

# Synthesis of Hybrid Se-SiNPs Nanoparticle for the Studies of Cellular Toxicity against Breast Cancer Cell Line MCF-7

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**Abstract:** A hybrid nanocomposite of biologically compatible silica nanoparticles (SiNPs), coated with selenium nanoparticles (SeNPs), was developed, and evaluated for cytotoxicity on the breast cancer cell line using MTT assay methods. The hybrid nanoparticles were characterized using DLS, pXRD, and SEM analytical techniques. The size and size distribution of SiNPs and composite Se-SiNPs nanoparticles were determined by the DLS experiment. The sizes of SiNPs and Se-SiNPs were found to be 23.85 nm and 89.9 nm in diameter, respectively. The Se-SiNPs hybrid nanomaterials exhibited higher cytotoxicity than pure SiNPs against the breast cancer cell line MCF-7. When compared to SiNPs, composite Se-SiNPs suppressed the growth of the breast cancer cell line MCF-7.

**Keywords:** Cytotoxicity, MCF-7 breast cancer cells, SiNPs, SeNPs, Se-SiNPs, nanocomposite

## Introduction

According to a report published by the World Health Organization in 2020, breast cancer is the most widely recognized disease for tumor-related death, with over 685,000 deaths every year. Breast cancer is becoming more prevalent worldwide, with approximately 2.26 million new cases diagnosed each year. Despite the existence of numerous treatment methods for breast cancer, such as radiotherapy and chemotherapy, these treatments have side effects on healthy cells.[1,2] Many recent studies have focused on the discovery of new drugs to treat breast cancer.[3,4] Although medicine has made significant progress, there are still several concerns that need to be addressed to improve cancer treatment. Nanotechnology is catalyzing a global revolution in the field of science.[5-9] Nanoparticles[10-12] possess well-defined and controllable physical and chemical properties, making them applicable in various health-related domains, including nutrition, medicine, targeted drug delivery, genetic engineering, and vaccine manufacturing.[13] They also exhibit significant potential for enhancing applications across diverse sectors, such as agriculture, consumer products, cosmetics, energy, and transportation.[14] Nanomedicines, which are

nanometer-sized compounds, enhance the bioavailability of drugs in tissues, potentially improving the efficacy of systemically administered chemotherapeutic drugs. Selenium (Se) is a crucial dietary micronutrient involved in various physiological functions[15, 16], including cancer prevention and immune response.[17] Selenium nanoparticles (SeNPs) exhibit the ability to selectively target cancer cells and exert synergistic anticancer effects[18,19], suggesting their potential in eliminating cancer cells. Silica nanoparticles (SiNPs) are another essential biocompatible and nontoxic nanoparticle, widely utilized in biological and other applications due to their stable chemical structure, high sensitivity, reactive surface, large surface-to-volume ratios, and manageable biodegradability.[20,21] Se and SiNPs find applications in various medical fields, including bioimaging, diagnostics, medication delivery, and photodynamic therapy. SiNPs have demonstrated cytotoxic effects in various cancer cell lines.[22-29] In this study, we aimed to prepare hybrid material Se-SiNPs, expecting to leverage their synergistic properties in the biotechnology and biopharmaceutical industry. Both SiNPs and SeNPs exhibit biocompatible effects with minimal harm in

physiological conditions. This marks the first instance of producing and using hybrid Se-SiNPs nanoparticles for evaluating their *in vitro* cytotoxic effects on the breast cancer cell line MCF-7. We conducted the assessment using a time and concentration-dependent MTT assay. The hybrid nanocomposite was developed by grafting 3-aminopropyl trimethoxysilane (APTS) into SiNPs and covering them with SeNPs. We characterized and studied this nanoparticle's cellular toxicity and anticancer efficacy on cancer cell lines, as depicted in Figure 1.

#### **Materials and methods:**

The chemicals and reagents, namely 3-aminopropyl trimethoxysilane (APTS), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, methyl-thiazolyl diphenyl-tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO), were procured from Sigma Aldrich and Hi-media. The cultured MCF-7 cell line was obtained from the Department of Chemistry at Delhi University.

#### **Procedure for preparation of nanocomposite Se-SiNPs:**

The protocol[30] for amine-functionalized SiNPs coated with SeNPs was modified in several steps:

1. Initially, 10.0 mg of SiNPs salt was dispersed in 10.0 mL of anhydrous ethanol using a sonicator operating at 20 kHz for 30 minutes.
2. Next, 1.0 mL of 3-aminopropyl trimethoxysilane was added dropwise to the SiNPs solution and stirred in a nitrogen environment for 12 hours at room temperature.
3. Following this, the solution mixture was subjected to centrifugation at 5000 rpm for 30 minutes, and the supernatant was discarded. The residue was washed five times with deionized water to remove any unreacted APTS.
4. Subsequently, the amine group was grafted onto the surfaces of SiNPs, resulting in SiNPs-NH<sub>2</sub>, which served as a linker for attachment (A).
5. Sample (A), consisting of white powder with the amine group grafted to SiNPs (SiNPs-NH<sub>2</sub>), was dissolved in Milli-Q water at a concentration of 1.0 mg/mL.
6. Sample (B), a pre-synthesized red powder of SeNPs at a concentration of 1.0 mg/mL, was dissolved in Milli-Q water.

7. Equal volumes (v/v) of both samples A and B were mixed, and the mixture was stirred at room temperature for 24 hours.

8. The resulting composite nanomaterial of Se-SiNPs had its solvent removed through centrifugation, and the samples were dried in powder form under vacuum conditions for subsequent characterization and utilization.

#### ***In-vitro* cytotoxicity and cell viability test with Se-SiNPs:**

##### ***Protocol of Cell Culture***

Breast cancer cell line MCF-7 was cultured and maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 20 µg/mL of penicillin, 100 µg/mL of streptomycin, and 10% fetal bovine serum (FBS). The culture was incubated at 37 °C with 95% air in a humidified, 5% CO<sub>2</sub> atmosphere of a BOD incubator. To increase the cell density, cells were subcultured every third day, starting with an initial density of 2×10<sup>5</sup> cells/mL.

##### ***Protocol of MTT assay***

The cultured cells (100 µL/well) were seeded in a 96-well cell culture plate (microtiter) and incubated at 37 °C overnight in a BOD incubator.

1. After seeding, cells with a confluence level of 60-70% were treated with various doses of compounds C1, C2, SR, and SSR (where C1 represents the control medium without cells, C2 represents the control medium with cells, SR represents cells cultured in a medium containing SiNPs, and SSR represents cells cultured in a medium containing Se-SiNPs), with concentrations ranging from 0-300 µM, which were diluted in cell culture media. These cells were then incubated for 48 hours after treatment.
2. MTT reagent (10.0 µL/well) was added at a final concentration of ≈0.5 mg/mL and incubated for 4 hours to form formazan.
3. To solubilize the formazan, 100 µL/well of DMSO was added to each well, and the solubilized formazan reagent was measured spectrophotometrically.
4. The absorbance of the purple-blue formazan dye was recorded at 540 nm with a reference wavelength of 690 nm to count the viable cells.

#### **Results and discussion**

The SiNPs (silica nanoparticles) were coated with SeNPs (selenium nanoparticles) using a novel method to prepare composite nanoparticles.[31,32] SiNPs (silica nanoparticles) were synthesized

applying the sol-gel chemical method[33,34] from sodium silicate. The synthesized SiNPs were characterized by DLS (Fig. 2), SEM (Fig. 3A), and energy dispersive spectroscopy (EDS) (Fig. 3B).

In our previous work,[30] we utilized *Agrobacterium* species to reduce sodium selenite, resulting in the production of selenium nanoparticles (SeNPs) from  $\text{Na}_2\text{SeO}_3$ , as illustrated in Figure 4A. To briefly summarize the process, the bacterial cell membranes were initially destabilized through steam penetration and then subjected to high-speed centrifugation. This approach yielded SeNPs with a larger percentage of particle sizes ranging from 200 to 300 nm in diameter, as shown in Figure 4C. The quantity of  $\text{Na}_2\text{SeO}_3$  metabolized by *Agrobacterium* (as depicted in Figure 4B) determined the amount of SeNPs generated, rather than the concentration of  $\text{Na}_2\text{SeO}_3$  in the solution. Dynamic light scattering (DLS) was employed to record the size distribution of SeNPs in the aqueous medium. Most of the nanoparticles fell within the diameter range of 100 to 350 nm, with a maximum size distribution of  $19 \pm 1$  nm. These nanoparticles were coated with biomolecular functional groups such as -OH, -COOH, -NH<sub>2</sub>, etc.

The hybrid nanoparticles were formed by combining white silica and red selenium nanoparticles in the presence of grafting agents APTS (3-aminopropyl trimethoxysilane). Since both SeNPs and SiNPs have negatively charged surfaces, an amine group from APTS was grafted onto the surfaces of SiNPs, creating SiNPs-NH<sub>2</sub> as a linker. This provided a platform for the attachment of SeNPs, resulting in the formation of composite nanomaterials.[35] Pre-synthesized SiNPs (silica nanoparticles) and SeNPs were utilized to develop the composite nanoparticle Se-SiNPs. However, it should be noted that the overall combination of nanoparticles led to an increase in the size of the composite nanoparticles. To achieve this goal, the hydroxy functionalities of SiNPs were initially converted to -NH<sub>2</sub> groups, which would act as a linker for the incorporation of biocompatible molecules in addition to the existing -OH functionalities on the SiNPs. In summary, the SiNPs were dispersed in anhydrous ethanol, and then a solution of 3-aminopropyl trimethoxysilane (APTS) was gradually added to the SiNPs solution. This mixture was stirred under a nitrogen atmosphere for 12 hours at room temperature, followed by centrifugation at 5000 rpm for 30 minutes. The supernatant was discarded, and the SiNPs were washed five times with deionized water to remove any unreacted APTS residue. Subsequently, the amine group was grafted onto the surfaces of SiNPs, resulting in SiNPs-NH<sub>2</sub>. This white powder of SiNPs-

NH<sub>2</sub> was then dispersed in deionized water at a concentration of 1.0 mg/mL, and the pH of the medium was adjusted to 7.0 by adding HCl/NaOH to create colloidal SiNPs-NH<sub>2</sub> in a neutral form.

In another container, a pre-synthesized red powder of SeNPs at a concentration of 1.0 mg/mL was dissolved in Milli-Q water. Both sets of nanoparticles were combined in equal volumes (v/v), and the mixture was stirred at room temperature for 24 hours. The solvent was subsequently removed through centrifugation, and the resulting samples were dried in powder form under vacuum conditions. This process yielded a nanocomposite nanomaterial known as Se-SiNPs, which was later used for various characterization and applications.

The DLS experiment determined the size and size distribution of SiNPs and nanocomposite Se-SiNPs, revealing that the polydispersity index (PDI) is 5.11 and 1.48, respectively, with a diameter of 23.85 nm for SiNPs and 189.9 nm for Se-SiNPs. The zeta ( $\zeta$ ) potential of SiNPs is -11.2 mV and that of Se-SiNPs is -1.35 mV, with negative charges on the surface of the nanoparticles.[36,37] It is assumed that the ability of hydrogen bond formation and electrostatic interactions of -OH and amine groups of SiNPs with functionalities of Se-nanoparticles facilitated the development of the nanocomposite (Fig. 5).

#### **Absorbance spectrum of SiNPs and nanocomposite Se-SiNPs:**

The formation of the Se-SiNPs nanocomposite was confirmed using absorbance spectroscopy.[38] SiNPs exhibited  $\lambda_{max}$  at 220 and 410 nm, whereas the Se-Si nanocomposite displayed  $\lambda_{max}$  at 220 and 455 nm in the absorbance spectra, indicating the successful preparation of the nanocomposite (Fig. 6).

#### **Powder XRD analysis of SiNPs and composite Se-SiNPs:**

The dried solid SiNPs and Se-SiNPs nanocomposite were subjected to XRD analysis to determine their crystalline pattern, with the scanning range set between 10-80° 2 $\theta$  angles. Figure 7 illustrates the PXRD patterns of SiNPs and the hybrid nanocomposite Se-SiNPs. In the PXRD pattern of SiNPs, no sharp peaks are observed, and a broad band centered at 22° indicates the amorphous structure of SiO<sub>2</sub>. In the Se-SiNPs PXRD pattern, the broad shoulder between 2 $\theta$  values of 20-28° corresponds to amorphous SiO<sub>2</sub>,[39,40] while the peaks at 2 $\theta$  values of 19°, 29°, 55°, and 59° correspond to the (100), (101), (112), and (202) lattice planes of the crystalline Se standard hexagonal phase, respectively (JCPDS No. 06-0362).[41] Therefore, in the PXRD pattern of Se-SiNPs, the standard peaks of hexagonal crystalline selenium were observed at lower values compared to the

standard peaks of hexagonal crystalline selenium at 23°, 56°, and 62°. This peak shifting may suggest an expansion to higher lattice parameters of SiNPs due to the interaction between silica and Se nanoparticles, as well as a change in the size of the SiNPs during composite formation with Se.[42] In the Se-SiNPs PXRD pattern, both amorphous silica nanoparticles and crystalline SeNPs exhibited well-defined diffraction peaks, confirming the formation of the nanocomposite. The highest peak signal corresponding to the (101) plane indicated that it is the predominant plane in Se-SiNPs. Overall, the XRD patterns demonstrated that the Se-SiNPs composite possessed a highly pure amorphous-crystalline structure.

#### **SEM images of SiNPs and nanocomposite Se-SiNPs:**

The sizes and shapes of SiNPs (SR) and composite Se-SiNPs (SSR) were assessed using their respective SEM images. These images revealed that the size of SiNPs is approximately 24 nm in diameter, and they have a spherical shape. In contrast, the composite Se-SiNPs were found to have a diameter of about 189 nm and an oval shape that overlapped with each other. This increase in size for the composite Se-SiNPs compared to the 24 nm diameter of SiNPs was observed within the 0 to 3.5 keV dispersive energy frequency range of the SEM equipment (Fig. 8).

#### **Cytotoxicity effects and cell viability study of SiNPs (SR) and nanocomposite Se-SiNPs (SSR)**

The cytotoxic effects of nanoparticles inhibited the proliferation of the MCF-7 breast cancer cell line, depending on time and concentration in the MTT assay.[43] The primary cause of treatment failure in cancer and the leading cause of death among women with breast cancer is the development of chemotherapy resistance by the cancer cells.[44,45] Nanoparticle-based treatments offer several advantages, including reduced toxicity and the ability to target cancer cells.[46,47] Composite and functionalized nanoparticles have been widely employed in anticancer therapy.[48] The MTT assay was employed to evaluate the cytotoxic effects of nanoparticles on MCF-7 cells in terms of inhibiting diseased cell growth and cell proliferation. Analogues of adenosine can inhibit cell proliferation and induce apoptosis in estrogen receptor-positive breast cancer. Throughout the incubation process, the infected cells retained their original morphology.[49] Multicellular organisms rely on programmed cell death to maintain their integrity and homeostasis.[50] The cytotoxicity of the developed nanoparticles was demonstrated in the treatment of the breast cancer cell line MCF-7 with malignancies.[51,52] The viability of MCF-7 breast cancer cell lines was reduced using

nanoparticles.[53-55] Cells were transplanted to produce formazan dyes by reducing the tetrazolium component (MTT).[56,57] Cell viability was tested in the concentration range of 1-300 µM to determine the anticancer activity of SiNPs and the composite Se-SiNPs against MCF-7 breast cancer cell lines after 48 hours of exposure. The percentage of cell viability was evaluated at various concentrations of nanoparticles. A graph illustrating the relationship between concentrations and cell viability inhibition was plotted based on these analytical findings (Fig. 9). Figure 9 reveals that cell viability remains nearly 100% when SR and SSR are present at concentrations up to 100 µM. This suggests that these nanoparticles exhibit negligible anti-cancer efficacy at concentrations up to 100 µM. However, as the concentration increases further, it becomes evident that cell viability in the presence of SSR is four times lower compared to its absence. This suggests that at higher concentrations, these nanomaterials can strongly bind to DNA, inhibiting cell proliferation and subsequent growth. Additionally, cell viability was determined to be 68% with SSR compared to 94% with SR at a concentration of 300 µM. Based on the results of the cell viability study, it can be concluded that SSR is a more effective anticancer agent than SR.

#### **Conclusions**

The nanocomposite Se-SiNPs were successfully synthesized, and their sizes, surface area, and physical properties were confirmed using analytical techniques such as DLS, absorbance spectroscopy, XRD, and SEM. *In vitro* MTT assay was applied to the MCF-7 breast cancer cell line to measure cytotoxic effects and cell viability in the presence of SiNPs and composite Se-SiNPs, separately. The research findings indicated that the nanocomposite Se-SiNPs were more effective than SiNPs. Increasing the concentration of the nanocomposite Se-SiNPs resulted in substantially higher cytotoxic and anticancer activity against the MCF-7 breast cancer cell line. When compared to SiNPs, nanocomposite Se-SiNPs inhibited the proliferation of the MCF-7 breast cancer cell line. Although clinical investigations are required to uncover their potential in the field of medical sciences as anticancer therapeutic agents, nanocomposite Se-SiNPs may be employed to treat breast cancer and offer the potential for treating other types of cancer and preventing mortality.

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**Figure 1.** Representation of the synthesis of hybrid nanoparticles (SSR)

**Figure 2.** DLS studies and histogram with zeta potential of synthesized SiNPs

**Figure 3.** (A) SEM images of synthesised SiNPs with a diameter of 24 nm and (B) element distribution by EDS.

**Figure 4.** (A) Yeast extract mannitol broth (YEMB) + agrobacterium and (B) YEMB + agrobacterium + Na<sub>2</sub>SeO<sub>3</sub> and (C) SeNPs in 0.1-1.0 M Na<sub>2</sub>SeO<sub>3</sub> solutions

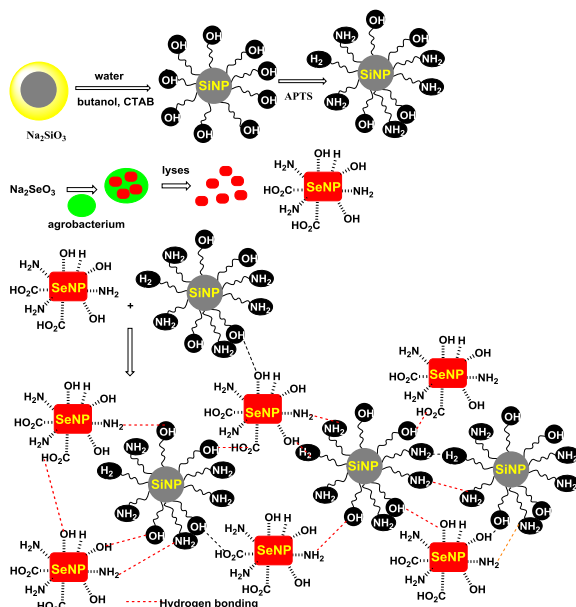
**Figure 5.** Dynamic light scattering (DLS) studies of Se-SiNPs and SiNPs (inset image)

**Figure 6.** The absorbance spectra of SiNPs and Se-SiNPs

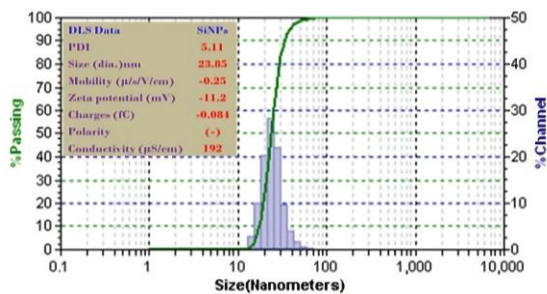
**Figure 7.** Powder XRD analysis of Se-SiNPs and SiNPs (inset image)

**Figure 8.** Scanning electron microscopy (SEM) images of the SiNPs (SR) and Se-SiNPs (SSR)

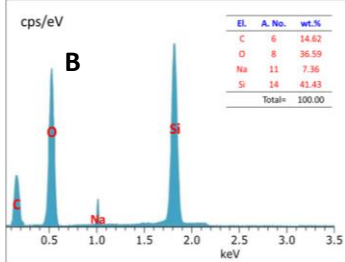
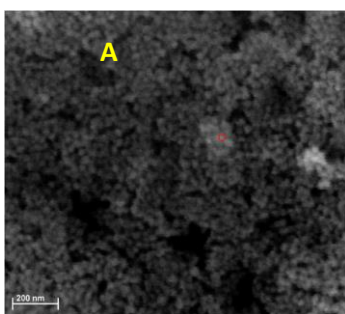
**Figure 9.** Cell viability study in the presence of SiNPs (SR) and Se-SiNPs (SSR)



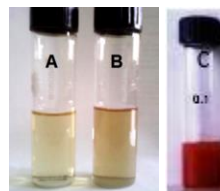
**Figure 1.** Representation of the synthesis of hybrid nanoparticles (SSR)



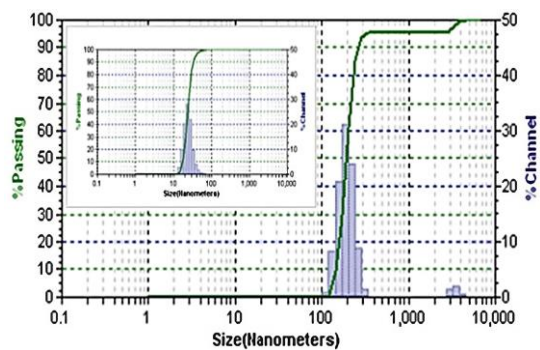
**Figure 2.** DLS studies and histogram with zeta potential of synthesized SiNPs



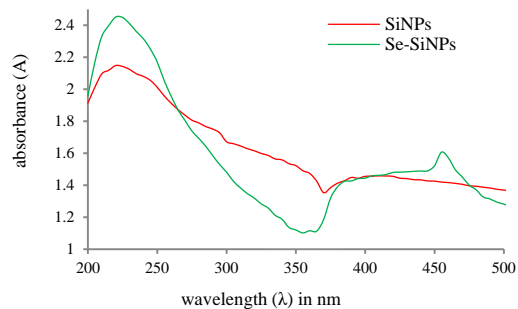
**Figure 3.** (A) SEM images of synthesised SiNPs with a diameter of 24 nm and (B) element distribution by EDS.



**Figure 4.** (A) Yeast extract mannitol broth (YEMB) + agrobacterium and (B) YEMB + agrobacterium + Na<sub>2</sub>SeO<sub>3</sub> and (C) SeNPs in 0.1-1.0 M Na<sub>2</sub>SeO<sub>3</sub> solutions

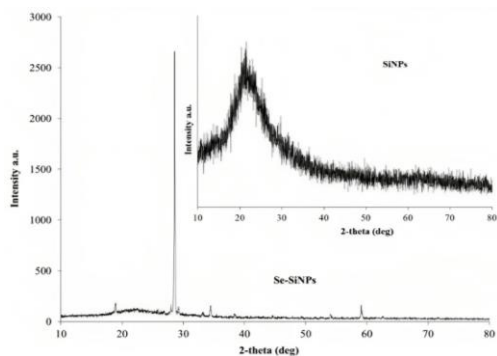


**Figure 5.** Dynamic light scattering (DLS) studies of Se-SiNPs and SiNPs (inset image)

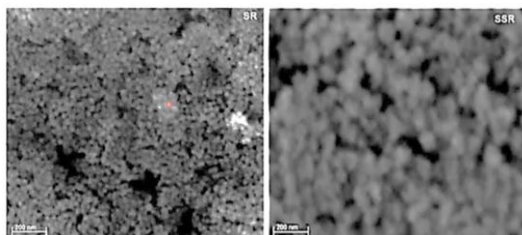


**Figure 6.** The absorbance spectra of SiNPs and Se-SiNPs

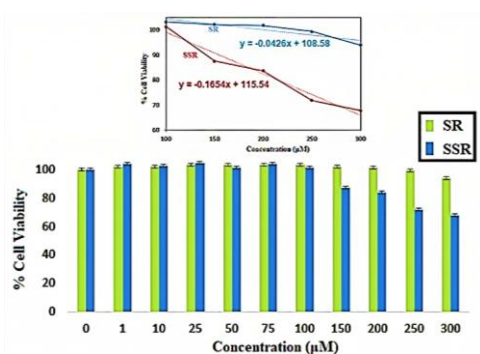




**Figure 7.** Powder XRD analysis of Se-SiNPs and SiNPs (inset image)



**Figure 8.** Scanning electron microscopy (SEM) images of the SiNPs (SR) and Se-SiNPs (SSR)



**Figure 9.** Cell viability study in the presence of SiNPs (SR) and Se-SiNPs (SSR)

### Biographies

**Anil Kumar** received his PhD from the School of Nanosciences, Central University of Gujarat, India in Nanoscience. He is currently a faculty member in the Department of Botany, Govt Tilak College, Katni MP. His research has focused extensively on the exploration of nanobiotechnology. He has published more than thirty research papers in various national and international journals.

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**Man Singh** is a senior professor in the School of Chemical Sciences, Central University of Gujarat. His research work includes the study of molecular engineering, molecular interaction, and surface chemistry. He is not only a devoted scientist but also invented several state-of-the-art instruments for measuring physicochemical properties of the molecules such as surface tension, viscosity, friccohesity etc. with the help of indigenous Survismeter, Econoburette and Visionmeter. These unique instruments glorify his dignity as a visionary scientist in the field of world science. He is the author and co-author of more than 220 scientific papers and a few books.

**Dhananjay Mondal** is an assistant professor in the School of Chemical Sciences at the Central University of Gujarat in Gandhinagar, India. Following his Ph.D. in synthetic organic chemistry from the National Chemical Laboratory in Pune, India, he spent more than four years in Canada and United States for pursuing advance research work in synthetic organic chemistry. His research interests include the exploration of synthetic routes of natural products, glycopeptidomimetics, and organo-nano conjugates for various biological applications. He has more than 55 research articles and six book chapters to his credit.