Research Article

Toxicity of Porous Silicon Nanoparticles on Liver of Mice

Kareem H. Jwaid[1*], Majid S. Jabir[2], Uday M. Nayef[3]
[1]Departments of medical of engineering ,University of Technology, IRAQ
[2]Departments of Applied Sciences biotechnology Branch, University of Technology, IRAQ.
[3]Departments of Applied Sciences Laser Physics Branch, University of Technology, IRAQ.
*Correspondent Author Email: karimuot@yahoo.com

ArticleInfo

Received
22 Feb. 2017

Accepted
12 Jun. 2017

Abstract

Nanoparticles are a special group of materials with unique features and extensive application in diverse fields. The present work demonstrates the toxicity impact of porous silicon nanoparticles (PSNPs) on kidney parameter which is prepared by electrochemical etching method. the synthesis of porous silicon nanoparticles are conformed by using structural and optical properties from through scanning electron microscope and atomic force microscopy techniques. The effect of toxicity of these nanoparticles on the liver parameters in laboratory animals use four groups each groups involve three duplicities was studied. Injected of porous silicon nanoparticles in the intraperitoneal at concentration of 1mg/kg. The results of biochemical assay Aspartate Amino-Transferase (GOT), Alanine Amino-Transferase (GPT), Alkaline Phosphatase (ALP) were compared with the control groups, for four weeks and then confirm a result was made with Histological study for section of liver. Results show no significant differences in levels (GOT, GPT, ALT) among the test groups *via* comparison with controls groups. This Result indicates no toxic effect of porous silicon nanoparticles' on kidney parameters.

Keywords: porous silicon nanoparticles, Toxicity, biochemical assay

الخلاصة

ان الاجسام النانوية هي المواد ذات مواصفات فريدة وتطبيقات كبيرة في مختلف المجالات. صممت الدراسة الحالية لمعرفة التاثيرالسمي لجسيمات السليكون المسامى النانوية المحضرة بطريقة التنميش الكهروكيميائي. تم الاستدلال على الحصول على الجسيمات النانوية للسيلكون المسامي باستخدام الخواص التركيبة والمظهرية وذلك باستخدام تقنيات AFMو SEM. تم دراسة تاثير تلك الجسيمات على الفعالية الكبدية للحيوانات المختبرية من خلال استخدام اربع مجاميع من الحيوانات التجريبية (الفئران) وكل مجموعة تحتوي على ثلاث مكررات, تم حقن الحيوانات المختبرية بمنطقة الغشاء البيرتوني بتركيز (1) ملغم /كغم. اجريت الاختبارات الكيميائية الحياتية (GOT, GPT, ALT)مقارنة مع مجموعة السيطرة ولمدة اربع اسابيع وتم تاكيد النتائج بالفحص النسيجي لمنطقة الكبد.اظهرت النتائج عدم وجود فروق معنوية في مستوى (GOT, GPT, ALT) بين مجاميع الاختبار بالمقارنة مع مجموعة السيطرة. النتائج تشير الى عدم وجود تاثير سمي لجزيئات السيليكون النانوية.

Introduction

Porous silicon nanoparticles (PSNPs) have been widely developed for different biomedical uses, for example cancer cell imaging, iosensors, drug delivery, radiother-apy and photothermal therapy because of their easy surface modification as well as optical and electronic properties [1]. PSNPs are presently used in the treatment of rheumatoid arthritis and cancer [2]. Usually, porous silicon (PS) has been considered inert and biocompatible. Nanoparticles possess single properties unlike from bulk-sized materials, involved volume ratios to large surface area, strong interaction with biological conditions, in height reactivity, and related to their less size. The studies on the toxicity of PSNPs have been described in vitro and in vivo [3]. Conflicting results have been demonstrated, depending on experimental conditions, such as particle size, cell lines, concentration, animal models and surface chemistry used [4]. Toxicity of PSNPs (13.5 nm) was also recognized in mice by intraperitoneal injection ,oral and intravenous,

Copyright © 2017 Authors and Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

respectively, viewing that tail vein injection of PSNPs had fewer toxicological effects compare oral or intraperitoneal injection [5]. Actually, record toxicity studies in vivo of PSNPs have been examined by intravenous injection or intraperitoneal, although its toxicity by oral exposure has not been widely explored. The application potential of PSNPs in therapeutic agents or oral delivery carriers has been focused on and requirement further verification on their oral toxicity [6]. Biokinetic activities of PSNPs, including tissue distribution design and oral absorption efficiency have not been well determined, which can provide major information on their potential toxicity and biomedical at harmless levels. The study was designed to comparative assessment *in vivo* toxicity of PSNPs in the liver of mice. Biochemical examinations at the four week after exposure were made.

Materials and Methodology

Porous silicon samples were prepared from single crystalline orientation of p-type silicon wafer (100) and with resistivity 1.5-4Ω.cm (Germany made) and the thickness of silicon wafer is (550±50μm). All chemicals, hydrofluoric acid, ethanol alcohol, were purchased from Sigma Aldrich (Malaysia) [7].

Before electrochemical etching method, the silicon wafers were washed with alcohol (ethanol) and action to remove dirt followed by dilute (24%) hydrofluoric PS layer wafer by free standing, washing and dry, milling system, mixed 0.1 g powder ps with (2.5 and 25) ml DDW, flittering 220nm, ultrasound system (4 hrs.; replicate 4 times) and posting by high power laser (Nd: YAG laser) [8].

A pulsed Q-switched Nd: YAG laser system at 1064nm wavelength with maximum energy 350mJ per pulse was used for treatment the samples. The output pulse duration is 9 ns and repetition rate 1 Hz. Beam diameter of 2.4 mm was used for post laser.

For scanning electron microscopy (SEM), SEM a collimated and focused beam of high energy electrons to produce images from a sample's surface were used. The PSNPs imaged by SEM microscopes (Nova –Nano SEM 430-USA) instrument was used and achieved in Department of Applied Science, University of Technology.

The morphological properties study of porous silicon (PS) sample gave a direct surface image of PS layer. Imaged *via*, (AFM) (CSPM-AA3000), instrument was used and achieved in department of Chemistry - University of Baghdad.

Albino male mice were supplied by the Biotechnology Research Centre (Al-Nahrain University). Their ages at the begging of experiments were 8-10 weeks, and their weights average was 35-45 grams. They were distributed in to five groups each group contains three mice, and each were kept individually in a separate plastic cage (details of these groups are given in the section of experimental design).

The animals were maintained at room temperature, and had free excess to food and water. In this experiment, 1 mg/kg was the dose of PSNPs, and assess the toxicity effects of this dose, the mice were distributed into five groups, and each group contains three animals (total: 15 mice):

Group I: Mice were administrated with physiological saline (Negative controls.)

Group II: (Injection PS NPs), Killing after a week.

Group III: (Injection PSNPs), Killing after two weeks.

Group IV: (Injection PSNPs), Killing after three weeks.

Group V: (Injection PSNPs), Killing after four weeks.

End of the experiment, mices were killed and the blood was collected *via* trans cardiac puncture (6 hrs.) after injection of the test compound. Serum collected by centrifuging blood sample at (6000) rpm for ten minutes and stored at (-20) C° keep used.

For the designed purpose, the following biochemistry tests such as Aspartate Amino-Transferase (GOT) Alanine Amino-Transferase (GPT) and Alkaline Phosphatase (ALP), and the sections of liver by a pathologist were done. For assessment of hepatic effects, Alanine Amino-Transferase GPT the enzyme activity of ALT was determined in the mouse liver tissue homogenate following the

enzymatic colorimetric method. For this purpose a commercial kit (Randox Company) was used. The absorbency was measured at 546 nm.

Aspartate Amino- Transferase GOT (AST) the enzyme activity of AST was determined in the mouse liver tissue homogenate following the enzymatic colorimetric method. For this purpose a commercial kit (Randox Company). The absorbency was measured at 546 nm.

Alkaline Phosphatase (ALP) the enzyme activity of ALP was assessed in mouse liver tissue homogenate using a commercial kit by Randox Company and the most commonly used method is that of Belfielda and Goldberg (1971), in which di-sodium phenyl phosphate is hydrolyzed with liberation of phenol and formation of sodium phosphate .The amount of phenol formed is estimated calorimetrically. The absorbency was measured at 510nm Calculations: The following equation was employed to assess the activity of ALP (Unit /ml):

$$\text{ALP} = \frac{\text{Sample absorb} - \text{Blank absorbance}}{\text{Standard absorbance}} \quad \ldots (1)$$

Statistical Analysis

Grouped data were statistically evaluated using $p \leq 0.05$ is considered significant. T-test and Values are representing as the mean Standard deviation (±S.D.) of the three triplicates of all investigate.

Results and Discussion

The surface morphology characterization of the PSNPs layers was studied by (AFM). Further the surface proprieties of the PS layers prepared *via* electrochemical etching was done as shown from the AFM graphs could be differentiated. A like sponge- structure was formed, as shown in Figure 1, 2 and3. The surface morphology of the PSNPs is known to be complicated and strongly depends on fabrication conditions. The etching time and current density used to control the shape and size of the final structures and histogram.

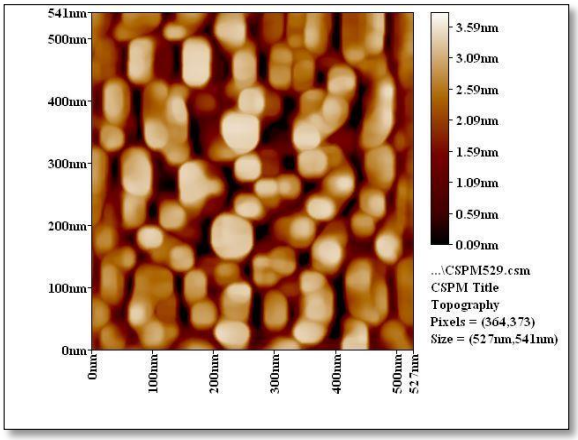

Figure 1: Show 2D AFM image for surface graphical of PSNPs, by electrochemical etching method and use laser pulse.

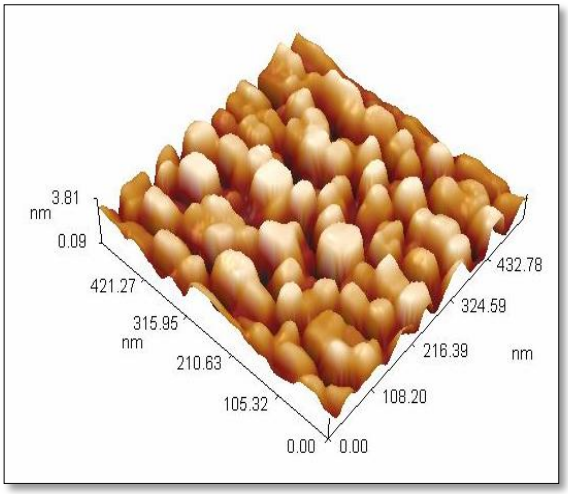

Figure 2: Show 3D AFM image for surface graphical of PSNPs, by electrochemical etching method and use laser pulse.

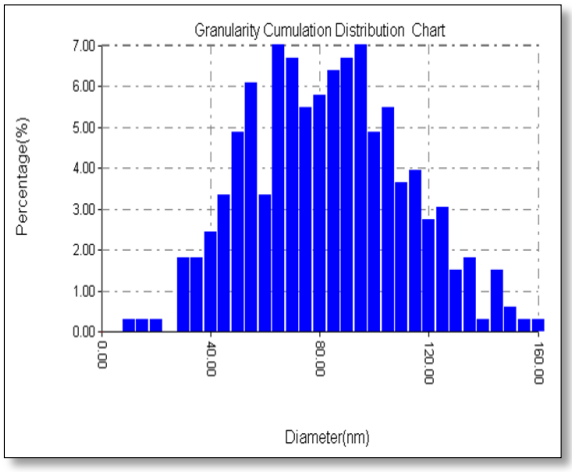

Figure 3**:** Show histogram image for surface graphical of PSNPs, by electrochemical etching method and use laser pulse.

Copyright © 2017 Authors and Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

Figure 4 shows the SEM image to determine the optimum morphology of the PS formed. The pores diameter, calculated by Image J software, ranged between 50 to 150 nm. The pores appear uniformly distributed throughout the structures suggesting an appropriated fabrication method. SEM image showed the uniform surface with some void spaces. Instead of spherical shapes elongated rod-like architecture with rough surface is noticed.

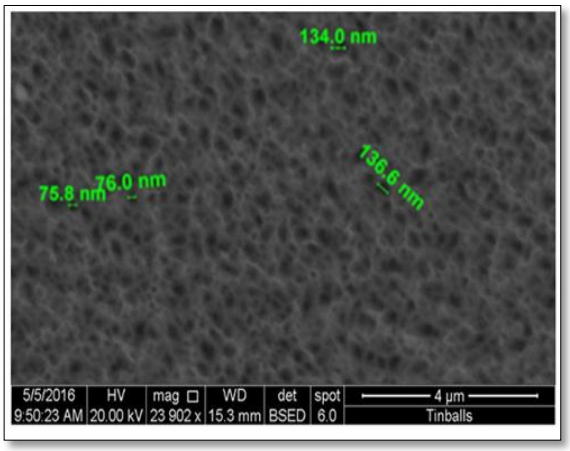

Figure 4: SEM image of porous silicon. Biochemical examination in mice treated with PS NPs at a dose of (1) µg/kg.

The results showed have mean values and standard deviations (SD) of GPT, GOT, and ALP, as shown in Figure 5, 6 and 7 respectively. The results are shown no difference among the groups and the variance was no statistically significant.

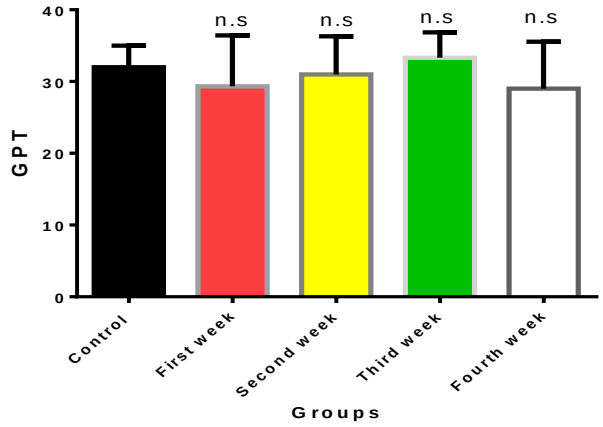

Figure 5: GPT level in mice treated with PS NPs at a dose of (1) µg/kg.

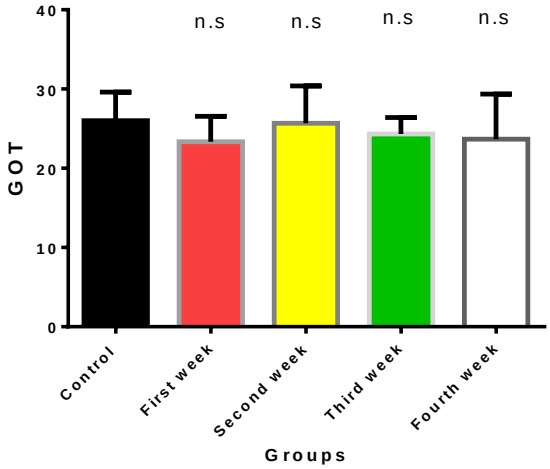

Figure 6: GOT level in mice treated with PS NPs at a dose of (1) µg/kg.

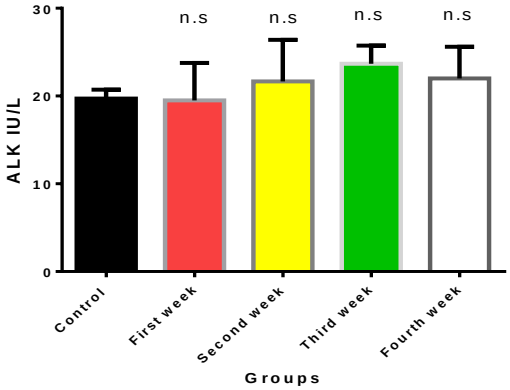

Figure 7: Biochemical assay serum markers (ALK) in mice treated with PS NPs at a dose of 1 µg/kg.

The results of histology are shown no differences between control group and treated groups. Figure 8 shows the normal structure appearance of liver in mice after PSNPs treatment, which consists of central vein and thread arrangements of hepatocytes.

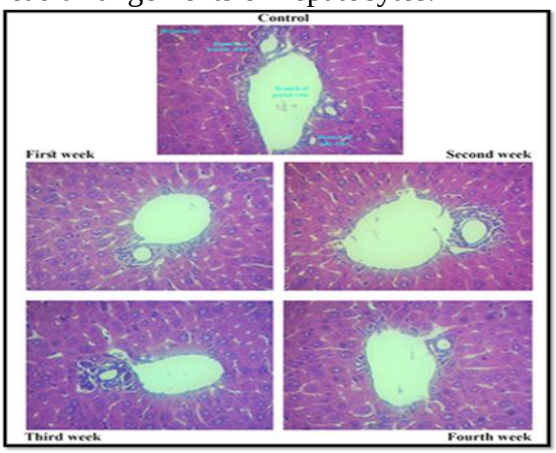

Figure 8: Histological examination of Liver after 1 week - 4 week intraperitoneal injection of PSNPs at dose 1 mg/kg.

Biocompatibility and biodegradability of PSNPs *in vivo* were examined. The PS NPs preparation is relatively non-toxic *in vivo* within the examination concentration range. This result compared with the slow removed normally observed for other kinds of inorganic nanoparticle [16]. By terminating a period of 4 weeks, *In vivo* studies, PS NPs (1 µg/ kg) were injected intraperitoneal into mice. However, the PS NPs collected in the organs are noticeably removed from the body within a period of first week and totally removed in four weeks. The mice of porous silicon nanoparticle internalization have been shown to be determined by particle size [17]. Some factors are thought to have a profound effect on the development of mediated toxicity nanoparticle. The key factor is nanoparticle diameter, which, at minimum for colloidal nanoparticle, inversely associated with the surface area. Special toxicological effects of PSNPs in mice were more established by histopathological investigation. Figure (5) shows that no atypical in histopathological findings were shown in liver after 4-week injection of PSNPs. This result clearly shows that PSNPs did not cause toxic effects. Pathological damage associated to the test materials was not shown in every treated group. Porous silicon nanoparticle used in the present study did no source severe toxicity in mice.

Conclusions

Toxicity of colloidal PSNPs was assessed in mice and *in vivo* after 4-weeks. Results injection that PSNPs did not cause cytotoxic effects after 4- weeks. *In vivo* testing indicated that PSNP did not cause toxicological effects on mice following 4 week consecutive injection. Further toxicity study of PSNP after long-period revelation is essential to confirm its low toxicity

References

[1] Xie, J.; Lee, S. and Chen, X., Nanoparticle-based theranostic agents. Adv Drug Deliver Rev . Vol (62), 2010, pp 1064-1079.

[2] Couvreur, P., Nanoparticles in drug delivery: past, present and future. Adv Drug Deliv . Vol(65), 2013,pp21-23.

[3] Choi, H. S.; Park, S.; Zhong, G. Renal clearance of quantum dots. Nature Biotech. Vol(25), 2007,PP1165-1170 .

[4] Wang, R. B.; Billone, P. S., and Mullett, W. M., Nanomedicine in action: an overview of cancer nanomedicine on the market and in clinical trials. J. Nanomater .2013, PP 629-681.

[5] Liu, Z.; Maniya, N. H.; Patel, S. R.,and Murthy, Z. V. P. Circulation and long-term fate of functionalized, biocompatible single-walled carbon nanotubes in mice probed by Raman spectroscopy .Proc. Natl Acad. Sci. USA. Vol (105), 2008, PP1410-1415.

[6] Ballou, B.; Lagerholm, B. C.; Ernst, L.; Bruchez, M. P. and Waggoner, A. S. Noninvasive imaging of quantum dots in mice. Bioconjugate Chem . Vol(15), 2004,PP79-86.

[7] Maniya, N. H.; Patel, S. R.; Murthy, Z. V. P., Electrochemical preparation of microstructured porous silicon layers for drug delivery applications. Super lattices and Microstructures. Vol (55), 2013, PP144-150.

[8] Yang, G. .Laser Ablation in Liquids Principles and Applications in the Preparation of Nanomaterials, Singapore Pan Stanford Publishing, USA. 2012. PP240-248.

[9] Kim, D.; Park, S.; Lee, J. H.; Jeong, Y. Y. and Jon, S. Antibiofouling polymer-coated gold nanoparticles as a contrast agent for *in vivo* X-ray computed tomography imaging. J. Am. Chem. Soc. Vol(129), 2007, PP7661-7665.

[10] Xie, G.; Sun, J.; Zhong, G.; Shi, L.; Zhang, D. Biodistribution and toxicity of intravenously administered silica nanoparticles in mice. Arch. Toxicol. Vol (84),2010,PP183–190.

Copyright © 2017 Authors and Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.