Research Article

Synthesis & Characterization of Silk/Surface Modified Silica Nanocomposites Using Sol - Gel Technique for Bone Applications

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ABSTRACT

Low bone mass (Osteoporosis) is a disease leading to bone fracture and is mainly resulted due to the imbalance in the rate of osteoblastic bone formation with respect to osteoclastic bone degradation. This study was intended to synthesize and characterize silk/surface functionalized silica. Nanosilica was extracted from Rice Husk (Agricultural waste) by sol gel technique and silk fibroin solution was extracted from *Bombyx Mori*. The composites of silica, silk/surface functionalized silica were then characterized using XRD, FTIR, particle size analysis. As a preliminary test, the toxicity of the composites were determined using antibacterial test and it was shown to be non toxic to bacterial cell lines. Further detailed studies are in progress to confirm the non-toxic behaviour of silk/surface modified silica composites.

Keywords: Osteoporosis, osteoblast, nanosilica, silk fibroin, composites.

INTRODUCTION

Osteoporosis is a most common bone disease which is characterized by reduced bone density, microarchitectural deterioration of bone tissues, and subsequent bone fragility along with the alteration of proteins which leads to fracture.[1] Osteoporosis results from imbalance in the rate of osteoblastic bone formation with respect to osteoclastic bone degradation. Bone fracture risk typically increase in the hip, vertebral, and distal forearm bones. These fractures are not only painful, but also leads to disability, further leading to the need for nursing in home care and increased mortality when compared to age matched populations.[2] Because of the high morbidity and mortality associated with osteoporotic fractures, treatment of osteoporosis prioritizes fracture prevention.[3]

The idea of new nanobiomaterials for bone regeneration has created new possibilities to develop novel therapeutic agents for treating osteoporosis. Various applications of Nanotechnology in medicine and other biotech areas involve the materials to design and interact with the body at various divisions of cellular scales with a higher degree of specificity, which surely is also being termed by targeted cells and tissue specific clinical applications that is broadly designed to achieve maximum therapeutic efficiency with minimal side-effects.[4]

The key component in tissue based engineering for bone regeneration is the scaffold that acts as a template for cellular interactions and for the formation of bone-extracellular matrix to provide structural support to the newly formed tissue. The scaffolds for bone regeneration should meet certain criteria to serve this function, including mechanical properties which are similar to those of the bone repair site, biocompatibility and biodegradability at a rate commensurate with remodeling.[5] Scaffolds serve primarily as osteoconductive moieties, since a new bone is being deposited by creeping substitution from adjacent living bone.

Silica based nanoparticles show good biocompatibility as they are non toxic in nature.

Deficiency of silica leads to detrimental effects such as skull and peripheral bone deformities, shortened joints, cartilage and collagen defects and mineral disruption in the femur and vertebrae.[6]

Nano sized silica particles are also used in cell targeting as well as in biosensing and bio imaging due to their high surface area- to volume ratio and the abundant availability of silanol groups (Si-OH).

Silica based nanomaterials are also being studied as skeletal repair materials due to their high bio activity and ability to bind strongly to the bone.

In the present study, nano silica was synthesized from Rice Husk, an agricultural waste, by sol-gel technique.[7]

Silk fibroin,which is isolated from *Bombyx Mori*. Silkworm, is a promising biomaterial which is used in this study for scaffold fabrication due to its widespread versatility as a mechanically robust, biocompatible, tissue engineering material.

Silk fibers are used as a matrix material for bone regeneration studies due to their high mechanical strength, good biocompatibility, and slow degradation rate that facilitates material substitution with newly formed tissue.[8]

In the present study, the goal was to synthesize nano silica from rice husk and to combine the useful biomaterial properties of silk and silica for composite organic/inorganic biomaterials for bone tissue applications.

MATERIALS AND METHODS

Synthesis of nanosilica from rice husk

All chemicals were purchased from Sigma-Aldrich (MO, USA) and were used without further purification. The agricultural waste, Rice Husk, was used for the extraction of nanosilica as described. Briefly, Rice husk was washed, cleaned and treated with EDTA and dilute HCl.

The treated Rice husk was then washed with double distilled water several times to remove all the acid and was allowed to dry at 110 °C for 12 hours.

The dried rice husk was then burned at 650-700°C for 4-6 hours to get rice husk ash (RHA). RHA was then treated with dil.NaOH and was heated at under constant stirring for 4 hours with a pH 9-10.

It was then filtered to get a clear and colourless solution of sodium silicate.[9]

Dilute HCl was then added to the sodium silicate solution to obtain silica gel which was then dried to obtain nano silica powder.

Synthesis of silk fibroin solution from *Bombyx Mori*. Silkworm

Cocoons from *B.Mori* silkworm was obtained from Sericulture lab, Bangalore.

The purification of silk was done through the degumming process followed by solubilisation in LiBr solution and dialysis of silk fibroin solution to obtain 8% of silk fibroin solution.[10]

Preparation of silk/nanosilica composites

Surface functionalization of silica was prepared by adding Aminopropyl triethoxy silane (APTES) to the silica gel in various concentrations.

APTES functionalized silica was then mixed with silk solution to obtain the final silk/silica composite.[11]

Characterizations

Scanning Electron Microscope (SEM) analysis was carried out to determine the surface morphology of nanosilica. Fourier transform infrared (FTIR) spectroscopy (FT-IR), Bruker Optics, Alpha-T, Germany) were carried out to determine the phase composition of the composites.[12]

Additionally, X-Ray Diffraction (XRD) and Particle size Analysis were characterized to determine the nature of the material as well as to determine the average particle size of the silica obtained.

Toxicity studies of the composites were observed using antimicrobial test.

RESULTS AND DISCUSSION

X-ray Diffraction (XRD) analysis

The samples prepared by sol-gel technique is characterized using X-ray diffraction for phase determination.

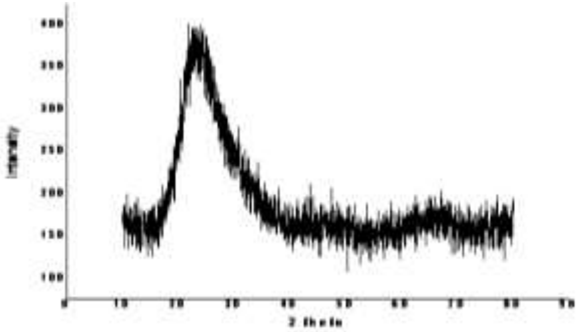

Figure 1: XRD of nanosilica after purification process

The XRD graph of nanosilica is shown in Fig. 1. After the purification process using acid and base treatment, a peak can be observed at 22° which shows the amorphous nature of purified nanosilica.[13]

Fourier Transform Infrared (FTIR) analysis

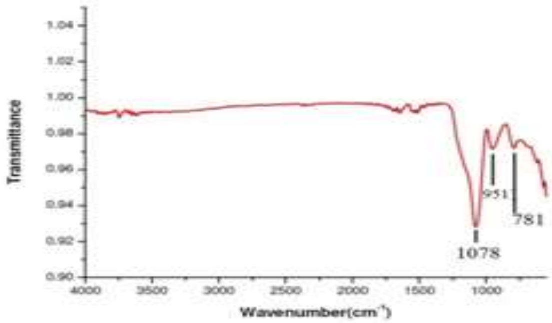

Figure 2: FTIR of silica

The synthesized nanosilica is characterized using FTIR. In Fig. 2, free silanol groups are present at 3000-3500 cm^{-1} which are due to the presence of –OH group. The presence of silica can be observed at 1000-1100 cm^{-1}. Si-O-Si groups are identified in the region of 500-1500 which also shows the presence of silica.[14] Fig. 3 shows the FTIR of Silk/Surface functionalized nanosilica composite.

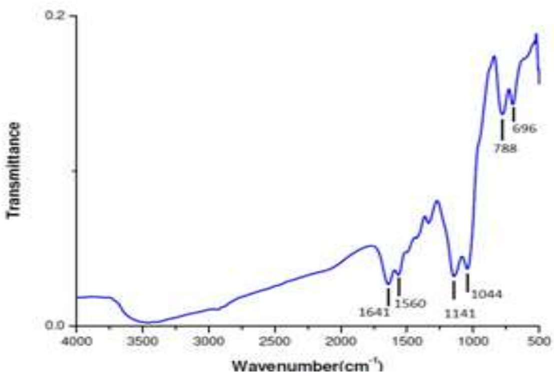

Figure 3: FTIR of Silk/ Surface functionalized silica composite

At 3000-3500 cm^{-1}, -NH groups are present, which indicates the presence of an amino group in silk.

At 1641 cm^{-1}, a strong band is observed along with another band at 1560 cm^{-1} which indicates the strong presence of $–NH_2$ groups.

At 500-1500 cm^{-1}, there is a shift in the transmittance region which indicates the surface functionalization of silk/silica nanocomposites.

Scanning Electron Microscopy (SEM)

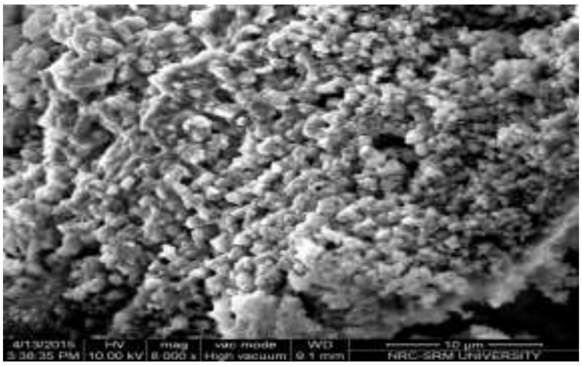

Figure 4: SEM of nanosilica

The surface morphology of silica composites and surface functionalized silica were analysed using Scanning Electron Microscope(SEM). The average size of silica nanoparticle which was synthesized using sol-gel process was determined to be 100-200nm. From Fig.4, the surface morphology of silica nanoparticles can be analysed. After the surface modification of APTES modified silica(Fig. 5), the average particle size determined was 80-100nm which shows a reduction in the size of the nanoparticle.[15]

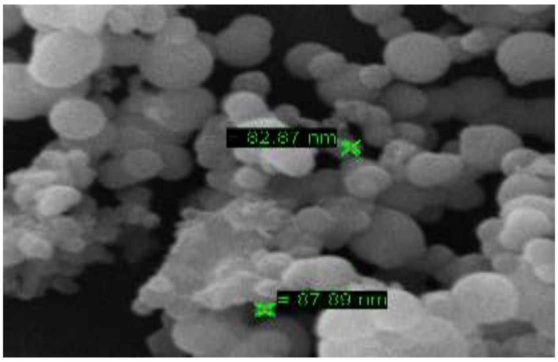

Figure 5: SEM of surface modified nanosilica

Particle size analysis

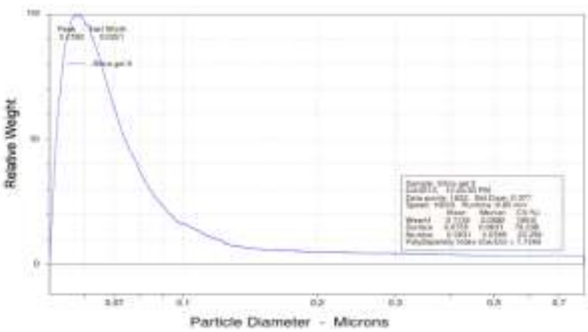

Figure 6: Particle size analysis of silica

The particle size analysis of silica was done using CPS disc centrifuge mechanism. The average particle size of silica particle determined was 50-60nm as shown in Fig. 6.[16]

Toxicity analysis using anti-bacterial test

A preliminary study was done inorder to test the toxicity of the composite using anti-bacterial test.

Amti bacterial activity of nano Silica (S1), Silk solution(S2), APTES functionalized nano silica (S3), APTES functionalized nano silica with silk(S4), Nano Silica/silk solution(S5) against *Pseudomonas*(gram-negative bacteria) (Fig.7), and *Streptococcus* (Gram-positive)(Fig.8). From the figures, it can be viewed that no zones were formed in any of the bacterial culture. This showed that the synthesized composites were non-toxic to the bacterial cells.[17]

Figure 7: Anti bacterial activity against *Pseudomonas* (gram-negative bacteria); S1: nanoSilica, S2: Silk solution, S3: APTES functionalized nano silica, S4: APTES functionalized nano silica with silk, S5: Nano Silica/silk solution, NC: Negative control, PC: Positive Control(Chloramphenicol)

Figure 8: Antibacterial activity against *Streptococcus* (Gram-positive); S1: nanoSilica, S2: Silk solution, S3: APTES functionalized nano silica, S4: APTES functionalized nano silica with silk, S5: Nano Silica/silk solution, NC: Negative control, PC: Positive Control(Chloramphenicol)

CONCLUSION

Amorphous nanosilica was extracted from Rice Husk using sol-gel process. The composites of silk and surface functionalized silica showed no zone inhibition for the antibacterial test which indicates that the synthesized composites were non-toxic to the bacterial cells. The anti-bacterial tests were implied as a preliminary test to check the toxicity of the compound. Silk/surface functionalized silica can be considered as an ideal composite for bone applications. Hence, with the incorporation of the nanocomposite into the bones, it can be a suitable candidate to strengthen the bones and to combat osteoporosis.

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