



# Selenium nanoparticles induce suppressed function of tumor associated macrophages and inhibit Dalton's lymphoma proliferation



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## ABSTRACT

Selenium Nanoparticle (SeNPs) is reported that it enhances and maintains optimal immune during infection and malignancies. To this end, we examined the role of selenium on TAMs whose anti-tumor function suppressed which favor tumor progression. BALB/c (H2d) strain of mice non-Hodgkin type of Dalton's cell line was used to check the role of carboxylic group induced, synthesized SeNPs on TAMs. Screening of IC50 value was done primarily trypan blue exclusion assay and 50% proliferation of DL cells inhibited 40 ng/ml to 50 ng/. Treatment also decreases  $\Delta\Psi_m$ , fragmentation of DNA of DL cells and arrest cells cycle in G1/G0 phase. Untreated TAMs cells showing suppressed expression of ROS, adhesion, phagocytosis, fusion and receptor profiling such as ICAM-1, CD47, CD172a. Which was induced more as compare to untreated group. SeNPs have potential to induce the anti-tumor function of TAMs whose anti-tumor function down-regulated pliable shifted towards tumor progression. It decreased the proliferation of DL cell by inducing apoptosis. Therefore, the synthesized SeNPs could be used for imaging diagnosis and cancer therapy which must be cost effective with negligible side effects shifted towards tumor progression. It decreased the proliferation of DL cell by inducing apoptosis.

## 1. Introduction

Selenium (Se) is an important micronutrient with several important aspects of human health such as cardiovascular health, prevention of neurodegeneration, proper thyroid hormone metabolism, preventing cancer progression and optimal immune responses [1]. It is a structural component of the active center of many antioxidant enzymes and functional proteins. Selenium mainly consists of two inorganic forms, selenite, and selenate [2]. Selenium containing protein (selenoproteins) plays an important role in chronic inflammation and initiation of immune response and thus regarded as good anti-cancer agent. The anti-cancer potential of the Selenium has been identify on different cancer cell line. Sufficient levels of selenium are important in regulating excessive immune responses and chronic inflammation [3–5]. Moreover, antioxidative effects of Se have been shown to protect phagocytic cells and surrounding tissues from oxidative radicals produced by the respiratory chain of neutrophils and macrophages during phagocytosis [6]. The deficiency of Selenium negatively affect immune cells activation during oxidative stress, protein folding and calcium flux [7,8].

It has been observed that CD34<sup>+</sup> originated lymphoid progenitor macrophages play an important role during encounter with the cancer

cells [9–11]. During tumor progression, macrophages migrated to the tumor site in the influence of vascular endothelial growth factor (VEGF), colony-stimulating factor (CSF)-1 and MIP-1 $\alpha$  present in to the cellular microenvironment [12]. Tumors are generally characterized by nutrient deprivation, hypoxia, acidosis and aberrant stroma, and consists both malignant and non-malignant cell types that include endothelial cells, fibroblasts, and various cells derived from the bone marrow. Tumor cells release several immunosuppressive factors and pro angiogenic cytokines [13,14] and growth factors, such as VEGF, TNF- $\alpha$ , TNF- $\beta$ , IL-1, IL-8 and bFGF (Basic fibroblast growth factor) that stimulate endothelial cell proliferation and promote the formation of [15–20] differentiated capillary tube. They also express a broad range of angiogenesis-modulating factors such as Matrix metalloproteinases (MMPs) and cyclooxygenase-2 that play a significant role in capillary formation and vascularization in the tumor masses. These tumor promoting factors further down-regulate macrophages function and change their functional phenotype M1 to altered M2 phenotype [15–24]. The M2 or tumor associated macrophages (TAMs) constitute about 10–50% of total tumor mass and serve as slaves for the tumor.

Previously, we have shown that TAMs are phenotypically and functionally altered populations of macrophages, characterized by

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decreased cytotoxic function, reduced surface receptor expression, and enhanced production of anti-inflammatory cytokines that polarizes the immune response to favors the growth of tumor cells [20–25]. Keeping the immunomodulatory function of selenium in mind, first we characterize and optimize the dose of chemically synthesized selenium nanoparticles (SeNPs) and use to induce TAMs isolated from DL-bearing mice. Results shows that selenium effectively induces the ROS generation, formation of macrophage polykaryon, expression of adhesion molecules (CD54 or ICAM-1), and fusion receptors (CD47 & CD172 $\alpha$ ) on TAMs. Further, we also found that SeNPs decrease the tumor cell proliferation.

## 2. Material and method

### 2.1. Reagents

Selenium powder, sodium sulphite, polyvinyl alcohol (PVA), MTT and Concanavalin-A were purchased from SigmaAldrich, Bangalore, India. RPMI 1640 culture medium was obtained from HiMedia, Mumbai, India. Foetal bovine serum (FBS) was obtained from Invitrogen, CA, USA. CD47, CD172a conjugated with FITC and CD14 conjugated with PE from eBiosciences, San Diego, CA, USA. LPS, DCFH-DA, PMA, RH-123, DAPI, Hoechst 33258 and Phalloidin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Na<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, formaldehyde, trypsin and acetone were purchased from Qualigens, Mumbai, India. Glutaraldehyde was obtained from Serva Electrophoresis, Heidelberg, Germany. All other chemicals otherwise stated were obtained from Qualigens.

### 2.2. Animals and tumor model

Inbred populations of BALB/c (H2d) strain of mice of either sex and at 8–12 weeks of age were used. Mice obtained from the Departmental Animal House, Banaras Hindu University, Varanasi, India, and housed in a pathogen-free specialized small animal facility with 12 h dark-light cycle. All the animals treated with utmost human care and had free access to food and water. For tumor system, healthy mice of either sex were injected intraperitoneally (i.p.) with  $1.0 \times 10^6$  non-Hodgkin type of Dalton's lymphoma (DL cells) in 0.5 ml sterile PBS and allow to grow for 18 days.

The experimental endpoint was 18th day of the tumor growth where more than 90% physical activity of the animals and ability to access food and water is lost. Mice were euthanized by cervical dislocation, a method authorized by Animal Ethical Committee, Banaras Hindu University Varanasi, India, and Animals Ethical Committee, Indian Council of Medical Research (ICMR), New Delhi for killing of experimental animals, and observed until all muscle activity and breathing has ceased for at least 120 s. No mice died before meeting the endpoints described.

### 2.3. Synthesis and characterization of SeNPs

#### 2.3.1. Synthesis of SeNPs by chemical method

SeNPs were synthesized as per previously reported method from our lab (8). In brief, Selenium powder (0.25 M) was added to the solution of sodium sulphate (0.50 M) in 100 ml of double distilled water and mixture was stirred at 70 °C for 9 h. A transparent sodium selenosulphate (Na<sub>2</sub>SeSO<sub>3</sub>) solution was obtained which was used as a precursor for the synthesis of the SeNPs. Sodium selenosulphate solution (0.002 M) was used in a separate Erlenmeyer flask and 0.005 M of acetic acid was added dropwise into sodium selenosulphate solution for the carboxylic group-induced synthesis of the SeNPs. Appearance of pink color preliminary confirmed the formation of the SeNPs. The synthesized SeNP was stabilized by 0.05 ml of 1% aqueous polyvinyl alcohol. This color indicated the synthesis of Se NPs in the solution. Further UV spectra were taken at different time interval. After synthesis

of selenium nanoparticle the whole supernatant was collected and centrifuged at 11,500 rpm for 15 min at 4 °C, supernatant was discarded and pellet was washed with distilled water thrice and final pellet suspended distilled water and sonicated, and lyophilized. After that powder was collected for performing analytical techniques.

#### 2.3.2. Ultraviolet-visible spectroscopic analysis

Synthesis of SeNPs by reducing the respective metal ions solution with Sodium citrate tribasic dehydrate (C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub>·2H<sub>2</sub>O) easily observed by UV–vis spectroscopy. The absorption spectrums recorded and quantify metal ion concentration using Hitachi U-2910 spectrophotometer in range from 300 to 1200 nm range. Synthesized SeNPs (200–350 nm) of diameter gave sharp peak in the visible region of the electromagnetic spectrum. Spectroscopy was performed at central facility of Department of Botany, BHU, Varanasi, India.

#### 2.3.3. Sonication and lyophilization

Samples were observed under UV–vis spectroscopy, synthesized sample further perform sonications which facilitate equal size fragmentation. In brief, sample were kept in the ice in 20 ml of centrifuge tube and provide ultrasonic vibration for the 2 min with the time gap of 30 s/cycle using SONIC, Vibra Cell sonicator at central facility of Department of Botany, BHU, Varanasi, India. Further sample was lyophilized using Christ Alpha 1–2 lyophilizer for preparation of powdered SeNPs for further standardization and characterization.

#### 2.3.4. Fourier transform infrared (FTIR) analysis

FTIR measurements of the powdered purified SeNPs were assessed using Perkin Elmer, Spectrum Two FTIR (Waltham, MA, USA) system in the diffused reflectance mode. In order to identify the possible reducing and stabilizing molecules in Sodium citrate solution, FTIR analysis was carried out. The spectral range from 400 to 4000 cm<sup>−1</sup> with resolution of 4 cm<sup>−1</sup> powder samples for the FTIR was similarly as for powder diffraction measurements. The FTIR spectra of base material (Sodium citrate solution) taken before and after synthesis of SeNPs were analyzed to study the possible functional for the formation of SeNPs.

#### 2.3.5. X-ray diffraction (XRD) analysis

To determine the structural characterization of SeNPs by using X-ray diffraction (X'Pert Pro PANalytical, ALMELO, Netherlands) at central facility of Department of Physics, BHU, Varanasi, India. To obtain XRD patterns of the SeNPs, NPs were subjected to XRD analysis at 45 kV and 40 mA with 2 $\theta$  in the range from 0° to 80° angle. This diffraction pattern is used to identify the specimen's crystalline phases and to measure its structural properties, size and orientation of crystalline profile.

#### 2.3.6. Thermogravimetric (TGA) analysis

The thermal stability of the SeNPs was measured by TGA. All samples were heated up to 950 °C with heating rate 30 °C/min. The initial weight loss up to 200 °C is attributed to the loss of physisorbed moisture. Thermal stability observation and characterization of samples were done at central facility of Department of Chemistry, BHU, Varanasi, India.

#### 2.3.7. Transmission electron microscopy (TEM) analysis

TEM analysis was performed to determine the morphology, size and shape of the selenium nanoparticles. TEM measurements were done by transmission electron microscope, Tecnai 20G2 FEI, Oregon, USA. The TEM grid was prepared by placing a drop of the bio-reduced diluted solution on a carbon-coated copper grid and later drying it under a lamp. TEM facility was using at central facility of Department of Physics, BHU, Varanasi.

#### 2.3.8. Scanning electron microscopy (SEM)

The surface observation of SeNPs samples were done by using

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