A Study of the effect of Porous Silicon Nanoparticles on Human Blood Components

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Abstract

The present work demonstrates the toxicity effect of(PSNPs)on blood components which are prepared by electrochemical etching and plus laser ablation (PLA) *Nd: YAG* laser method. We conformed the synthesis of porous silicon nanoparticles by using structures and optical properties through measuring absorbance of color and scanning electron microscope techniques. The study of toxicity effect of these nanoparticles on the blood human parameters (*in vitro)* used complete blood count (CBC). The results of hematology parameter (HCT-PCV); (PLT); (HGB –Hb); (RBCs); (WBCs); Count type white blood cells)are compared with the control groups, Our results shows no significant differences in levels (HCT-PCV); (PLT); (HGB –Hb) ; (RBCs); (WBCs); Count type white blood cells) between the test groups when compared with control groups. This result that there indicates no toxic effect of porous silicon nanoparticles in hematology parameter (*in vitro)*.

Keyword: porous silicon nanoparticles ,toxicity, blood cell.

Introduction

Biomedical nanoscience has an enormous similarity to get profits in analysis, treatment and identification of different diseases, with fewer sideways effects and well feature of life for the patients. [1] Porous silicon nanoparticles (PSNPs) have interesting optical properties, such as strong optical absorption and highly photo thermal effect with surface plasm on resonance. Their distinctive size-dependent characters make these supplies suitable for the supplement of biomolecules like antibodies /proteins, DNA and treatments. PS nanoparticles have recognized some promising effects, such as gene therapy vector [2] antitumor [3] antiviral [4] anti-inflammatory[5] and antibacterial effects[6]. However, most studies committed to PS nanoparticles have limited their experiments to *in vitro* toxicological assessments [7], Nonetheless of the management pathway the transit of PS nanoparticles through the blood flow are noticeable. [8] Subsequently most of the future uses of PS NPS are based on systemic administration, investigates on their interaction with human blood

components are highly needed. The knowledge of the degree to which damage on red blood cells following exposure to PS NPs subsidizes to the general toxicity of PS NPs is still incomplete [9]. The aim of study is the effect of porous silicon nanoparticles on human blood Components by using complete blood count (C.B.C) and studed toxicity of them.

Materials &Methods

- **Preparation of Porous silicon nanoparticles**

Porous silicon samples have been produced by the electrochemical etching process silicon wafers (100) oriented, boron doped, diameter(7.6cm), resistivity (1.5-4Ω).cm and thickness (550±50)nm. The used electrolyte was 1:1 (v/v) hydrofluoric acid (24%) and ethanol(99.9%).The Porous silicon films are obtained by anodizing the Si – wafers for 40 min with current density of (80mA/cm2). Where the HF container made from Teflon because the Teflon has high resistance from HF and to avoid any chemical reaction with HF. And the rubber O-ring is used before the upper part of cell. The latter has a central circular of ($5.5cm^2$) to allow touching the silicon wafer, and the two electrodes are used to apply current across the cell. The lower one is stainless still foil below silicon wafer and the upper one is gold mesh connected with the HF solution. The method is utilized for removing the porous layer from Si substrate by application of current density of (250 mA) /cm2 for 30 sec . After etching process the sample is rinsed with ethanol and pentane and dried film are then milled with ball mill to obtain nanoparticles. And washed twice with double distilled H_2O. The nanoparticles then dissolve with deionized water and put into the ultrasound device for 4 hours. The nanoparticles are then emitted through a filter that passes nanoparticles size up to the 1μm. Finally, nanoparticles prepared treated with post-laser under conditions (Nd -YAG laser Q-switching with wavelength (1064 nm), laser energy(350mJ) for the purpose to obtaine smaller size for silicon nanoparticles.

Characterization of PSNPs

Ultraviolet-visible Spectroscopy (UV-Vis).

UV-Vis spectrophotometer (Metertech, SP8001 spectrophotometer, Japan) is employed for the optical analysis of PSNPs, and colloided at different preparation conditions within the spectral range(250-1100 nm) for PSNPs,the instrument was used and achieved in Department of Applied Science, University of Technology

Scanning Electron Microscopy (SEM).

A scanning electron microscope (Nova-Nano SEM 430-USA) uses a collimated and focused beam of high energy electrons to produce image from a sample's surface. The PSNPs imaged by SEM microscopes

instrument was used and achieved in Department of Applied Science, University of Technology.

***The study of the Effect of PSNPs on Human Blood Components* by:-**

- ***Complete Blood Count test (C.B.C)***

Blood samples were collected from ten healthy human (men) (25 to 40 years old) in EDTA tubes in order to prevent clotting . Blood samples were treated with PSI at concentration (10µg/ml) for 1hr [10] compared with untreated samples. Followed by complete blood count test (C.B.C) and blood film. This experiment is done at AL-Always hospital. Baghdad.

- ***Staining Blood Film Sample:-***

1. Take drop of blood (control &test) in to slid glass and smear blood.
2. Giemsa stain (2 - 5 mint).
3. Washing & dried (10 mints).
4. Examined microscopically (100X) to inspect the effected PSiNPs on blood cell. This experiment done at in blood laboratory, biotechnology, department of Applied Science, University of Technology, Baghdad, Iraq.

Statistical analysis

Student's t-test was applied to all data and the difference between them was accepted to be statistically significant when $p < 0.05$.

Results & Discussion

The UV-Visible absorption spectrum of the synthesized composite nanoparticles prepared by electrochemical etching for pours silicon targets of measured in the wavelength region (250 – 1100 nm). Figure (1) shows the optical absorption spectra of pours silicon nanoparticles at concentration (10)µg/ml, that solutions containing PSiNPs produce a characteristic absorption peak in the 450 nm at consentraction10µg/ml, which represent the sharpest absorption peak PSiNPs was observed in 450 nm because Nano size less. UV-Vis spectroscopy is commonly used to examine the size and shape of nanoparticles in aqueous suspensions [11].

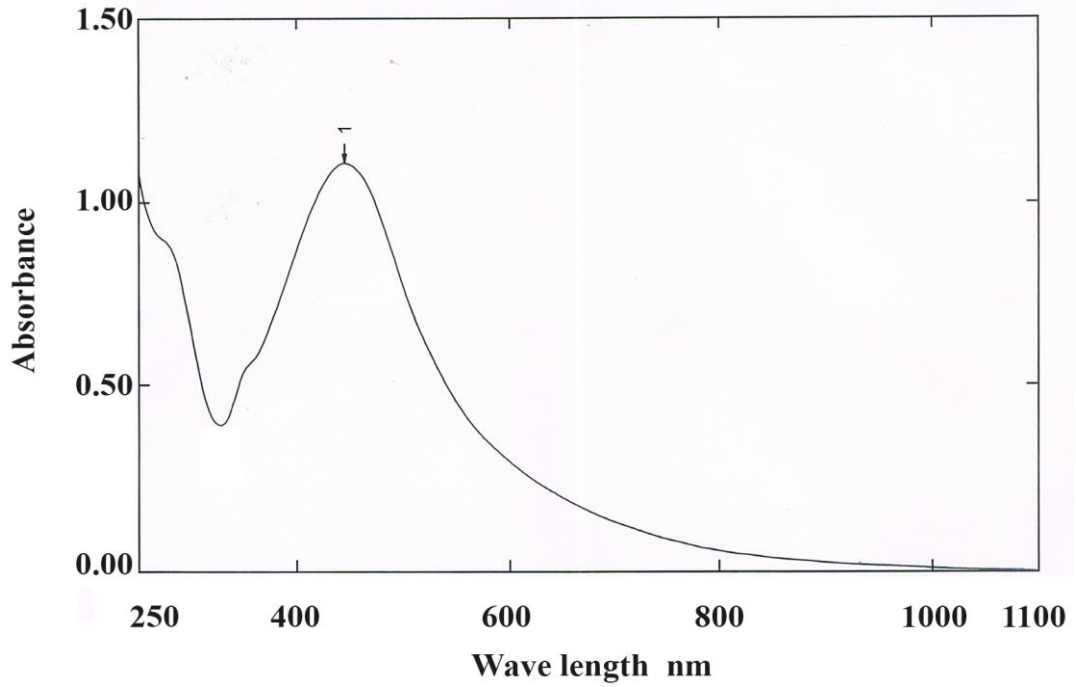

Fig. (1) UV-Vis spectroscopy of PSiNPs prepared by electrochemical etching method and laser ablation at concentration (10)µg/ml

Figure (2) shows the SEM image that determines the optimum morphology of the PSiNPs formed. The pores diameter, calculated by Image J software, ranged between (10 to 50 nm). An average pore distribution between (18 to 70 nm)in the histogram graph at concentration (10)µg/ml and average diameter(38.27 nm). The pores appear uniformly distributed throughout the structures suggesting an appropriated fabrication method and with some void spaces. Instead of spherical shapes elongated rod-like architecture with rough surface is noticed.

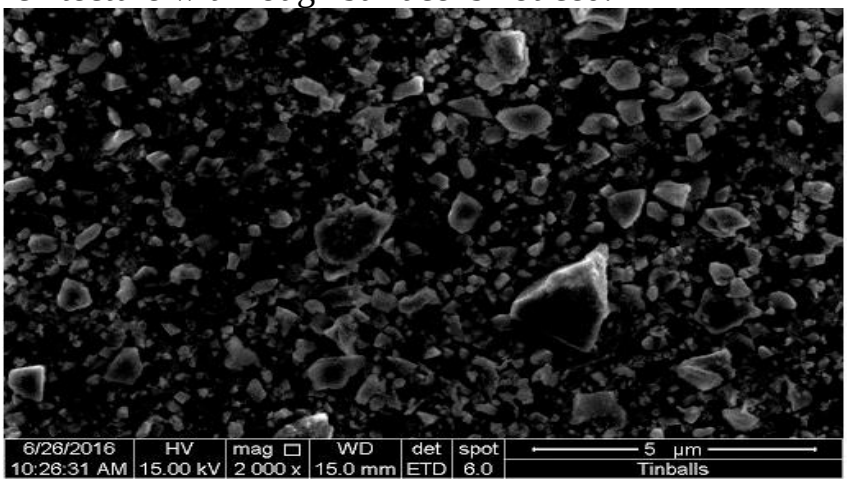

Fig. (2). SEM image of porous silicon nanoparticles prepared by electrochemical etching method and laser ablation at concentration (10)µg/ml

We studied the effect of PSiNPs on major human blood components in *vitro*, after 1hr incubated blood samples(control &test) at 37ºC. These examinations were not different either within or between groups. Blood parameters in Complete Blood Count (C.B.C.) at a concentration of (10 µl/ml) from PSNPs show the changes in hematological examination in the blood of human treated with PSiNPs the difference was not statistically significant . The results show mean values and SD of (**A**) HGB; (**B**) hematocrit; (HCT);(C) platelets (PLT); (**D**) red blood cells (RBC); (**E**) white blood cells (WBC); (**F**) count type white blood cells (WBCc). The results show no differences in treated and non- treated blood samples figures (3, 4, 5, 6, 7 and 8) respectively.

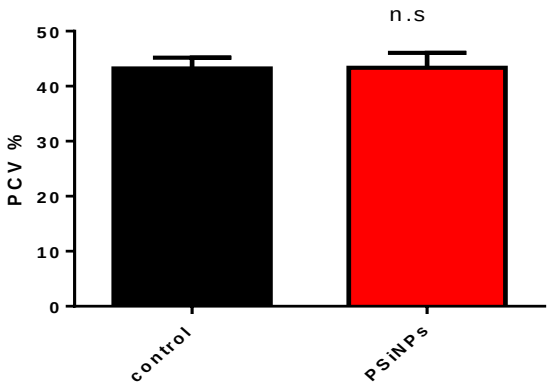

Fig. (3) PCV level in blood samples in presence and absence of PSNPs

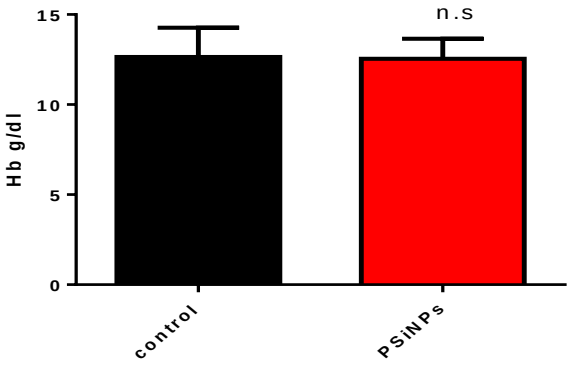

Fig. (4) Hb level in blood samples in presence and absence of PSNPs

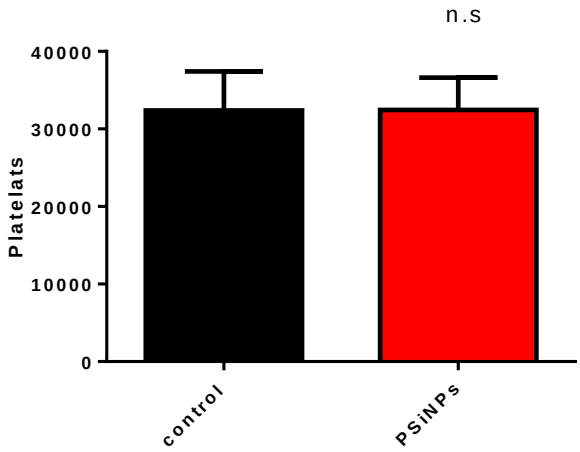

Fig. (5) Platelets count level in blood samples in presence and absence of PSNPs

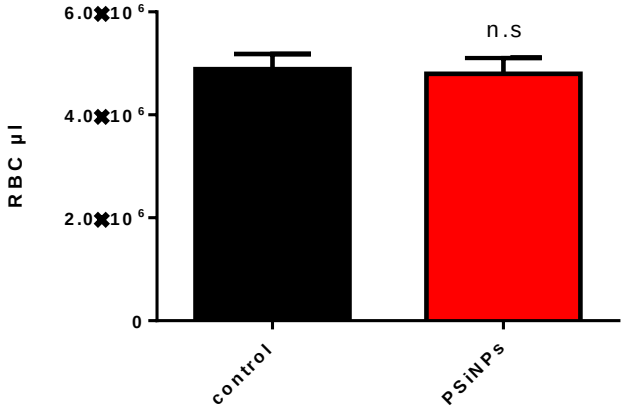

Fig. (7) RBCs count level in blood samples in presence and absence of PSNPs

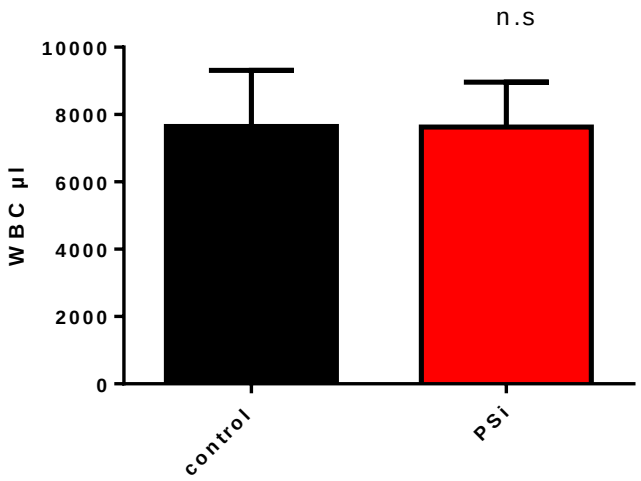

Fig. (6) WBCs count in blood samples in presence and absence of PSNPs

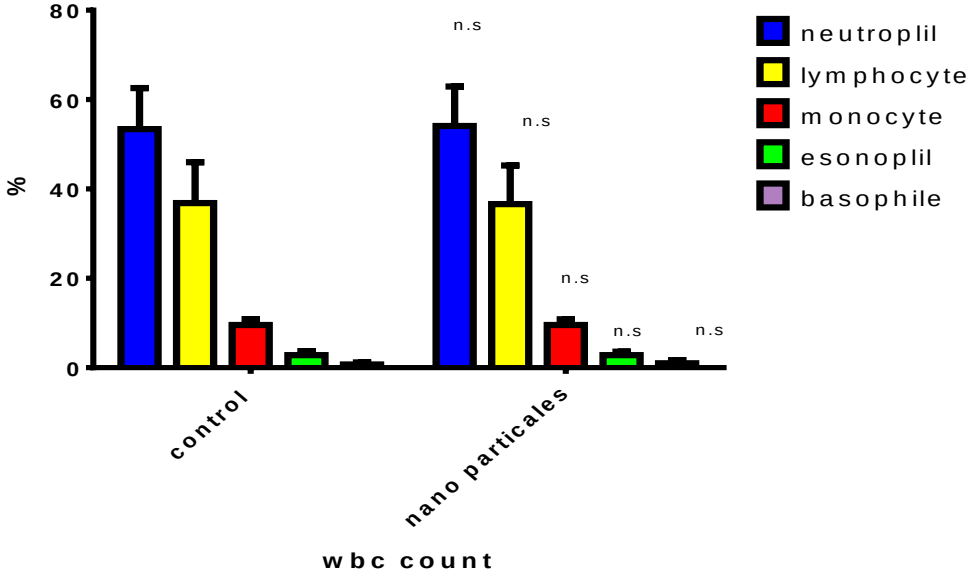

Fig. (8) Percentage of WBCs in blood samples in presence and absence of PSNPs.

We studied the effect of PSiNPs on blood cell components in *vitro*, after 1hr incubated blood samples (control &test) at 37ºC by staining blood Film and examined microscopically (100X) to inspect the effected PSiNPs on blood cell.

These examinations were not different either within or between groups show in figure(9).

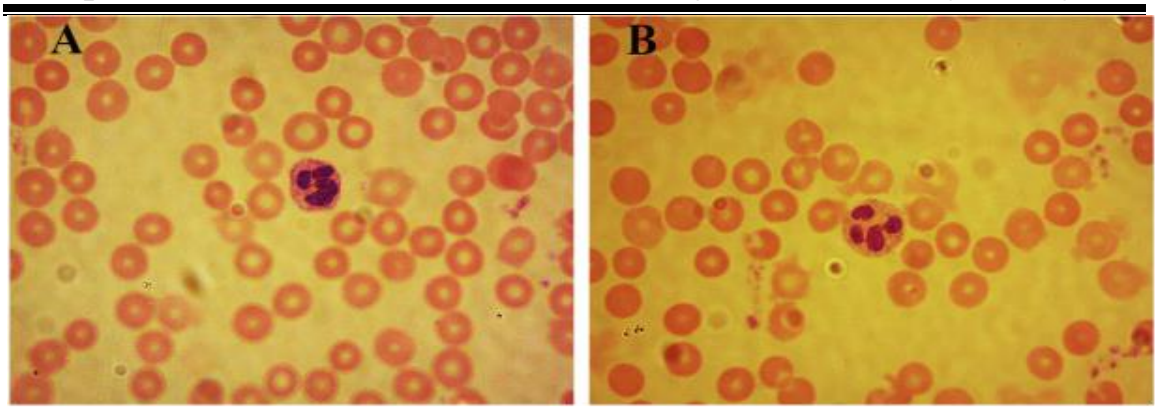

Fig . (9). A. Morphology of RBCs and WBCs in control group. , B. Pours silicon nanoparticles treated group .

The previous studies to assess the of PSNPs toxicity in *vitro* have been done [12]. The toxicity of PSNPs is dependent powerfully on physicochemical properties such as shape, chemical purity, particle size, porosity surface chemistry and solubility [13]. The nanoparticle chemistry, size, shape, agglomeration, surface and surface functionalization are major factors that influence the biokinetics of PS nanoparticles and consequently, their cytotoxicity. The mechanisms of antimicrobial activity of PS nanoparticles and their toxicity to human tissues are not fully characterized. [14] Blood cells are the main cells carrying oxygen to tissues. Using an *in vitro* system and human blood cells we calculated the capacity of cells incubated with PSnanoparticles to release increased quantities of ATP in response to acute contact. The results show an increase in ATP concentration following PS NPs treated. related to the increase in concentration. When Blood cells were rapidly to PSNPs, ATP release increased. There is no toxicity mechanisms obtained *in vitro* that may be compared with those likely to occur *in vivo*, as in *in vitro* studies one bolus dose is delivered, which is usually rationally high. [15] When PSNPs are administered in the blood stream, red blood cells are one of the first biological moieties being affected. Therefore, we design our study from a clinical viewpoint (ex: when Ag Nps are administered in systemic circulation) [16]. For a better kind of the concentration range, it was compared to previous studies [17]. Human blood exposure to PSiNPs, either accidental or considered when its biomedical properties are exploited, is unavoidable. Therefore, it is important to understand the parameters affecting PSNPs toxicity, especially in mammalian cells [18].

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دراسة تأثير الجسيمات النانوية للسيليكون المسامي على مكونات دم الانسان .

الخلاصة :-

صممت الدراسه الحاليه لمعرفة التاثيرالسمي للجسيمات النانوية للسليكون المسامى المحضرة بطريقة التنميش الكهروكيمياوي و الليزر النبضي (الانديك) على مكونات دم الانسان. تم الاستدلال على الحصول على الجسيمات النانوية للسيلكون المسامي باستعمال الخواص التركيبة والبصرية وذلك من خلال قياس الامتصاصية اللونية واستعمال تقنيات المجهر الالكتروني الماسح. تم دراسة تاثير تلك الجسيمات النانوية للسيليكون المسامي على كريات دم الانسان باستعمال جهاز العد الكامل لمكونات الدم مقارنة مع مجموعة السيطرة باستخدام انابيب الاختبار الحاوية على المادة المانعة للتخثر .اظهرت النتائج عدم وجود فروق معنوية في مستوى مكونات الدم بين مجاميع الاختبار بالمقارنة مع مجموعة السيطرة. تشير النتائج الى عدم وجود تاثير سمي لجسيمات السيليكون النانوية لمكونات الدم خارج الجسم الحي .

الكلمات المفتاحية :- السليكون المسامي النانوي ,السمية ,خلايا الدم .