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Gold Nanoparticles: Preparation, Characterization, Cytotoxicity, and its Impact on Knitted Fabrics

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ABSTRACT

In this study, we synthesized gold nanoparticles (AuNPs) from Auric-chloride ($HAuCl_4$) by using tri sodium citrate as reducing agent. Their particle size range from 5-25 nm. Ultra violet spectrophotometry (UV-vis.) and Transmission Electron Microscope (TEM) used to characterize the synthesized AuNPs. Fabrics structure from REP and INTERLOCK made from cotton, PET and Lycra were used to be in medical purposes after treatments with AuNPs. The fabrics were treated with the prepared AuNPs with three concentrations 10, 20 and 25 mM to determine the best effective treated fabric structure from Atomic absorption and ultra violet protection factor (UBF) to be applied to toxicity test in vitro. Cytotoxicity of the prepared gold nanoparticles were evaluated using cell viability assay from MMT and IC_{50} values and these results confirm that we can use AuNPs safely in contact medical treatment with skin.

Keywords: Gold nanoparticles, Preparation, Characterization, Cytotoxicity, Fabric structure

INTRODUCTION

Nanotechnology has been used to establish new trend for produce small, fast and cheap materials and devices in nanometer range. In the smart devices, the most compatible nanomaterials were gold nanoparticles (AuNPs). It is suitable nanomaterial for bio imaging, biomedical therapeutics and bio diagnostic tools [1,2].

Colloidal gold nanoparticles are widely used in research fields because of their amazing properties [3,4]. There are several methods used for gold nanoparticles preparation. The most common one is reduction of Auric-chloride ($HAuCl_4$) in the presence of tri sodium citrate, which generates AuNPs in 15-20 nm size range. Metal nanoparticles paly important rules many different areas such as photonics, photography, catalysis, biological labeling and textiles [5]. The properties of metal nanoparticles determined by its shape, size, composition and structure. In some cases, AuNPs prepared from silver nano boxes as follow:

$$3Ag_{(S)} + HAuCl_{4\,(aq.)} \longrightarrow Au_{(S)} + 3AgCl_{(aq.)} + HCl_{(aq.)}$$

AuNPs has been used in several research fields such as for enhancing devices memory [6] and electronics printing inks [7].

AuNPs are used for treatment of cotton and wool fabrics to impart them permanent coloration of valuable textiles [2]. There are huge growth of nanoparticles preparation to its usage in many fields such as textiles and biomaterials. In addition AuNPs acts as anti-aging factor for skin protection [4].

In this work, we aimed to synthesize gold nanoparticles with minimal cytotoxicity on skin cells as follow: first, we prepare gold nanoparticles by reduction of auric-chloride ($HAuCl_4$) in the presence of tri sodium citrate followed by characterization of the prepared AuNPs using UV-Vis. and TEM imaging. Then treatment of fabrics with the prepared AuNPs to impart it biological properties medications followed by characterization of this treated fabrics to confirm the bind of AuNPs with fabrics by using FTIR, SEM and EXD it. Finally, study evaluation of cytotoxicity of fabrics treated with AuNPs to monitor the extent of use these nanoparticles in different medical fields.

MATERIALS AND METHODS

Materials

Gold (III) chloride (Aldrich, USA). Trisodium citrate, from Sigma, USA.

All other chemicals were used without further purification at analytical grades. Two types of knitted fabric construction based on rib and interlock produced from blended cotton combed yarn with 8% Lycra and cotton/Polyester (PET) blended fiber with 8% Lycra (Table 1).

Table 1: Fabric samples specifications

Materials	Description	Thickness (mm)	Weight (g\m^2)
1-Interlock fabrics from threated cotton (8% lycra)	Untreated	1.01	316.77
2-Interlock fabrics from combed cotton (8% lycra)	Untreated	0.97	321.55
3 Interlock fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	Untreated	1.09	314.33
4-. Interlock fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	Untreated	1.17	331.88
5. Interlock fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	Untreated	0.71	212.72
6-Interlock fabrics from compacted cotton (8% lycra)	Untreated	1.39	418.1
7-Interlock fabrics from Polyester (8% lycra)	Untreated	1.23	355.1
8-rib fabrics from threated cotton (8% lycra)	Untreated	0.84	258.66
9-rib fabrics from combed cotton (8% lycra)	Untreated	0.84	261.33
10-rib fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	Untreated	0.83	217.99
11-rib fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	Untreated	0.83	217.88
12-rib fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	Untreated	0.68	173.44
13-rib fabrics from compacted cotton (8% lycra)	Untreated	0.79	237.44
14-rib fabrics from Polyester (8% lycra)	Untreated	0.64	184.88
Treated fabrics			
15-Interlock fabrics from threated cotton (8% lycra)	Concentration 1	1.06	383
16-Interlock fabrics from combed cotton (8% lycra)	Concentration 1	1.04	375
17-Interlock fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	Concentration 1	1.37	394
18-Interlock fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	Concentration 1	1.24	364
19-Interlock fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	Concentration 1	0.73	192
20-Interlock fabrics from compacted cotton (8% lycra)	Concentration 1	1.45	436
21-Interlock fabrics from Polyester (8% lycra)	Concentration 1	1.44	435
22-rib fabrics from threated cotton (8% lycra)	Concentration 1	0.86	297
23-rib fabrics from combed cotton (8% lycra)	Concentration 1	0.87	308
24-rib fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	Concentration 1	0.79	233
25-rib fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	Concentration 1	0.79	217
26-rib fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	Concentration 1	0.65	139
27-rib fabrics from compacted cotton (8% lycra)	Concentration 1	0.65	217
28-rib fabrics from Polyester (8% lycra)	Concentration 1	0.76	200
29-Interlock fabrics from threated cotton (8% lycra)	Concentration 2	1.02	361
30-Interlock fabrics from combed cotton (8% lycra)	Concentration 2	1.03	375
31-Interlock fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	Concentration 2	0.82	206
32-Interlock fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	Concentration 2	1.25	386
33-Interlock fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	Concentration 2	0.78	211
34-Interlock fabrics from compacted cotton (8% lycra)	Concentration 2	1.53	436
35-Interlock fabrics from Polyester (8% lycra)	Concentration 2	1.41	442
36-rib fabrics from threated cotton (8% lycra)	Concentration 2	0.90	292
37-rib fabrics from combed cotton (8% lycra)	Concentration 2	0.91	311
38-rib fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	Concentration 2	0.83	233
39-rib fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	Concentration 2	0.79	236
40-rib fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	Concentration 2	0.73	183
41-rib fabrics from compacted cotton (8% lycra)	Concentration 2	0.81	225
42-rib fabrics from Polyester (8% lycra)	Concentration 2	0.67	189
43-Interlock fabrics from threated cotton (8% lycra)	Concentration 3	0.98	383
44-Interlock fabrics from combed cotton (8% lycra)	Concentration 3	1.03	375
45-Interlock fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	Concentration 3	1.20	394
46-Interlock fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	Concentration 3	1.23	364
47-Interlock fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	Concentration 3	0.78	192
48-Interlock fabrics from compacted cotton (8% lycra)	Concentration 3	1.47	436
49-Interlock fabrics from Polyester (8% lycra)	Concentration 3	1.30	453
50-rib fabrics from threated cotton (8% lycra)	Concentration 3	0.85	297
51-rib fabrics from combed cotton (8% lycra)	Concentration 3	0.85	308
52-rib fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	Concentration 3	0.39	233
53-rib fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	Concentration 3	0.78	217
54-rib fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	Concentration 3	0.67	139
55-rib fabrics from compacted cotton (8% lycra)	Concentration 3	0.77	217
56-rib fabrics from Polyester (8% lycra)	Concentration 3	0.65	200

METHODS

Preparation of gold nanoparticles

Gold nanoparticle were prepared by reduction of sodium citrate method as mentioned where [2,8] with some slight modifications as follow: Gold (III) chloride stock solution (1%) used to prepare 15, 20 and 25 mM respectively, followed by heating to 95°C under stirring on magnetic stirrer with heater. To this boiling solution add tri sodium citrate (1.5% w/v), and continue stirring until give red colour. Then we stored this solution at 4°C to be ready for use.

Finishing of fabrics with gold nanoparticles

The prepared gold nanoparticles (AuNPs) were applied on washed and dried fabrics using pad-dry-cure method. 30 × 30 cm of fabrics were immersed in the gold nanoparticles (AuNPs) (0.05-0.5 g/ml) solution that contains 1% acrylate binder than left for 30 min., followed by 100% wet up pad dry for 5 min at 80°C, followed by thermo-fixation for at 140°C for 3 min, followed by washing and drying.

Characterizations of AuNPs and its treated fabrics

- UV–vis spectroscopies of AuNPs were record on Shimadzu (UV-2450) to confirm the presence of AuNPs in the reaction medium at range 510-560 nm.
- The UV-protection factor (UPF) demonstrates the ratio of sunburn-causing UV measured without and with the protection of the fabric. The UPF of untreated and finished fabric samples (size 3 cm × 3 cm) was determined according to the Australian/New Zealand standard (AS/NZS 4399-1996: Sun protective clothing-Evaluation and classification) using UV-Shimadzu 3101PC spectrophotometer at wavelength of 280-390 nm, which includes the UVB (280-320 nm) and the UVA (320-400 nm) according to the following equation:

$$UPF_i = \frac{\sum_{\lambda=280}^{400} E_\lambda \times S_\lambda \times \Delta\lambda}{\sum_{\lambda=280}^{400} E_\lambda \times S_\lambda \times T_\lambda \times \Delta\lambda}$$

Where: E_λ=Relative erythemal spectral effectiveness, S_λ=Solar spectral irradiance, T_λ=Average spectral transmission of the specimen, and Δ_λ=Measured wavelength interval (nm) Regarding UV−protection categories, fabrics are classified to good, very good, and excellent if their UPF values range from 15-24, 25-39 and above 40 (40+) respectively.

- TEM images of AuNPs were recorded on JOEL 8400 to evaluate its shape and particle size.
- SEM and EDX of the treated fabrics recorded on JXA 840A, Japan.

The test was carried out at the Central unit for analysis and scientific services at National Research Center.

Evaluation of cytotoxicity of AuNPs

Cytotoxicity of the prepared AuNPs on A-549 cells were evaluated via cell viability test using MMT method (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) and determination of values of IC_{50} [9,10].

RESULTS AND DISCUSSION

Preparation of gold nanoparticles

The prepared gold nanoparticles from 5-45 nm particles size range were found stable for long period without stabilizing agents [11]. 1% w/v, Sodium citrate were used to reduce from gold (III) chloride, 20 mM to colloidal solution of gold nanoparticles with 15-25 nm range [12,13]. The optimum temperature for citrate reduction was found 97°C to produce gold nanoparticles with normal distribution [14].

Transmission electron microscopy

TEM images of gold nanoparticles (AuNPs) illustrated in Figure 1 for both different concentrations resulting in different particles size dispersed nanoparticles distribution. The whole nanoparticles diameter showed in TEM images were used to determine the particles size in AuNPs colloidal. All these images showed that these nanoparticles were normal distributed with particle size average from 15 to 25 nm. In addition, from TEM images we found that AuNPs has monodispersional state because of repulsion force generated from citrate ions layer with negative charges, which results in formation of spherical AuNPs.

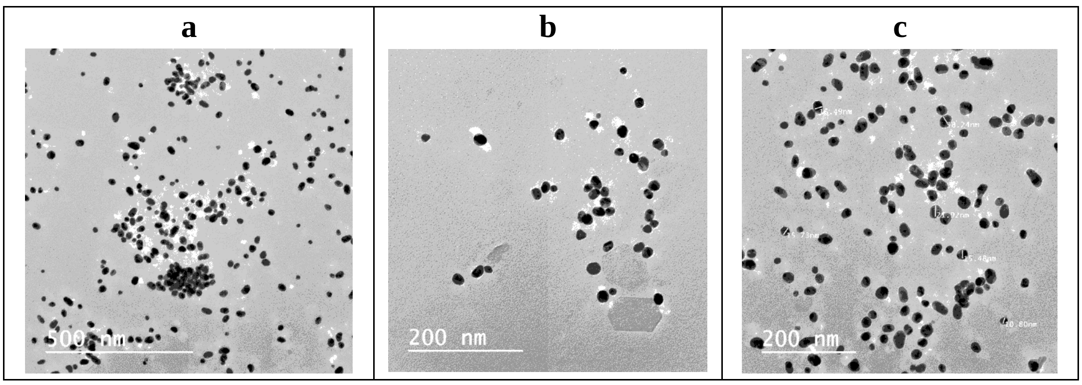

Figure 1: TEM images of gold nanoparticles (AuNPs)

UV-VIS spectrophotometry

UV-Vis spectrophotometry used to confirm the formation of AuNPs by appearing peaks at 510-560 nm. We found that as the particle size increase the absorption peaks shifted to longer wavelength as shown in Figure 2. Gold (III) chloride solution shows maximum absorption peaks at (360-390 nm) while gold nanoparticles form nanospheres at peaks from range 510-560 nm to resonance of surface plasmon of AuNPs which optimized at 520 nm giving brilliant red colour of AuNPs.

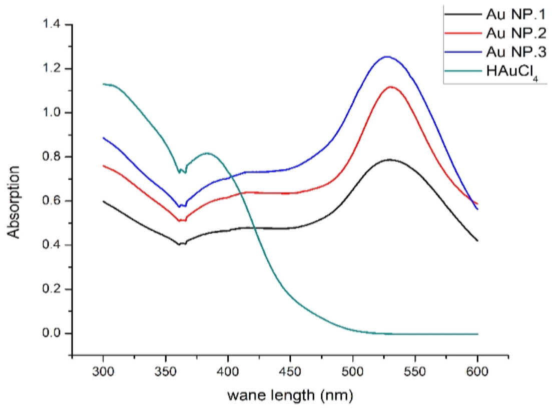

Figure 2: UV-Vis absorption of gold nanoparticles (AuNPs) Vs. Gold (III) chloride ($HAuCl_4$)

Table 2: Atomic absorption and UV-protection factor (UPF) of untreated and treated fabrics

Description		Atomic absorption (mg/dl)	UPF
1-Interlock fabrics from threated cotton (8% lycra)	Untreated	0.000	3.57
2-Interlock fabrics from combed cotton (8% lycra)	Untreated	0.000	3.89
3-Interlock fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	Untreated	0.000	3.81
4-Interlock fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	Untreated	0.000	4.47
5-Interlock fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	Untreated	0.000	6.11
6-Interlock fabrics from compacted cotton (8% lycra)	Untreated	0.000	10.35
7-Interlock fabrics from Polyester (8% lycra)	Untreated	0.000	6.78
8-ribb fabrics from threated cotton (8% lycra)	Untreated	0.000	3.22
9-rib fabrics from combed cotton (8% lycra)	Untreated	0.000	3.62
10-rib fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	Untreated	0.000	3.63
11-rib fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	Untreated	0.000	3.99
12-rib fabrics from 50:50 compacted cotton/ Polyester blended (8% lycra)	Untreated	0.000	5.02
13-rib fabrics from compacted cotton (8% lycra)	Untreated	0.000	9.35
14-ribbing fabrics from Polyester (8% lycra)	Untreated	0.000	4.56
15-Interlock fabrics from threated cotton (8% lycra)	15 mM	1.203	8.211
16-Interlock fabrics from combed cotton (8% lycra)	15 mM	1.006	8.947
17-Interlock fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	15 mM	0.874	8.763
18-Interlock fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	15 mM	0.970	10.281
19-Interlock fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	15 mM	2.076	14.053
20-Interlock fabrics from compacted cotton (8% lycra)	15 mM	2.525	23.805
21-Interlock fabrics from polyester (8% lycra)	15 mM	0.878	15.594
22-rib fabrics from threated cotton (8% lycra)	15 mM	1.023	5.313
23-rib fabrics from combed cotton (8% lycra)	15 mM	0.855	5.973
24-rib fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	15 mM	0.743	5.9895
25-rib fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	15 mM	0.825	6.5835
26-rib fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	15 mM	1.765	8.283
27-rib fabrics from compacted cotton (8% lycra)	15 mM	2.146	15.427
28-rib fabrics from polyester (8% lycra)	15 mM	0.746	7.524
29-Interlock fabrics from threated cotton (8% lycra)	20 mM	2.045	10.81
30-Interlock fabrics from combed cotton (8% lycra)	20 mM	2.226	11.78
31-Interlock fabrics from 50:50 combed cotton /Polyester blended (8% lycra)	20 mM	1.486	11.54
32-Interlock fabrics from 50:50 threated	20 mM	1.617	13.51

cotton/Polyester blended (8% lycra)			
33-Interlock fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	20 mM	3.529	18.53
34-Interlock fabrics from compacted cotton (8% lycra)	20 mM	3.841	31.35
35-Interlock fabrics from polyester (8% lycra)	20 mM	1.493	20.54
36-rib fabrics from threated cotton (8% lycra)	20 mM	1.203	8.46
37-rib fabrics from combed cotton (8% lycra)	20 mM	0.874	9.52
38-rib fabrics from 50:50 combed cotton/Polyester blended (8% lycra)	20 mM	1.021	9.54
39-rib fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	20 mM	0.979	10.47
40-rib fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	20 mM	1.882	13.26
41-rib fabrics from compacted cotton (8% lycra)	20 mM	1.150	24.55
42-rib fabrics from Polyester (8% lycra)	20 mM	0.551	11.98
43-Interlock fabrics from threated cotton (8% lycra)	25 m M	3.750	46.67
44-Interlock fabrics from combed cotton (8% lycra)	25 m M	3.020	50.65
45-Interlock fabrics from 50:50 combed cotton/Polyester blended (8% lycra)	25 m M	3.620	42.65
46-Interlock fabrics from 50:50 threated cotton/Polyester blended (8% lycras	25 m M	4.550	39.32
47-Interlock fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	25 m M	3.630	64.39
48-Interlock fabrics from compacted cotton (8% lycra)	25 m M	4.010	75.65
49-Interlock fabrics from polyester (8% lycra)	25 m M	3.002	66.95
50-rib fabrics from threated cotton (8% lycra)	25 m M	2.980	39.65
51-rib fabrics from combed cotton (8% lycra)	25 m M	1.990	33.02
52-rib fabrics from 50:50 combed cotton/Polyester blended (8% lycra)	25 m M	2.330	20.33
53-rib fabrics from 50:50 threated cotton/Polyester blended (8% lycra)	25 m M	1.540	19.89
54-rib fabrics from 50:50 compacted cotton/Polyester blended (8% lycra)	25 m M	1.982	25.12
55-rib fabrics from compacted cotton (8% lycra)	25 m M	2.540	61.23
56-rib fabrics from Polyester (8% lycra)	25 m M	2.013	32.33

Finishing of fabrics with gold nanoparticles, atomic absorption and UV-protection factor (UPF)

Table 2 shows that all treated fabrics contains gold nanoparticles and the percent increase as the concentration of treated samples increase at constant weight pickup.

This table shows both atomic absorption and UPF of treated and untreated fabrics with AuNPs with three different concentrations to obtain the optimum condition for ultraviolet protection factor of these treated fabrics. From these data we found that the treated fabrics process higher protection for UV radiation and could be used for medical treatment from rheumatoid disease after explosion to sun light at the same time to get the least effective amount from treated fabrics to minimize the toxicity of AuNPs.

EDX analysis

Scanning electron microscope (SEM/EDX) analyses were show in Figure 3, which shows the presence of Au nanoparticles in the fabrics. The surface morphology of the treated fabric with Au nanoparticles appears as smooth surface with deposit of the nanoparticles. It is clear that the prepared Au nanoparticles is more homogenous and regular distribution on the surface and has higher intensity peaks, on the other hand the Au nanoparticles EDX analysis indicate that the content of Au (0.49, 0.29) (Au weight 0.91) and has lower intensity peaks [15].

The observation of the Au nanoparticles coating shows that the surface texture appears to have dense and low porosity (The choice samples were interlock). In case of ripe fabrics the nanoparticles coated the fibers and appear to be uniform in size.

The coated fabric with Au nanoparticles film was formed and firmed on the surface of the sample. It is evident that experimental and reaction conditions did not alter the morphology surface on using Au nanoparticles. The Au nanoparticle was strongly attached to the fibers due to very strong electrostatic or chemical interactions between the Au nanoparticles and the fabric [15].

Cytotoxicity of AuNPs suspensions

To study the effect of gold ions concentration present in AuNPs suspensions on their toxicity, A549 cells were treated for 24 h with three different batches of AuNPs suspension, which contained the same concentration of AuNPs (0.5-2.0 µg/ml) in three GNP types. Epidermis cell line selected for cytotoxicity test for Au NPs prepared from HAuCl4 solution. We prepared 3-solution concentration from Au NPs: 2.5 µg/ml, 10 µg/ml and 40 µg/ml.

Cytotoxicity evaluated using two protocols: IC_{50} and MTT

Table 3 shows that the IC_{50} of gold nanoparticles prepared by chemical reduction using citrate method. But its toxicity reduces in AuNPs treated with fabrics than powder itself. In this study, Au^+ decreased mitochondrial activity more than AuNPs with almost two fold difference in IC_{50} values as shown in Table 2, which agreed with previous studies of many researchers [15-18].

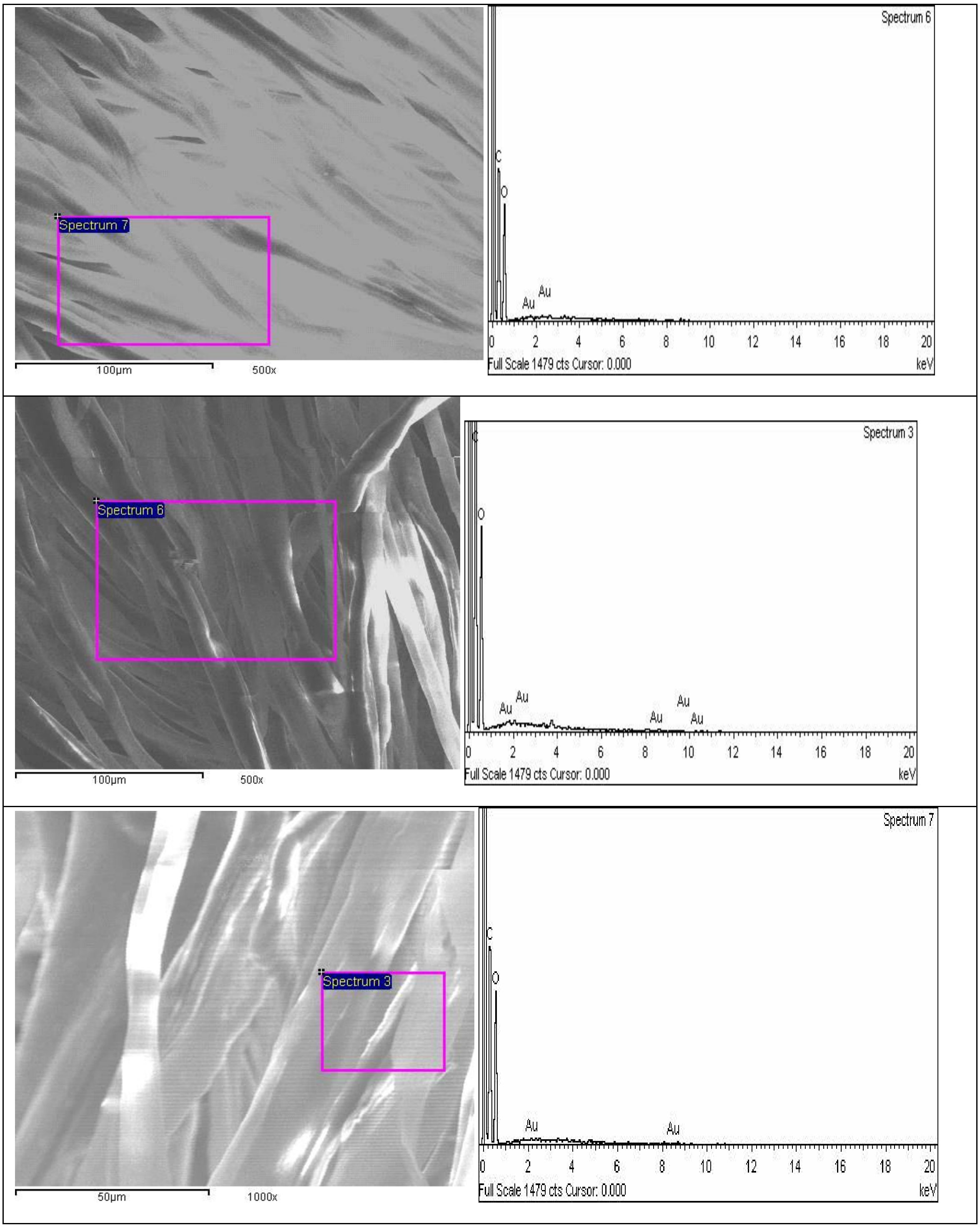

Figure 3: SEM and EDX spectra cotton treated with AuNPs

Table 3: IC_{50} A549 cell line after exposing to HAuCl4, AuNP1 (NP Powder), AuNP2 (NP loaded Interlock) and AuNP3 (NP loaded Ripe) (for 24 h)

Material	**IC_{50}**
$HAuCl_4$ (1 mM)	0.21 µg/ml
Au NP1 (NP Powder)	0.32 µg/ml
Au NP2 (NP loaded Interlock)	2.10 µg/ml
Au NP3 (NP loaded Ripe)	3.21 µg/ml

MTT assay used to measure the cell viability expressed in the decrease in mitochondrial activity (Table 4). A reduction in mitochondrial function of A549 cells exposed to the three AuNPs types prepared.

Table 4: MTT test: (metabolic activity of the mitochondria)

Material	MTT expressed in viable cells	
	After 3 h	**After 24 h**
$HAuCl_4$ (1 mM)	18.6	2.8
Au NP1 (NP Powder)	14.2	1.9
Au NP2 (NP loaded Interlock)	52.3	46.2
Au NP3 (NP loaded Ripe)	76.2	64.2

CONCLUSION

AuNPs were prepared by reduction of $HAuCl_4$ in the presence of tri sodium citrate with particle size within range 5-25 nm. UV absorption and TEM used to characterize these AuNPs. Fabrics structure from REP and INTERLOCK made from cotton, PET and Lycra were used to be in medical purposes after treatments with AuNPs. The fabrics were treated with the prepared AuNPs with three concentrations 10, 20 and 25 mM to determine the best effective treated fabric structure from UBF to be applied to toxicity test *in vitro*. Cytotoxicity of the prepared gold nanoparticles were evaluated using cell viability assay from MMT and IC_{50} values and these results confirm that we can use AuNPs safely in contact medical treatment with skin.

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