamboo dried leaves was burnt at 1023 K at the indigenous high temperature muffle furnace for 3 h. Then, the obtained ash was weighed in digital microbalance (AUX- 220, Shimadzu, Japan). The dry weight percentage of nanosilica from BLA is measured with the help of the following relation:

$$\text{Dry weight percentage }(\%) = \frac{\text{Weight of the ash (g)}}{\text{Weight of leaves (g)}} \times 100 \quad (1)$$

Elemental analysis

Different parts (leaf and stem) of the bamboo were amassed and the SiO_2 deposition was quantified employing X-ray fluorescence (XRF) spectrometry (EDX-720, Shimadzu, Japan). Qualitative and quantitative elemental study of young and mature leaf, and stem ash of bamboo samples were carried out using XRF. The powder samples were examined directly at 10 mm/5 mm of focussing on Mylar thin film without any sample preparation and destruction.

Cytotoxicity study

The silica nanoparticles used for biomedical applications are ultimately affects human cells; hence, they were screened to ascertain the biocompatibility of the particles via cytotoxicity assay against osteoblast-like MG-63 cell line. The MG-63 cell line was purchased from National Centre for Cell Sciences, Pune, India. The cells were cultured in RPMI (Roswell park memorial institute)-1640 medium supplemented with 10% heat-inactivated foetal bovine serum, 3% L-glutamine, 100 U mL^{-1} penicillin G and 100 µg mL^{-1} streptomycin grown at 310 K in a humidified atmosphere of 5% CO_2 in air. When the cells attain 80–90% confluence, they were seeded into 96-well microtitre plate at a density of 1×10^3 cells per well for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. After 24 h of incubation, filter-sterilised nano silica at different concentrations from 1 to 500 µg mL^{-1} were loaded in wells, and again incubated at 310 K for 48 h. Then, 80 µg mL^{-1} MTT solutions were added into each well and incubated for 4 h. At the end of incubation, 1 mL of dimethyl sulfoxide was added to reduce the formazan crystals into pink colour. Then, the optical density (OD) of the test samples was read at 570 nm spectrophotometrically (U-2900, Hitachi, Japan)

Table 1 Elemental compositions of bamboo leaf ash and rice husk ash

Analyte (%)	Young leaf powder	Mature leaf powder	BLA (young)	BLA (mature)	RHA
K	39.793	45.091	27.527	18.545	26.764
Si	35.337	27.835	36.469	49.881	60.550
S	8.841	11.662	9.009	10.105	0.071
Ca	14.468	11.579	23.230	20.006	1.901
P	0.736	3.145	0.793	1.080	9.497
Fe	0.496	0.306	1.916	0.173	0.684
Ti	0.172	0.106	0.464	0.093	0.116
Mn	0.086	0.097	0.178	0.085	0.366
Cu	0.046	0.049	0.047	0.009	0.013
Zn	0.018	0.022	0.082	0.016	0.025
Sr	0.008	–	0.174	0.008	0.006
Zr	–	–	0.008	–	0.001

with 630 nm as the control. The treatment induced morphological changes are observed under microscope (Carl Zeiss PrimoVert^{TM} Inverted Microscope, Germany) to explore the particle biocompatibility with MG-63 cell line.

Results and discussion

The percentage composition of young and old leaves, BLA and RHA is examined from the X-ray florescence spectra as listed in Table 1. The yield percentage is comparatively less in the younger leaves than the mature ones. Therefore, the green and mature leaves are burnt so as to obtain the residue from bamboo leave and compared with RHA. It is obvious from the fact that accumulation and distribution of silica bodies present in bamboo leaves are of large intensities especially in mature leaves on a dry weight basis (Motomura et al. 2002).

HR-SEM results (Fig. 2) exhibit the accumulation pattern of siliceous compounds in leaves. Wide and dense deposition of silica is confirmed through the reflection of silica in mature leaves. This microscopic observation supports the elemental composition analysed from XRF analysis. The silica content is found to be high in RH (60.55%) than bamboo (49.88%). However, the demand for rice husk in different industrial applications like feed and energy etc. (Yalcin and Sevinc 2001; Ramchandra Pode 2016), an alternate cost effective source for silica can be considered from bamboo leaves for the mass production of silica nanoparticles. Burning of the bamboo leaves are comfortable for an industry to attain consistent burning since it is a light weight material.

In the first stage, bamboo leaves are fired in an open atmosphere and then in a muffle furnace reactor to acquire ash containing the white amorphous silica. The indigenous reactor set-up aids the continuous processing of the biomass into silica particles at large scale. A mass percentage (20%) is acquired as white ash from the bamboo leave powder after burning. Metal impurities are removed during acid leaching of BLA under magnetic blending for 1.5 h. Then, the colourless extract of Na_2SiO_3 is found with the treatment of NaOH. Silica gel is formed when the Na_2SiO_3 solution is allowed to the treatment of Con.H_2SO_4. The white silica powder is attained after calcinations at 723 K. The schematic representation of synthesis route using the high temperature tubular furnace reactor set-up during the extraction of silica nanoparticles from BLA is given in Fig. 3. The chemical reaction rate and reaction kinetics are calculated for the preparation of silica nanoparticles. Reaction rate for the entire extraction process for producing white transparent silica precipitate from BLA is calculated as 0.286 g h^{-1} based on the following formula:

$$\text{Reaction rate} = \text{quantity of product produced (g)}/\text{time interval (h)} \quad (2)$$

The following are possible chemical reaction occurs during the extraction process from bamboo leave biomass:

Bamboo leave ash (BLA) + HCl	Leaching (1 h) →	BLA+ Metal impurities	
BLA + 4NaOH	Alkali extraction (2 h) →	$2Na_2SiO_3 + 2H_2O$	(2^{nd} order)
$2Na_2SiO_3 + 2H_2SO_4$	Acid Precipitation (30 min) →	$Si(OH)_4 + 2Na_2SO_4$	(2^{nd} order)
$Si(OH)_4$ (Wet)	Drying at 373 K (6 h) →	$Si(OH)_4$ (Dry)	(1^{st} order)
$Si(OH)_4$ (Dry)	Calcination at 723 K (2 h) →	$SiO_2 + 2H_2O$	(1^{st} order)

The physico-chemical characteristics of the prepared silica nanoparticles are broadly evaluated. X-ray diffraction spectra of the prepared silica powder reveal a highly amorphous nature, which corresponds with the strong broad band localized at 22° (2θ) (Fig. 4a). The existence of crystalline minerals was not discovered in the spectra with devoid of contaminants like sodium sulphate and other metals. These pure amorphous particles are highly favoured the agro-biotechnological application as it is considered safe for humans by the World Health Organization (WHO). Hence, it has the demand to be extensively used in materials such as fillers, pharmaceuticals, catalysts, and chromatography (Bakaev and Pantano 2009).

The specific functional groups correspond to silica nanoparticles are viewed from the FTIR absorption spectra which illustrates the purity of silica nanoparticles (Fig. 4b). The peak at 467 cm^{-1} corresponds to bending vibrations of Si–O–Si groups. The observed sharp peak at 809 cm^{-1} and the short peak at 619 cm^{-1} reveal the existence of Si–O

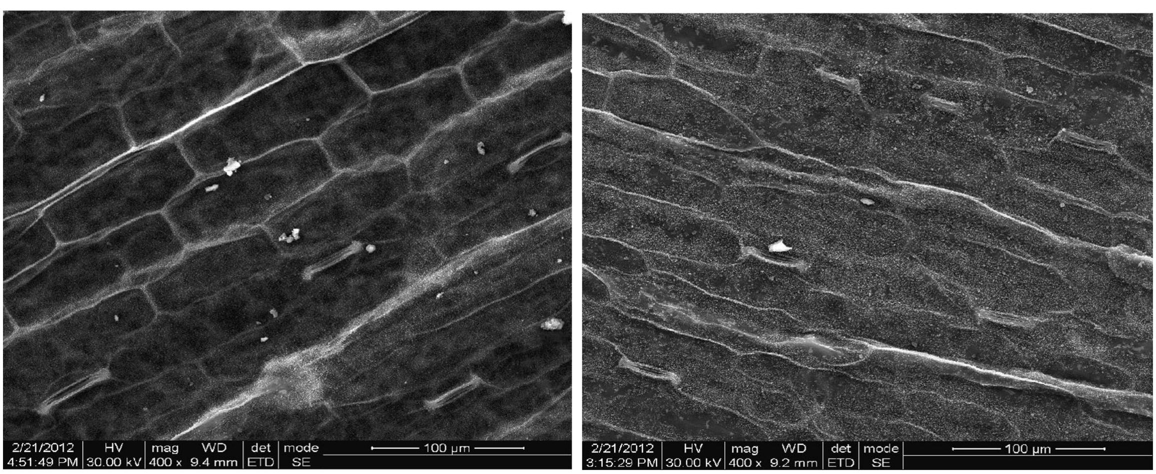

Fig. 2 HRSEM image of the young and mature leaf blades of bamboo

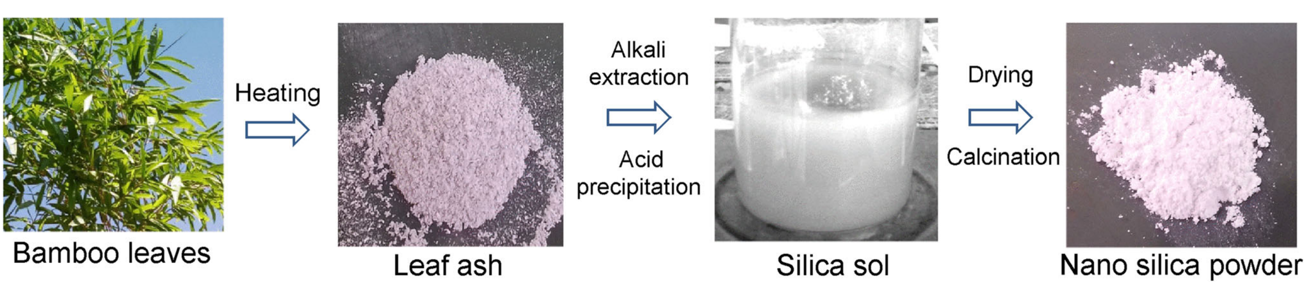

Fig. 3 Schematic representation of synthesizing silica nanoparticles from bamboo leaf biomass

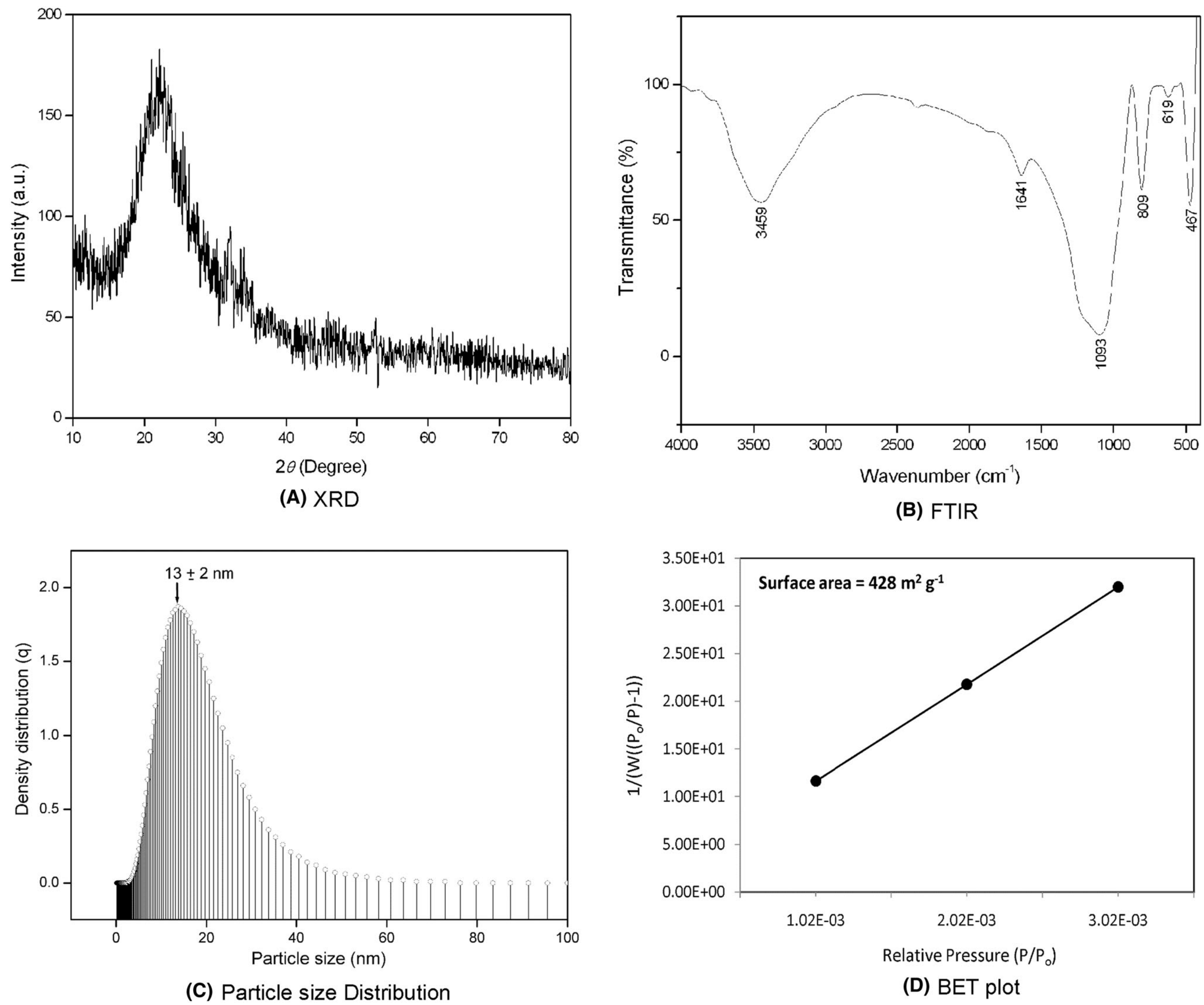

Fig. 4 Structural and textural characteristics of the prepared silica powder from bamboo leaf biomass

bending vibrations. The peak shift seen from 1052 to 1093 cm^{-1} assigned to an asymmetric siloxane (Si–O–Si) stretching vibration (Suriyaprabha et al. 2012) signifies the arrangement of bonding structures of Si and O atoms towards smaller particle size, i.e. nanoscale (Rahman et al. 2009). A broad band in the range of 3200–3600 cm^{-1} is due to the silanol –OH groups and adsorbed water. The peak arrangements of FTIR spectrum are not found to have significant difference in the prepared nanosilica from bamboo and rice husk. However, the reduction in particle size is observed in bamboo.

The research of particle size distribution through dynamic light scattering displays the broad distribution range of particles from 3 (d_{10}) to 60 (d_{90}) (Fig. 4c). The mean size distribution of the particles is found to be 13.8 ± 2 nm. BET surface area of the silica particles is found to be very high (428 m^2 g^{-1}) as observed from the plot (Fig. 4d) derived from nitrogen absorption–desorption process. As derived from our previous survey records, it is clear that this is distribution is comparatively larger when compared to RH derived silica nanoparticles (360 m^2 g^{-1}). The TEM image of the sample displays the transparent spherical silica particles in the size range of 10–60 nm (Fig. 5a) and their selected area energy diffraction (SAED) pattern (inset of Fig. 3a) reveals the diffusive ring pattern corresponding to amorphous silica which is also confirmed from XRD spectra. Moreover, these characteristics of SNPs are confirmed with the results obtained for rice husk in our previous studies (Suriyaprabha et al. 2012; Palanivelu et al. 2014). The scanning electron micrograph of the organized silica powders illustrates the smaller and discrete particles with slight aggregation and displays spherical morphology as seen in Fig. 5b. The EDX spectrum of the corresponding SEM observation confirms the purity of silica nanoparticles

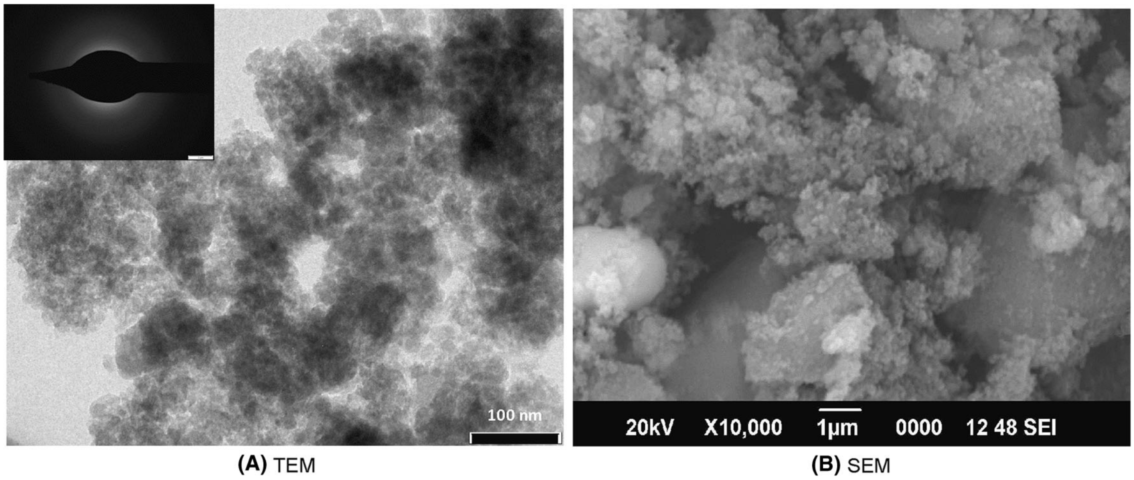

Fig. 5 Scanning and transmission electron micro graphical image of the extracted silica particles

as 99.1%. Therefore, the endeavour for producing high purity and high surface area silica particle from BLA for biomedical applications are more beneficial and it can be further used to demonstrate precise and sensitive results.

On the other hand, the usage of the bamboo residue for practical applications is lower while the demand for rice husk is high for different applications (Ramchandra Pode 2016). Their purity and dry weight percentage are compared with the silica which is mined from the RH. The yield percentage (%) of silica from BLA gained in the present analysis is 50.2% as obtained from this following equation:

$$\text{Yield percentage (\%)} = \frac{\text{Quantity of silica nanoparticles synthesized (g)}}{\text{Quantity of BLA used (g)}} \times 100 \quad (3)$$

It is interesting to note that the yield percentage of RH derived silica is higher (60.1%) than BLA. However, the bamboo waste is merely low cost resource yielding high purity (99%) than RHA (97%) as evidenced from our previous investigations (Suriyaprabha et al. 2012; Palanivelu et al. 2014). The production cost of amorphous silica nanoparticles varies from 700 to 6000 INR based on the purity grades (Industrial, agricultural and food Grade) of the silica and precursor. While comparing the rice husk as a source for silica, bamboo reduces the cost of production by neglecting the investment for buying rice husk from industry because matured and senescence bamboo leaves is littered as a waste in forest. Hence, 40% of the expense for the source materials is reduced while using bamboo leaves for nanosilica production. In addition, synthesized silica nanoparticles from BLA possess high surface area with improved purity percentage than the particles produced from RHA. The observed difference in silica accumulation between two plants of different parts is also in line with the previous statement that the ability of plants to accumulate Si varies greatly between species (0.1–10% of shoot dry weight). Furthermore, different parts of the same plant can illustrate large differences in Si accumulation (Hodson and Evans 1995; Currie and Perry 2007).

The morphological changes in MG-63 cell lines with the treatment of different concentrations of silica nanoparticles reveal the non-significant variations (Fig. 6) and cell death which is also confirmed by MTT assay. Concentration of particles above 100 μg mL^{-1} shows an increased accumulation of silica within the cells causing cell detachment, and aggregation of particles in culture media as seen in Fig. 6. The cytotoxic response of osteoblast cells to the treatment of different concentrations of silica nanoparticles

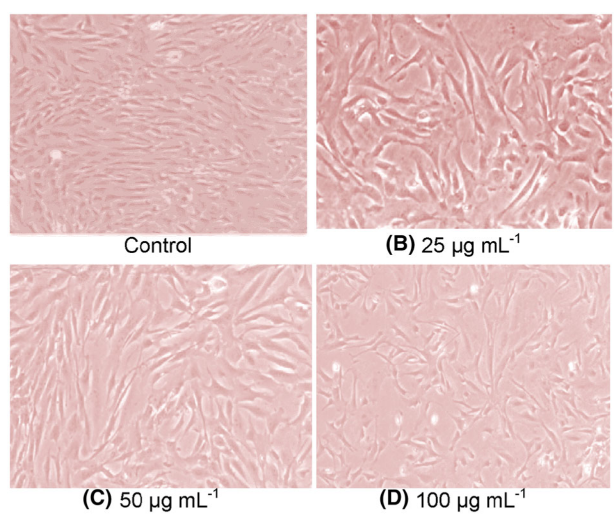

Fig. 6 Morphological changes with the treatment for silica nanoparticles in MG-63 cell line

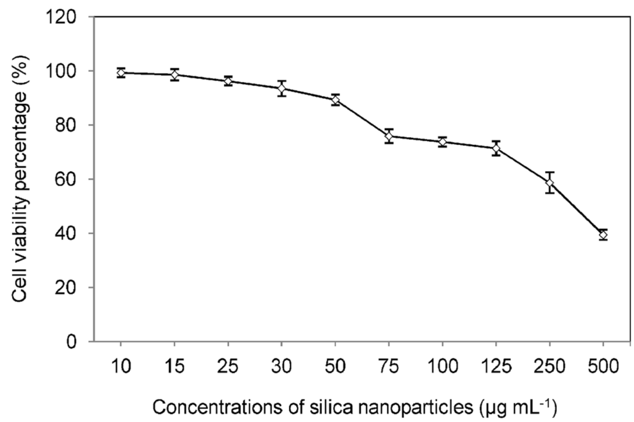

Fig. 7 Percentage of cytotoxicity as a function of treatment of silica nanoparticles

through MTT assay are shown in Fig. 7. From the figure, the total cell viability percentage of MG-63 cells under different concentrations is elucidated against 100% control samples. Above 100 μg mL^{-1}, cell viability tends to decrease significantly. This may be due to the formation of particle agglomeration in RPMI medium as well as interference during the cell proliferation at high particles concentration which results in decreased cell viability. Conventionally synthesized amorphous nano silica (500 and 50 nm) exhibits their potency against Bronchial epithelial lung cells confirming the suitability for pharmacological applications (Skuland et al. 2014). Hence, the in vitro cytotoxic responses of MG-63 cell line to bamboo derived amorphous silica nanoparticles are not showing any toxicological responses exhibiting good biocompatibility which affirms the use of silica nanoparticles for drug delivery applications in future. This cytotoxic study also supports the previous investigations made by Huang et al. (2014) on the use of chemically synthesized silica nanoparticles for drug delivery applications. It is an added advantage that use of cost effective silica at nanoscale prepared from bamboo waste is biocompatible at a very high concentration of treatment to the living system.

Conclusion

Low energy bio-chemical processing for extraction yields high purity (98.9%) amorphous silica nanoparticles in the particle range of 10–60 nm. The availability and production cost of bamboo leave biomass is comparatively lesser than rice husk biomass even though it has higher silica content. The result of the morphological changes and cytotoxic response of silica nanoparticles to osteoblast cell lines reveals the good biocompatibility for further medical applications. As silica is considered to be the one of the most necessitate nanomaterials for biomedical, agricultural and electronic applications, this cost effective technique is an ideal method to devise a model strategy for mass production of biogenic silica nanoparticles.

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