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In Vitro Cytotoxicity Study of Pt Nanoparticles Decorated TiO₂ Nanotube Array

Shaymaa R. Baqer^{1*} *Abdulkareem M. Ali Alsammaraie*² *Mahasin Alias*¹
*Mohammad M. F. Al-Halbosiy*³ *Amaal S. Sadiq*¹

¹Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad Iraq

²Department of Chemistry, College of Science, University of Baghdad, Baghdad-Iraq,

³Biotechnology Research Center, Al-Nahrain University, Baghdad-Iraq,

*Corresponding author: *shyma0213@gmail.com, karim.alsamuraee@gmail.com, mahasinfa_chem@csw.uobaghdad.edu.iq, ma8jed@yahoo.com, amaalsameer74@gmail.com

*ORCID ID: <https://orcid.org/0000-0002-7150-165X>, <https://orcid.org/0000-0002-3983-1608>, <https://orcid.org/0000-0002-3375-1797>, <https://orcid.org/0000-0003-1373-4188>, <https://orcid.org/0000-0002-5664-4767>

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Abstract

Titanium dioxide nanotubes were synthesized by anodizing Ti sheets in the ethylene glycol solution and were covered in Pt nanoparticles onto the surface of TiO₂NTs using electrodeposition method from using five derivatives of Mannich base Pt complexes which have been used as precursor of platinum. The mean size, shape, elemental composition of the titanium dioxide nanotubes and platinum deposited on the template were evaluated by different techniques such as field emission scanning electron microscope (FE-SEM), transmission electron microscopy (TEM), X-ray diffraction pattern (XRD), and energy dispersive X-ray (EDX) technique. From all these analyses, the TiO₂NTs prepared and Pt nanoparticles deposited on it were identified. The diagnoses proved that all the Pt nanoparticles have a size less than 50 nm. The MCF-7 cancer cell lines and WRL68 normal cell lines were treated with concentration 800, 400, 200, 100, 50, 25, 12.5 μg/ml of TiO₂NTs and Pt/TiO₂NTs(1) and (2) for 48 hours using MTT assay. IC₅₀ and inhibition rate were calculated. The result shows that the Pt/TiO₂NTs have more inhibition effect on cancer cell lines than TiO₂NTs array.

Key words: Electrochemical deposition, Platinum nanoparticles, Titanium dioxide nanotubes

Introduction:

Most recent research on titanium dioxide nanotubes interested in the doping or deposition of metal ions like chromium, iron, cobalt, nickel, copper, palladium, platinum, silver, and zirconium (1) and like boron, carbon, nitrogen and fluoride as non-metal in addition to using the metal oxides such as manganese dioxide had increased its applications in several fields (2). Among the varied nanostructured oxide materials, special attention has been directed toward TiO₂ nanotubes as result of developing some feature like low-cost and it has a large surface area compared to the volume (3). The titanium nanotubes are used as catalysts in accumulation boiling, photocatalysis (4), electrochromic device (5), resistant to corrosion (6), H₂ gas generation (7), solar cells (8), sensors, memory device (9), catalyst support (10), wastewater. Conjointly consistent with several

researchers, the titanium oxide nanotubes could have been employed in drug-eluting stents and for the native unleash of antibiotics, drug delivery in cancer and tumor therapy. It is also widely used in dental implants and bones (11). Using titanium nanotubes in nanomedicine has a promising future in treating many diseases because improving cell adhesion, growth and differentiation (12,13), as well as its use in drug delivery. Later findings proved the strong relation between the cell responses and nanotube dimensions (14). Some of methods used to improve the performance of TiO₂NTs are to load nanotubes with some antibiotics such as vancomycin (15) or decorate the surface of TiO₂NTs with different nanoparticles such as gold (16) and silver (17). Platinum medicine is still one of the most important treatments for

cancer, one of the most effective materials in the treatment of human cancers in particular (18).

Platinum nanoparticles have many properties that can be used in practical applications, including the manufacture of electronics and internal electrodes (19), durable proton exchange membrane fuel cells and biology and biochemistry applications (20). In this study five Mannich base Pt(IV) complexes were used as a source of platinum instead of platinum salt ($H_2PtCl_6 \cdot 6H_2O$) because the ligand prevents the aggregation of Pt nanoparticles when decorated on the surface of nanotubes. In this research titanium nanotubes were prepared and different sizes of Pt nanoparticles deposited on the surface of titanium nanotubes using different deposition times 3 minutes when using PtL_1, PtL_2, PtL_3 and PtL_5 and 6 minutes when use PtL_4 at the fixed concentration to show the effect of the size and density of platinum nanoparticles and their effect on inhibition of cancer cells compared to nanotubes alone by using MTT method in 620nm. This research aims to synthesis Pt nanoparticles of different size from Mannich base platinum complexes as a source of platinum after deposition on TiO_2 NTs and study the cytotoxic effect of these nanomaterials on breast cancer cell lines.

Material and Methods

All chemicals were purchased from commercial sources $H_2PtCl_6 \cdot 6H_2O$ (99.9%), S-(1-(benzothiazole-2-ylamino) methyl]-H-benzimidazole-2-yl)4- nitrobenzothioate (L_1), S-(1-(Pyrazine-2-carboxamido) methyl)-1-H-benzimidazole-2-yl)4- nitrobenzothiolate (L_2), N-((2-((Morpholinomethyl)thiol)-1H-benzimidazol-yl)methyl) pyrazine-2-carboxamide (L_3), 2-(Morpholin-N-methyl)mercapto-1H-benzimidazole (L_4), S-(1-Morpholinomethyl)-1-benzimidazol-2-yl)4-nitrobenzothioate (L_5). NH_4F (99.5%), ethylene glycol 99.8% and Ti, Pt foil (99.6, 99.99%) and thickness 0.25mm. Solvents and reagents were used as received. The nanostructures were characterized by SEM, TEM, XRD, EDX, FT-IR. Transmission Electron microscopy (TEM) was recorded on Philips CM (10). EDS. Atomic weight and atomic number of all prepared nanoparticles were carried out by energy dispersive X-ray spectroscopy (EDS) XFlah6-10 Detector –Bruker. X-ray diffraction was measured using Shimadzu ray 6000.

Preparation of Complexes

Preparation of Trichloro S - (1 -(benzothiazole - 2 -ylamino) methyl]- H -benzimidazole-2-yl)4-nitrobenzothioate Patinum (IV).

Chloride.0.5hydrate (PtL_1), Trichloro S-(1-((pyrazine-2-carboxamido) methyl)-1-H-benzimidazole-2-yl) 4-nitrobenzothioate Palatinum (IV). Chloride Ethanol (PtL_2), Trichloro N-((2-((Morpholinomethyl)thiol)-1H-benzimidazol-yl)methyl) pyrazine-2-carboxamide) Platinum (IV). Chloro. Hydrate (PtL_3). Dichlorobis (2-(Morpholin-N-methyl)mercapto-1H-benzimidazole) Platinum(IV). Dichloride .Hydrate (PtL_4). Dichloro Bis S-(1-Morpholinomethyl)-1-benzimidazol-2-yl)4-nitrobenzothioate Platinum (IV). Dichloride.Dihydrate (PtL_5). The Pt complexes were prepared according to the literature (21, 22) The Mannich bases reaction occurs in ethanol with platinum salt, 1:1 and 1:2 molar ratio for L_1, L_2, L_3 and L_4, L_5 respectively. The mixture was then refluxed for (3 hours.); the color solid complexes were formed, and then filtered, washed with ethanol and dried in desiccator

Preparation of TiO_2 Nanotubes

Titanium dioxide nanotubes was prepared according to the literature (8). Titanium foils were cut into the suitable size (1×2 cm²). A direct current power supply (matrix E3612A) was utilized as the voltage source for the anodization. The anodization process was executed in a homemade plexiglass cell with two electrode arrays; titanium foil as the working electrode and Pt mesh utilized as the counter electrode in constant potential at 25°C. The distance between the substrates and the counter-electrode was approximately 1.5 cm. Degreased by sonication in detergent, deionized (DI) water, ethanol and acetone respectively for 10 minutes dried in an oven at 100 °C for 15 minutes. For the anodization process, the electrolyte used was 0.5 wt% ammonium fluoride (NH_4F), (99.5%) in anhydrous ethylene glycol (99.8% of purity at room temperature. The anodized substrate was then soaked in a water bath at 40 °C for 20 minutes to remove the organic electrolyte. The anodization was performed for one hour at 40 V. After the occurrence of the anode, annealing in the oven with a temperature at 550°C was done (8).

Preparation of PtNPs/ TiO_2 NTs

Platinum nanoparticles were deposited onto the annealed TiO_2 by using an electrochemical (reduction) method at a constant potential in a typical two-electrode system with the prepared TiO_2 nanotube as the working electrode, Pt sheet as the counter electrode. The electrolyte solution was prepared by dissolving the 2mM from five complexes $PtL_1, PtL_2, PtL_3, PtL_4$ and PtL_5 in 100

ml mixture solvent (dimethyl formamide DMF, ethanol, deionized water (1:1:1)). Electrodeposition time was set at 3 minutes, while the PtL₄ at 6 minutes while the electrodeposition voltage was fixed at 7 V and pH=5.5. The prepared Pt modified TiO₂NTs was washed several times with deionized water for 3 minutes to remove the residue of the solutions that are not deposited above the template, and then dried in air.

Cytotoxic Assays

Cytotoxicity effect of TiO₂NTs and PtNPs when deposition on TiO₂NTs on MCF-7 and WRL68 cancer cell line, and normal cell lines were done in a sterile area using the biosafety conditions of the airflow cabinet, MCF-7, WRL68 cell lines used in this study were equipped from Biotechnology Center/Al-Nahrain University. The cells were cultured in (MEM) modified eagles medium with serum ((100 U/ml) of antibiotic, ((100 µg)) of streptomycin/ml in incubator with (5% CO₂ at 37 °C). The survival or death of cells were determined using (3-(4,5- dimethylthiazole-2-yl)-2,5-diphenyl Tetrazolium bromide ((MTT)) which is diagnosed by using spectrophotometer. Plated in 96-well sterilized microliter-plates at a density of (1×10⁵ cells/well). After twenty-four hours, Cells were treated with different concentrations of prepared compounds starting from the lowest concentration and incubated in (5% CO₂) atmosphere with high humidity. After forty eight hours of compounds exposure, the cells were incubated with (0.5 mg/ml, MTT) distilled water for another four hours at thirty-seven degrees. 10% of salt (sodium dodecyl sulphate) then incubated for two hours. Absorption was measured at the wave length 620 nm on a multi-well ELISA plate reader (23).

Results and Discussion

Hitachi S-4160 Field emission scanning electron microscope (FE-SEM) was utilized to diagnose the surface morphology of TiO₂ nanotubes template **Fig. 1**(A,B,C,D,E,F). Template was scratched with a steel blade so as to observe the nanotubes of the side, as shown in **Fig. 1** (A1,B1,C1,D1,E1,F1). The process of anodizing led to the arrangement of nanotubes vertically. Generally, the nanotubes had lengths in the range 3 - 5 µm, and average diameters 83 nm, range from (51.8-95.7) .There were no differences when compared the observed morphology of the annealed crystalline nanotubes and transmission electron microscopy **Fig. 2**. TiO₂NTs may serve as the active sites or platform to deposit nanocrystals and able to promote unidirectional charge transport due to the one dimensional feature of the

nanotubes. The aggregated Pt nanoparticles formed for (PtL₄) were larger than the other particles upon electrodeposition at 6 minutes as depicted as in **Fig. 1** (B, B1). While other which observed in **Fig. 1** (C,C1,D,D1,F,F1,E,E1), the Pt nanoparticles were dispersed uniformly on the tube mouth of the TiO₂NTs at 7V, 2 mM and 3 minutes, some Pt nanoparticles were found to have embedded into the TiO₂NTs. However, Pt nanoparticles prepared at 7 V, 2 mM for 6 minutes, became larger than Pt synthesized at 3 minutes (24). The EDX unmistakably demonstrates that Pt, Ti and O are the major elements of composition which assures the existence of Pt decorated on TiO₂NTs substrate as appear in **Fig. 3**.

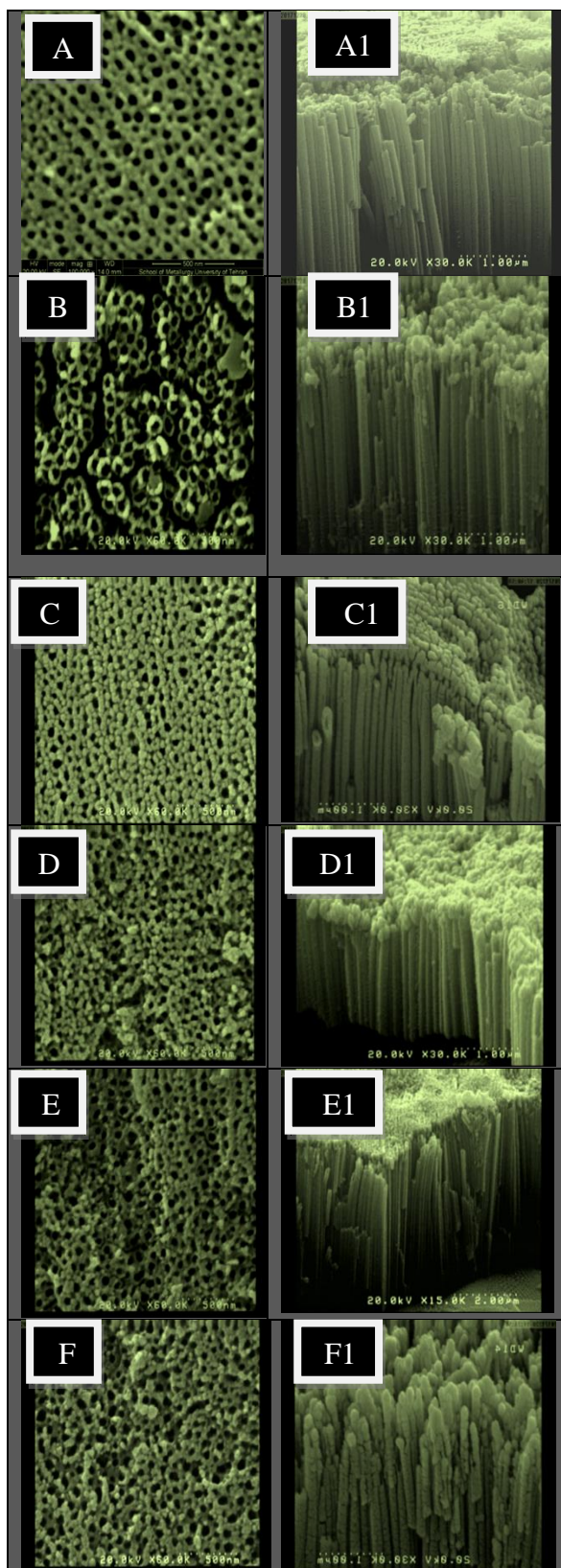


Figure 1 . FE-SEM images: A, A1 TiO₂NTs surface and cross section; B, B1 ,C ,C1 ,D ,D1 ,E ,E1 ,F ,F1 Pt/TiO₂ NTs surface and cross section B ,B1 at 7 Vol.,2mM 6 minutes C ,C1 ,D ,D1 ,E ,E1 ,F ,F1 at 7V, 2mM at 3 minutes

Field emission scanning electron microscope (FE-SEM) was supported by transmission electron microscope (TEM) technique and similar results have been shown .TEM images of the TiO₂NTs and Pt/TiO₂NTs are summarized in Fig. 2. both scans, show similar results in size and shape of nanotubes in average diameter (83nm) and nanoparticles (less than 50 nm) and deposition of platinum nanoparticles on the internal and external walls of TiO₂NTs, Fig. 2 a and b.

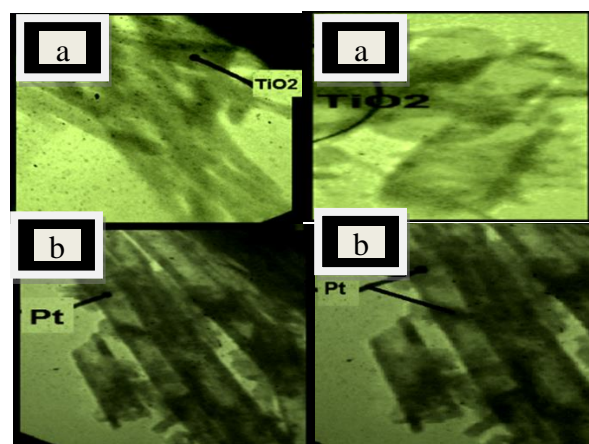


Figure 2. (a) TEM images of TiO₂NTs; and (b) Pt/TiO₂NTs

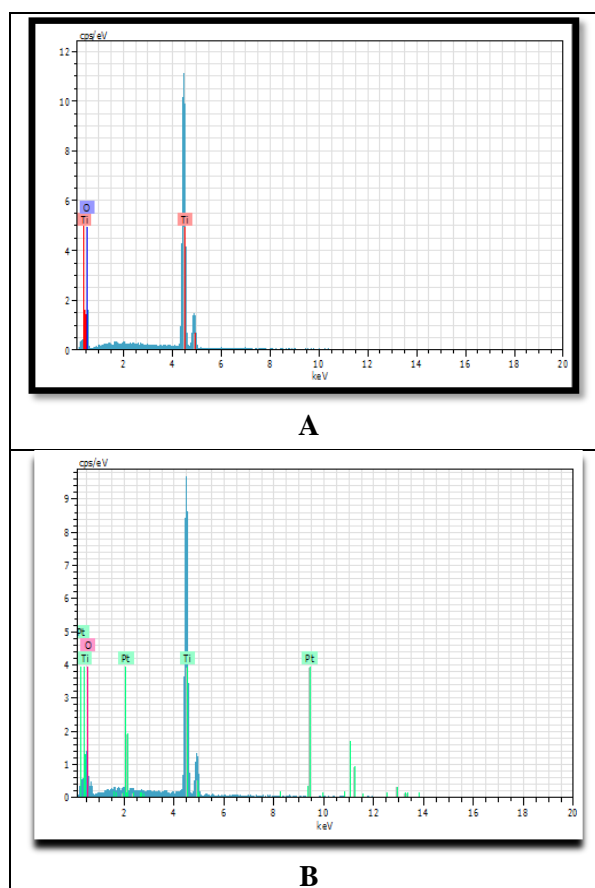


Figure 3. EDX (A) TiO₂NTs template, (B)Pt_{1,2,3,4,5}/TiO₂NTs

XRD analysis was used to confirm the crystal phases of TiO₂ nanotubes and the Pt-nanoparticles. The results are shown in Fig. 4, when the sample was heated at 550⁰C, only anatase phase was detected (25). The XRD patterns of TiO₂ nanotubes and Pt/TiO₂NTs prepared at 2mM. Plain TiO₂NTs were polycrystalline in nature with the existence of hexagonal structure and anatase phase, the XRD pattern exhibited the presence of titanium(JCPDS No. 44-1294), anatase (JCPDS No. 21-1272), diffraction peaks of TiO₂ 2θ=25.44, 38.20, 48.29, 54.22, 55.30, 62.82, 70.48 and 75.58o can be attributed to the (101), (004), (200), (105), (211), (204), (220) and (215) lattice planes of anatase TiO₂, respectively (25). Crystallite size value of TiO₂ was calculated 59.6 nm. A comparison of XRD patterns/Pt samples was shown in Fig.4,only anatase phase of TiO₂ was observed for all samples, because of the high intensity of the TiO₂ peaks and overlapped with Pt nanoparticles peak (due to the large TiO₂ crystallite size) compared with XRD of the sample(26).

The crystallite size of the TiO₂ nanotubes can be calculated by applying Debye–Scherrer’s equation as below (27):

$$D = \frac{0.94\lambda}{\beta \cos \theta}$$

where

D= Represents the mean size of crystalline

λ =Represents the wavelength of X-ray

β= Represents the line broadening in radians

Θ= Represents the Bragg angle

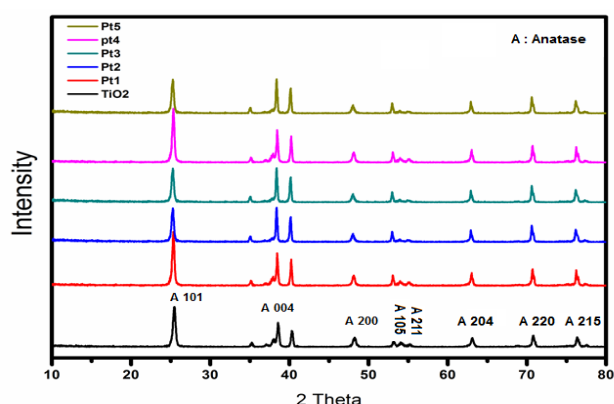


Figure 4. X-ray diffraction pattern of synthesized TiO₂NTs and other Pt nanoparticles decorated on it

Interpretation of Cytotoxic Assay Results

Cells toxicity was evaluated by (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl Tetrazolium bromide ((MTT)) method. Cultured MCF-7 were treated with TiO₂NTs and Pt/TiO₂NTs at concentration (800, 400, 200, 100, 50, 25 and 12.5μg/ml) for 48 hours. **Table 1** shows the statistical results, and the value of IC₅₀ for MCF-7 cancer cell lines and WRL68 normal cell lines. According to IC₅₀ test, the concentration of Pt \TiO₂NT that was required for 50% inhibition of MCF-7 and WRL68 cell inhibition was calculated. All data were expressed as means±standard deviations (SD). The statistical analysis was performed using Independent Samples Test (2-tailed (t-test)) at confidence levels of 95%.

The results in **Table 1** when deposited the Pt nanoparticles have different grain size on the surface of titanium nanotubes to modify it, and when we compare the values of IC₅₀ for the three compounds Pt\TiO₂NTs (1), Pt\TiO₂NTs (2), and TiO₂NTs, the following are concluded:

1-The nanomaterial Pt\TiO₂NTs (1) has platinum of particle size between 22-32 nm which has an inhibitory effect more than platinum of a particle size between 30-45 nm on MCF-7 cell line.

2-When comparing values IC₅₀ of the three nanomaterials Pt\TiO₂NTs (1), Pt\TiO₂NTs (2) and TiO₂NTs, it has been observed that the modification of the titanium-nanotubes surface by different nanoparticles size of platinum, which has a particle size of less than 50 nm, has toxicity against MCF-7 higher than titanium nanotubes alone Pt\TiO₂NTs(1)>Pt\TiO₂NTs (2)> TiO₂NTs.

3-When comparing values IC₅₀ for the two cell lines MCF-7 and WRL68 of the three nanomaterials Pt\TiO₂NTs (1), Pt\TiO₂NTs (2) and TiO₂NT, it was observed that the toxicity of these nanomaterials towards cancer cells were much higher than that of normal cell lines **Fig.5**.

Table 1. Statistical data and IC₅₀ Values of Pt/TiO₂NTs(1), Pt/TiO₂NTs(2) and TiO₂NTs on cancer (MCF-7) cell lines and normal (WRL68) cell lines in time of exposure 48 hrs

Conc. µg/ml	(Inhibition rate%(means ±standard deviation± SD)					
	Pt/TiO ₂ NT(1)		Pt/TiO ₂ NT(2)		TiO ₂ NT	
	MCF-7	WRL68	MCF-7	WRL68	MCF-7	WRL68
800	72.40±0.172975	29.22±.091198	59.56±0.502162	37.22±0.217785	49.20±0.136163	34.40±.0577697
400	66.56±0.167097	8.66±0.445073	52.15±0.469597	9.50±0.430155	43.38±.0122317	12.89±0.867106
200	51.97±0.132842	5.63±0.235530	48.80±0.912652	5.40±0.460815	30.97±0.884123	8.84±0.511783
100	37.97±0.136704	4.40±0.330807	39.70±0.302108	5.30±0.484152	20.94±0.051394	7.30±0.237557
50	20.85±0.597523	4.32±0.000577	22.30±0.389295	3.34±0.401623	15.80±0.705143	5.98±0.256689
25	15.34±0.154717	3.13±0.262543	11.26±0.267275	3.13±0.421099	10.50±0.45254	4.30±0.436534
12.5	8.22±0.240563	3.08±0.896177	5.13±0.008737	3.08±0.125819	7.03±.0417440	3.00±0.325140
IC ₅₀	191	431	156	450	212	406

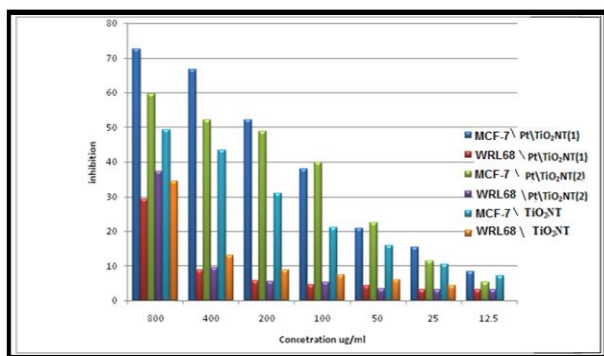


Figure 5: The percentage inhibition rate in MCF-7 cell line after treatment with TiO₂NTs, Pt/TiO₂NTs (1) and Pt/TiO₂NTs(2) , 48 hrs compared to normal WRL68 cell line

The viability of the cell depends on the environment or the dominant medium in order to achieve the best response, including cell adhesion or migration and proliferation. Biological effectiveness depends largely on several factors, the most important of which are chemical and physical properties, including surface area, particle size shape and purity of the phase in addition to the concentration of nanoparticles (28, 29).

Therefore a number of reasons have been suggested to inhibit the growth of cancer cell lines, including the Pt-high surface density of nanoparticles which was found to be incompatible with MCF-7 cell adhesion and proliferation (28,29). Therefore, it is important and desirable to find an optimal surface density of Pt nanoparticles to be decorated on TiO₂NTs including the nanoparticle and nanotube diameters that effectively kill bacteria, cancer cells and remains favorable to the normal cells.

The reason may be releasing platinum nanoparticles from Pt/TiO₂NTs and the breakdown of DNA (30), or maybe attributed to inhibition of

cancer cells incorporated the nanostructure into the cells; form aggregates in the cells and inhibit migration and proliferation of cancer cells (31).

Conclusions:

Electrodeposition was applied to synthesize Pt/TiO₂NTs. The regular crystalline with single-phase formation (anatase). The experiential methods Powder XRD, FE-SEM, TEM, EDX analytical techniques confirmed the presence of TiO₂ NTs in anatase phase and Pt nanoparticles decorated on it. In vitro cytotoxicity test has been carried out using the MTT assay method in wave length 620 nm. The study proved that the toxicity of the titanium nanotubes toward cancer cell lines (MCF-7) increased by deposition platinum nanoparticles on it. Also from IC₅₀ Value proved that these prepared nanomaterials have very low toxicity toward normal cell lines.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

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دراسة السمية الخلوية خارج جسم الكائن الحي لجسيمات البلاتين النانوية المزخرفة لصفائف الانابيب النانوية للتيتانيوم ثنائي الاوكسيد

شيماء رجب باقر¹ عبد الكريم محمد علي السامرائي² محاسن الياس¹ محمد محمود فرحان¹
الطيبوسي³ امال سمير صادق¹

¹قسم الكيمياء، كلية العلوم للنبات، جامعة بغداد، بغداد، العراق
²قسم الكيمياء، كلية العلوم ، جامعة بغداد، بغداد، العراق
³مركز بحوث التقنيات الاحيائية ، جامعة النهرين ، بغداد ، العراق

الخلاصة:

تم تحضير الانابيب النانوية لثنائي اوكسيد التيتانيوم بأنودة صفائح التيتانيوم في محلول الايثيلين كليكول و تمت تغطية سطحها بجسيمات البلاتين النانوية بطريقة الترسيب الكهربائي باستخدام خمس مشتقات من معقدات البلاتين لقواعد مانخ التي استخدمت كمصدر او بأدء للبلاتين .تم تقييم متوسط الحجم والشكل وتركيب العناصر لانابيب التيتانيوم داي اوكسيد النانوية وجسيمات البلاتين المترسبة عليها بتقنيات مختلفة مثل المجهر الالكتروني الماسح (FE-SEM) ، المجهر الإلكتروني النافذ (TEM) ، نمط حيود الأشعة السينية (XRD) و الأشعة السينية المشتتة للطاقة (EDX). من كل هذه الفحوصات ، تم تشخيص TiO₂NTs وجسيمات البلاتين النانوية المودعة عليها وقد اثبتت الدراسة ان جميع جسيمات البلاتين النانوية ذات حجم أقل من 50 نانومتر. تم معاملة خطوط الخلايا السرطانية MCF-7 وخطوط الخلايا الطبيعية WRL68 بتركيز 800 ، 400 ، 200 ، 50 ، 25 ، 12.5 ميكروجرام / مل من TiO₂NTs و(1) ،(2) Pt \ TiO₂NTs لمدة 48 ساعة باستخدام اختبار MTT. بالإضافة الى ذلك تم حساب IC₅₀ ومعدل التثبيط لهم. تظهر النتيجة أن Pt \ TiO₂NTs لها تأثير تثبيط أكبر على خطوط الخلايا السرطانية من صفيف TiO₂NTs.

الكلمات المفتاحية: الترسيب الكهروكيميائي ، جسيمات البلاتين النانوية، الانابيب النانوية لثنائي اوكسيد التيتانيوم