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Synthesis and Biodistribution Study of Biocompatible 198Au Nanoparticles by use of Arabinoxylan as Reducing and Stabilizing Agent

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Abstract:	Radioactive gold-198 is a useful diagnostic and therapeutic agent. Gold in the form of nanoparticles possesses even more exciting properties. This work aimed at arabinoxylan-mediated synthesis and biodistribution study of radioactive gold nanoparticles (198AuNPs). The particles were synthesized by mixing suspension of arabinoxylan with H198AuCl4 without use of any additional reducing and stabilizing agents. An aqueous suspension of arabinoxylan was added to a H198AuCl4 solution, which resulted in reduction of Au3+ to 198AuNPs. Biodistribution was studied in vitro and in rabbit. The particles having exceptional stability were readily formed. Highest radioactivity was recorded in spleen after 3 h followed by liver, heart, kidney and lungs after i.v. administration. After 24 h the activity was not detectable in spleen; it accumulated in liver. However, after oral administration the activity mainly accumulated in colon. In serum proteins the distribution was: α1-globulin 6.5%, α2-globulin ~2%, β-globulin ~1%, γ-globulin 0.7% and albumin 0.7% of the administered dose. This indicates a low protein binding implying high bioavailability of the particles. The cytotoxicity study showed that the particles were inactive against HeLa cell line and A. tumefaciens. Highly stable 198AuNPs reported in this work have potential for targeting colon. They show affinity for globulins, the property that can used in study of immune system.

The Editor
BTER

Dec 30, 2018

Dear Sir,
I am submitting a research paper "Synthesis and Biodistribution Study of Biocompatible 198Au Nanoparticles by use of Arabinoxylan as Reducing and Stabilizing Agent" for publication in BTER. This has the approval of all the authors. The work describes synthesis of radioactive gold nanoparticles by use of arabinoxylan – biomaterial isolated from from ispaghula (*Plantago ovata*) husk. The particles are non-toxic, free from hazardous reducing and capping agents and exhibit potential for imaging and diagnosis.

The paper is solely being submitted to this journal. This work does not involve any conflict of interest.
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Thanks and regards.

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Response to reviewer's comments

The responses are in red in the manuscript and in here.

Reviewer 2

Comment

The authors still need to provide a statistics section in the materials and methods. The authors added info on N and P values in the figure legends but provided no information about statistical methods or software used to analyze the data (e.g. T tests, ANOVA, etc).

Response

The section "**Statistical Analysis"** has been added at page 13 as the last paragraph in **"Materials and Methods".**

Synthesis and Biodistribution Study of Biocompatible ^{198}Au Nanoparticles by use of Arabinoxylan as Reducing and Stabilizing Agent

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Synthesis and Biodistribution Study of Biocompatible ^{198}Au Nanoparticles by use of Arabinoxylan as Reducing and Stabilizing agent

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Abstract
Radioactive gold-198 is a useful diagnostic and therapeutic agent. Gold in the form of nanoparticles possesses even more exciting properties. This work aimed at arabinoxylan-mediated synthesis and biodistribution study of radioactive gold nanoparticles (^{198}AuNPs). The particles were synthesized by mixing suspension of arabinoxylan with $H^{198}AuCl_4$ without use of any additional reducing and stabilizing agents. An aqueous suspension of arabinoxylan was added to a $H^{198}AuCl_4$ solution, which resulted in reduction of Au^{3+} to ^{198}AuNPs. Biodistribution was studied *in vitro* and in rabbit. The particles having exceptional stability were readily formed. Highest radioactivity was recorded in spleen after 3 h followed by liver, heart, kidney and lungs after *i.v.* administration. After 24 h the activity was not detectable in spleen; it accumulated in liver. However, after oral administration the activity mainly accumulated in colon. In serum proteins the distribution was: α_1-globulin 6.5%, α_2-globulin ~2%, β-globulin ~1%, γ-globulin 0.7% and albumin 0.7% of the administered dose. This indicates a low protein binding implying high bioavailability of the particles. The cytotoxicity study showed that the particles were inactive against HeLa cell line and *A. tumefaciens*. Highly stable ^{198}AuNPs reported in this work have potential for targeting colon. They show affinity for globulins, the property that can used in study of immune system.

Keywords Hemicelluloses • Arabinoxylan • Gold nanoparticles • Targeted delivery • Radioactive gold nanoparticles

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Introduction

AuNPs are being widely studied for their potential applications in therapy, diagnosis, drug delivery and imaging due to their unique properties such as inertness, ease of synthesis, functionalizability and peculiar optical properties [1–8]. The efficiency of NPs in biomedical imaging largely depends on their optical properties. The size dependent optical properties of AuNPs, including linear surface plasmon resonance, fluorescence and Raman scattering, make them ideal candidate for organ imaging and optical sensors [9–11]. The particles having size 3 – 40 nm absorb at 510 – 530 nm with extinction coefficients to the order of 10^{11} mol^{-1} cm^{-1}, that is much higher than those of conventional dyes used for imaging. In case of particles > 40 nm the scattering-to-absorption ratio is several orders higher than those of fluorescent dyes [12, 13]. On account of these properties AuNPs have been widely investigated for diagnostic imaging [4, 14]. AuNPs have also been investigated for their use in photothermal destruction of cancerous cells due to their excellent thermal conductivity [1, 15, 16].

In the present work we hypothesize that AuNPs encapsulated in arabinoxylan (AX), a swellable hemicellulosic material, would exhibit organ specific uptake and if the gold is radioactive they can be used for radioimaging. ^{198}Au is a radioactive isotope of gold that emits β-particles and γ-rays of energies 0.412, 0.68 and 1.09 MeV. The γ-radiation of 0.412 MeV energy is highly suitable for imaging human organs. On the other hand β-particles have been successfully employed for treating prostate tumours [17–25].

Generally AuNPs are synthesized by chemical methods involving use of highly toxic reducing agents such as sodium borohydride, hydrazine or formaldehyde [26–28] and capped with stabilizing agents. Although the particles are thoroughly washed but the process does not rule out the presence of residual amount of the toxic reducing agents. For biomedical

applications the particles need to be absolutely free from toxic materials. In order to ensure this the use of toxic materials in the synthesis of NPs has to be eliminated. Recently, highly biocompatible and biodegradable materials have been identified as reducing and stabilizing agents. These include hemicelluloses, which can simultaneously reduce Au^{3+} to Au^{0} and stabilize them for more than three years [29, 30].

In the present study we have used AX from ispaghula (*Plantago ovata*) husk for the synthesis and stabilization of radioactive ^{198}AuNPs coupled with their biodistribution *in vitro* and *in vivo*. AX has the potential to deliver encapsulated drugs at colon as this material is insoluble in acidic and soluble in alkaline media [31]. Thus when administered orally the AX-encapsulated AuNPs are expected to remain intact in stomach (pH < 2) and pass onto intestine where they get dissolved and hydrolyzed due to alkaline pH and the presence of micro flora there [32]. They are not digested in the small intestine and can easily pass to colon where they can be partially digested and release encapsulated particles. Moreover, the hydrophilic nature of AX and its high affinity toward mucosal surfaces would assist the encapsulated particles to be released for longer periods of time at the specific site [33]. Thus we hypothesize that AX-encapsulated ^{198}AuNPs are biocompatible and suitable for diagnosis and therapy of colon related malignancies.

Materials and Methods

Materials

The materials used in this study were: gold (ARY Gold, Lahore, 99.99%), NaOH, HNO_3 and HCl, Extrapure® from E. Merck, Germany; L-(+)-arabinose, D-(+)-galactose, D-glucose, D-(+)-xylose, L-rhamnose monohydrate, galacturonic acid, Citric acid, from E. Merck, Germany;

oligosaccharides β-(1-4)-D-xylotriose, β-(1-4)-D-xylotetraose, β-(1-4)-D-xylopentaose, and β-(1-4)-D-xylohexaose used as GPC standards were from Megazyme (Sydney, Australia); reactant free AuNPs (30 nm) stabilized in phosphate buffer, Coomassie Brilliant Blue R-250, dimethyl sulfoxide (DMSO), glycerol, bovine serum albumin (BSA), human serum albumin (HSA), neutral red, glacial acetic acid, *l*-cysteine, disodium hydrogen phosphate, sodium nitrate and sodium dodecyl sulfate (SDS), were of analytical grade from sigma Aldrich (USA); *A. tumefaciens*, HeLa cells (ATCC: CCL 2) from Flow Labs (London, UK); Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) from Gibco (USA); complement protein C_3 kit was from Orion Diagnostica (Finland). AX (molar mass 3.17×10^6 Da) was a gift from Dr. Shazma Massey of FC College Lahore, isolated according to the reported method [34]. The AX sample was recharacterized by elemental analysis, monosaccharide composition, ATR-FT-IR and gel permeation chromatography (GPC). Nanopure® water was used throughout this work. All the chemicals were used without further purification.

Preparation of $H^{198}AuCl_4{\cdot}3H_2O$

The gold foil (0.050 g) was irradiated with a neutron flux of 8×10^{13} $n{\cdot}cm^2{\cdot}s^{-1}$ for 30 min at the research reactor of Pakistan Institute of Nuclear Science and Technology, Islamabad. The irradiated gold was dissolved in aqua regia (150 µL) at 100 °C, evaporated near to dryness, followed by addition of HCl (0.01 M) and evaporation near to dryness. The evaporation process was repeated three times and the remaining mass was left at room temperature for crystallization of $H^{198}AuCl_4{\cdot}3H_2O$.

Synthesis of ^{198}AuNPs

The ^{198}AuNPs were synthesized as reported earlier for non-radioactive AuNPs [29]. Briefly, $H^{198}AuCl_4 \cdot 3H_2O$ (0.0393 g, 0.1 mmol) was dissolved in water (100 mL); to 20 mL of this solution 20 mL of AX-suspension (0.1 % in water) was added under vigorous stirring for 2 h at 50 ºC. A successive change in color from pale yellow to red was observed in 25 min, indicating the formation of ^{198}AuNPs, however the stirring was continued for further 2 h to ensure complete reduction. The particles were isolated by centrifugation at 35000 rpm for 30 min, re-dispersed in water using an ultrasonic bath, washed with water, centrifuged again and dialyzed to free them from unreacted materials.

Characterization

The AX sample used in this work was recharacterized according to the reported methods [34]. Elemental analysis was carried out by CHNS analyzer (Vario MICRO V1.4.2; Elementar Analysen Systeme, GmbH, Germany). Monosaccharide composition was determined by HPLC using Dionex ICS 3000 system, consisting of CarboPacPA20 column (0.4×150 mm) and electrochemical detector, according to a reported method [35] after acid hydrolysis [36]. ATR-FT-IR spectrum was recorded by Cary 630 FTIR Spectrometer (Agilent, USA). GPC was performed by Agilent 1200 series (Agilent, Germany) system using PL aquagel-OH mixed column (8 µm; 7.5×300 mm) and refractive index detector (G1362A). Water containing 0.1% $NaNO_3$ was used as eluent (flow rate: 1.0 mL min^{-1} at 70 ℃) and injection volume was 10 mL.

Data were analyzed by the Chem-Station GPC Data Analysis Rev. A.02.02 (Agilent, Germany). Pullulan and dextran were used as calibration standards.

The AX-encapsulated ^{198}AuNPs samples were diluted four times with water, filtered through 0.22 µm membrane and SPR spectra recorded on Pharmaspec UV-1700 spectrophotometer (Shimadzu, Japan) in 300–800 nm range using AX suspension as the reference. The SPR absorption at 526 nm was used to determine the particle size and concentration of Au was determined by using molar absorptivity value of 3.36×10^9 at 526 nm [37]. Stability of the particles was studied in human serum by recording SPR spectra 1 h, 2 h, 48 h and 60 h after appropriate dilution with water.

The pXRD spectra of AX-encapsulated ^{198}AuNPs were recorded on Bruker D8 Discover (Germany) diffractometer using monochromatic Cu Kα radiation ($\lambda = 1.5406$Å) operating at 40 kV and 30 mA. The data were collected over a 10–80° 2θ range. The size of particles was calculated from the highest intensity peak in the XRD spectra by use of Debye–Scherrer equation ($D = 0.9\lambda/\beta\cos\theta$) [38].

For transmission electron microscopy (TEM) an ultrasonically dispersed solution of the NPs was placed on a carbon coated grid and images were obtained by JEM-1200EX (JEOL, Japan) microscope at an accelerating voltage of 120 kV.

Particle Size Distribution and Zeta Potential

Particle size and zeta potential were determined by NanoZS90 HPPS 5001 zetasizer (Malvern, UK). The disposable cuvette was filled with the sample and allowed to equilibrate for 2-3 min at

21°C before the measurement. Ten replicates of each sample were measured and an average value was reported.

For zeta potential (ζ) measurements the capillary cell (DTS 1060) was flushed with water and filled with sample avoiding air bubbles in the capillary. Ten replicates were measured and the average values were reported.

Cell Viability and Cytotoxicity

Cytotoxicity of the AX-encapsulated ^{198}AuNPs was determined by Neutral Red (NR) [39] and potato disc assays [40]. Briefly, HeLa cells were cultured as monolayers into 96-wells plate, containing approximately 1×10^4 cells in each well, DMEM culture medium supplemented with 10 % of FBS, glucose (4.500 g L^{-1}) and 1 % of penicillin-streptomycin. The plate was incubated at 37 ℃ for 24 h under 5% CO_2 and 90% humidity. To the cultured cells, three blanks (one excluding the AX and ^{198}AuNPs, the other containing AX (6.70 µg mL^{-1}) and excluding ^{198}AuNPs), third one excluding AX and containing ^{198}AuNP (18.0 µg mL^{-1}) and varying concentrations of AX-encapsulated^{198}AuNPs (18.0, 9.0 and 4.5 µg mL^{-1}) were added in 1:1 ratio and the plate was incubated again for 24 h under similar conditions. After incubation the medium was drained, cells washed with PBS, the NR dye added and the plate incubated for further 2 h. The surplus NR was washed out by freshly prepared de-staining solution (glacial acetic acid-ethanol-water, 1:49: 50) and the culture was incubated for further 10 min. The optical density of the released NR dye was measured at 570 nm. Relative cell viability was calculated as:

$$\text{Cell viability (\%)} = \frac{\textbf{sample absorbance}}{\textbf{control absorbance}} \times 100$$

The potato disc method was also used to assess the cytotoxicity of ^{198}AuNPs. For this *A. tumefaciens* was grown on Luria broth medium for 48h at 28°C in shaking incubator. Various concentrations of ^{198}AuNPs (1.8, 9.0 and 4.5 µg mL^{-1}) and of AX suspension (67.0 µg mL^{-1} were tested. Inoculum was prepared by mixing each dilution (100 µL) with bacterial culture (100 µL). Negative control was prepared by mixing of water (100 µL) and *A. tumefaciens* (100 µL). Roxithromycin (100 ppm: 100 µL) was used as positive control. Red-skinned potatoes were purchased from a local market and surface sterilized by using 10 % bleach solution. Cylinders of surface sterilized red skinned potato were made with the help of sterilized borer. The 5 mm thick discs of these potato cylinders were cut and placed on solidified agar plates (10 discs per plate). Inoculum (50 µL) was poured on the surface of each disc of respective concentration as well as controls. The discs were examined after 21 days of incubation under dissecting microscope after staining with Lugol's solution (potassium iodide-iodine-distilled water in 10:5:7 ratio). The number of tumours per disc was counted. The doses for the cytotoxicity tests were chosen by considering the radioactivity suitable for imaging. Percentage inhibition for each concentration was determined by using the following formula.

$$\% \text{ Inhibition} = \frac{\text{No of tumour with sample}}{\text{No of tumour with control}} \times 100$$

Biodistribution Studies

Biodistribution experiments were planned to be carried out *in vivo* after intravenous and oral administration to healthy rabbits, in tumour-bearing mouse, and *in vitro* among serum proteins as follows.

Biodistribution after Intravenous Administration of ^{198}AuNPs in Rabbit

These experiments were conducted at Institute of Nuclear Medicine and Oncology hospital, Lahore. The AX suspension containing ^{198}AuNPs equivalent to 14.0 µg mL^{-1} Au having ~1000 µCi mL^{-1} activity was prepared by appropriately diluting the stock suspension. The dose (0.5 mL), adjusted to pH 7 by use of HCl (0.1 M) or NaOH (0.1 M) solutions, was filtered through 0.22-µm membrane (Polycarbonate, Sterlitech, USA) and injected into the ear vein of healthy rabbits (n = 4; local breed, age 12-16 weeks, average weight 900 ± 50 g). The animals were anesthetized by intramuscular ketamine injection (50 mg kg^{-1}) before administration of the dose. The administered dose was determined by subtracting the activity remaining in the syringe from the total taken in the syringe.

Whole body images of the animals were acquired in dynamic mode (for 15 min with 1 min interval) and at 5, 60, 120, 180 min and 24 h post-injection by use of large field-of-view dual-head gamma camera (Infinia Hawkeye®, GE Healthcare, Milwaukee, USA) fitted with a high resolution and high energy collimator. The images were acquired from anterior, posterior, left lateral, right lateral, dorsal and ventral views. After re-anesthetization at 180 min and 24 h post-injection the animals were sacrificed and organs of interest and remaining carcass were collected, weighed and counted for the radioactivity in a well-type Beckman 8000 gamma counter (Beckman, Brea, CA). Approximately 75% of the total blood volume was withdrawn by cardiac

puncture and counted for radioactivity. The radioactivity in the sample and an aliquot of the injection mixture was determined and reported as % injected dose g^{-1} of the tissue.

Biodistribution After Oral Administration of ^{198}AuNPs in Rabbit

A suspension of AX-encapsulated^{198}AuNPs containing 20.0 µg mL^{-1} Au having ~1200 µCi mL^{-1} activity in saline (2.00 mL) adjusted to pH 7 was administered orally to the rabbits (n=4). The animals were anesthetized by intramuscular ketamine injection (50 mg kg^{-1}) and fixed on a wooden board. A flexible cannula was placed in the upper third of the oesophagus and AX-encapsulated ^{198}AuNPs suspension (2.0 mL) was gently instilled and images were recorded from anterior, posterior, left lateral, right lateral, dorsal and ventral sides at 5 min, 0.5, 1, 2, 24, 48 and 96 h post-administration.

In vitro Distribution of ^{198}AuNPs in Serum Proteins

Serum samples (0.5 mL, n = 3) from healthy human were vortex-mixed with equal volumes of AX-encapsulated ^{198}AuNPs equivalent to 14.0 µg mL^{-1} Au having ~1000 mCi mL^{-1} suspension and a buffer mixture (Tris, 2 SDS, 10 % glycerol and 0.0125 % bromophenol blue; pH adjusted to 6.8 with HCl or NaOH solution). The samples were incubated at 37 ℃ for 1h and subjected to SDS-polyacrylamide gel (SDS-PAGE) analysis at 50 mA/gel [41]. The gel was stained with a staining solution coomassie brilliant blue R-250 in (water-methanol-glacial acetic acid, 50:10:0.25) for 10 min. The gel was de-stained in methanol-acetic acid-water solution

(250:100:650) until protein fractions appeared clear. The strips were dried for 2–3 min in air then at 70 ℃ in an oven for 30 min. The bands were cut (3 × 1 mm^2) and counted for radioactivity.

Hemocompatibility Study

Hemocompatibility of AX-encapsulated ^{198}AuNPs was studied by direct exposure for 3 h of fresh blood (0.5 mL) from healthy humans (n=4) to ^{198}AuNPs (0.2 mL) equivalent to14.0 µg mL^{-1} Au having ~1000 mCi mL-1 at 37 ℃. Optical images were taken at 0, 5, 10, 30, 60 and 120 min by Euromax iScope (Holland) microscope; AX mucilage was used as the control.

Complement activation

Complement fixation test was performed by turbidimetric method [42] by measuring the depletion of complement protein C_3 on incubation with the AX suspension and AX-encapsulated ^{198}AuNPs separately. The AX suspension (0.2 mL) and ^{198}AuNPs equivalent to14.0 µg mL^{-1} of Au were incubated for 1 h at 37°C with of citrated blood (0.2 mL). The final concentration of the ^{198}AuNPs in the assay was adjusted to 7.0 µg mL^{-1}. The assay was performed in triplicate according to the protocol provided by the kit manufacturer.

Statistical Analysis

The data was analyzed by employing the student t-test and one way-ANOVA where appropriate using Statgraphics® Centurion 18 (Statgraphics Technologies, Inc., USA) software with $p < 0.05$ significance level. The graphs were plotted by use of MS Excel® 2010.

Results and Discussion

AX from ispaghula is a well-established food ingredient and herbal remedy for bowel disorders. It is a well-characterized highly branched hemicellulosic material soluble in alkaline and insoluble in acidic media [43]. The AX used in the present work was found to be similar to the previously reported material [34] as revealed by elemental, monosaccharide, ATR-FT-IR and GPC analyses (Table 1). Other than ispaghula AX is found in a variety of cereals grains including rice, wheat, corn, rye, oat, barley and sorghum. It is also part of several plant cell walls such as bamboo and pangola grass [44]. It has a linear Xyl*p* backbone, partially substituted with α-L-Ara*f* residues on O-3 or O-2 or both of the Xyl*p* units (Scheme 1) [45]. It is composed of arabinose and xylose which are reducing sugars. The monosaccharides are released on partial hydrolysis of the polysaccharide in acidic environment provided by the gold salt, $H^{198}AuCl_4 \cdot 3H_2O$, in the present work. The cyclic structure of the monosaccharides is known to exist in equilibrium with the aldose form that affords reduction of Au^{3+} to Au^0 in the reaction mixture. This process is outlined in Scheme 1.

Our previous experience with AX for synthesis of non-radioactive AuNPs [29] prompted us to use this material for synthesis of ^{198}AuNPs. Among 36 radioisotopes of gold ^{198}Au is the most common isotope used for diagnosis and radiotherapy due to its appropriate half-life of 2.7 days [22]. Therefore, we attempted to synthesize ^{198}AuNPs using AX as reducing and capping agent for diagnostic imaging.

Synthesis and Characterization of ^{198}AuNPs

The ^{198}AuNPs were successfully prepared according to the method described in Materials and Methods section. It was observed that AX reduced gold ions to NPs within 25 min at 50°C. The optimal time and temperature were determined through preliminary experiments. The reduction process was witnessed by colour changes from yellow to purple and finally to ruby red. The reaction was monitored by recording the SPR spectra in the 350-800 nm range (Fig. 1a). The position of the absorption band at 526 nm indicates that the particles are spherical in shape having an average size of 30 nm [37]. The optimum conditions to obtain the SPR band at 526 nm were: amount of mucilage (0.1 %) 16 mL/40 mL, amount of $H^{198}AuCl_4$ (0.1 mM) 20 mL/40 mL, pH 8, time 25 min and temperature 50°C.

The XRD spectra (Fig. 1b) revealed characteristic face-centered cubic phase (JCPDS File No. 87-0720). The peaks were assigned to (1 1 1), (2 0 0), (2 2 2) and (3 1 1) planes of the nanocrystalloids. The intensities of the peaks suggest that the NPs were mainly oriented along (1 1 1) plane. The average size (n = 3) of the particles calculated from the XRD spectra by use of Debye–Scherrer equation were found to be 28 ± 3 nm. The TEM image (Fig. 1c) revealed that the particles were spherical in shape with size range of 25–30 nm. The DLS analysis provides information about hydrodynamic diameter and polydispersity index (PDI) of NPs. The hydrodynamic diameter thus determined was 101.4 ±1.2 at pH 8. The relatively larger diameter by DLS indicates that the particles were covered with the AX film which swell on contact with the buffer solution [46]. The ζ value -23.5 ± 3.2 indicated that the particles should be stable for a couple of months [47]. However, the particles synthesized by use of AX in the present work exhibited exceptional stability extending over three years, which suggests that the AX plays a role

in imparting exceptional stability to the particles. The PDI value of 0.322 reveals a narrow size distribution (Fig. 1d).

Cell Viability and Cytotoxicity Study

Biocompatibility of the AX-encapsulated ^{198}AuNPs was assessed by the NR uptake assay that provided a quantitative estimation of the number of viable cells in the culture. It is one of the widely used tests for the purpose [39]. It was found that incubation of HeLa cells with AX, AX-encapsulated ^{198}AuNPs at different concentrations and ^{198}AuNPs and did not affect the viability and morphology of the cells significantly (Fig. 2a-d) indicating their non-toxic nature. Thus, the AX-encapsulated ^{198}AuNPs can be used for *in vivo* diagnostic studies.

In order to rule out the interference of ^{198}AuNPs with neutral red assay a secondary cytotoxicity test was performed by potato disc assay. In this assay the tumour was induced by *A. tumefaciens*, which is considered histologically similar to that in animals and humans [40, 48] A negligible inhibition i.e., < 5 % was observed (Fig. 2e) suggesting the non-toxic nature of particles.

Biodistribution After Intravenous Administration of ^{198}AuNPs in Rabbit

In order to validate the *in vivo* study, stability of the AX-encapsulated ^{198}AuNPs was checked *in vitro* in biologically relevant media [49] including 10% NaCl, 0.2M cysteine, 0.2M histidine and 0.2M HSA at physiological pH for the time required to complete the study, i.e. 60 h. Typical spectra in HSA on time scale are shown in Fig. 3b. The AX-encapsulated ^{198}AuNPs suspensions

were found to be stable in all these media for that time. It was observed that there was no appearance of the band around 320 nm, characteristic of Au^{3+} compounds [50]. This clearly suggests that the particles do not ionize in the period under investigation.

Biodistribution data and gamma-camera images obtained after i.v. administration of AX-encapsulated^{198}AuNPs at fixed intervals of time are exhibited in Fig. 4. Highest uptake of ^{198}AuNPs after 3 h, was found in the spleen followed by liver, heart, muscle, kidneys and lungs (Fig. 4a). After 24 h the activity was not detectable in spleen and was significantly higher in liver.

Biodistribution After Oral Administration of ^{198}AuNPs in Rabbit

The gamma-camera images obtained after oral administration of AX-encapsulated ^{198}AuNPs are shown in Fig. 5. It can be seen that the activity did not move from stomach even after 24 h. This means that the particles are firmly encapsulated by AX, which coagulated there being insoluble in acidic medium. This effect was verified when the activity moved on oral administration of 1% sodium bicarbonate solution (5 mL) and accumulated in colon and remained confined there up to last observation at 96 h. These results suggest that the AX-encapsulated ^{198}AuNPs may be used for a drug delivery at colon or as diagnostic probes. At colon there is a host of enzymes including glycosidases and xylosidases, which would hydrolyse AX [51] in addition to a basic pH there. Thus, this study presents AX as a highly biocompatible capping agent for AuNPs for their use in medicine. A problem associated with other colon-specific drug delivery systems [52] is that a major drug loss occurs before they reach the ileocecal junction.

In vitro Distribution of ^{198}AuNPs in Serum Proteins

Five proteins bands separated after incubation of healthy human serum with AX-encapsulated ^{198}AuNPs are shown in Fig. 6 and distribution of radioactivity in these bands is depicted as bar graph (Fig. 6). The highest affinity of gold was recorded for α_1-globulin (6.5%) followed by α_2-globulin (~2%), β-globulin (~1%), γ-globulin (0.707%) and albumin (0.66%). Binding of gold with globulins from auranofin, an oral gold drug for rheumatoid arthritis, has also been reported previously [53, 54]. These results suggest that globulins can be labelled with radioactive gold by use of AX-ecapsulated^{198}AuNPs. Globulins play important role in inflammatory and malignancy processes. Therefore, the ^{198}Au-labelled globulins may find some useful applications as diagnostics and therapeutics.

Hemocompatibility Study

This study was carried out to assess the morphological effects of AX-encapsulated^{198}AuNPs on blood cells. No haemolysis was observed, whereas rouleaux effect (Fig. 7) was there with AX without NPs. The rouleaux effect is commonly observed with polysaccharides and polyols [55] and is not a deleterious effect. Mechanism of such type of aggregation of erythrocytes is not yet fully understood.

Complement activation

The amount of C_3 in blood before incubation was 128 %. After incubation with AX and ^{198}AuNPs separately, the amount of C_3 was 125 ± 6.35 % and 129 ± 5.14 %, respectively. These results clearly indicate that AX and ^{198}AuNPs do not activate the complement system. Thus the AX-encapsulated ^{198}AuNPs appear to be biocompatible [50].

Conclusions

This study demonstrates that ^{198}AuNPs synthesized by use of AX (a food material) as the reducing and dispersing agent are non-toxic to HeLa cells and *A. tumefaciens* and biocompatible. The AX-encapsulated ^{198}AuNPs exhibited different biodistribution *in vivo* depending upon the route of administration. After oral administration they accumulated at colon in rabbit after passing through stomach and produced good quality gamma images. After i. v. administration to rabbit and mouse they accumulated in liver. Thus, the AX-encapsulated ^{198}AuNPs appear to have potential for use as diagnostic and therapeutic agents. Since ^{198}Au is both γ and β emitter, these particles show a promise for use in image-guided nanoparticle therapies of cancer and advanced drug delivery devices. In diagnostics gamma imaging is preferred because of its lower limit of detection as compared to other techniques. These properties have been imparted to the particles by the presence of AX.

Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

Ethical Approval for use of human blood and animals The study protocols regarding use of human blood and animals were reviewed and approved by Institutional Review Board of Forman Christian College Lahore. Written informed consent was obtained from the blood donors (healthy human adults). Guidelines provided in Good Clinical Practice by ICH, World Medical Association Declaration of Helsinki and APA Committee on Animal Research and Ethics (CARE), USA were followed.

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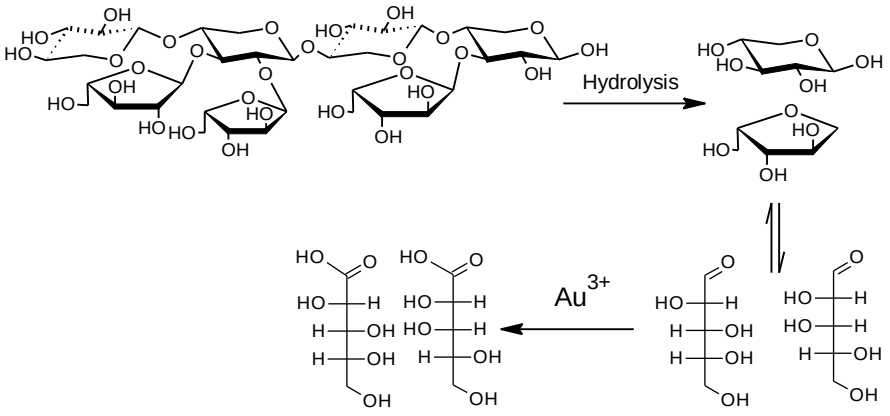
Figures



Scheme 1 AX undergoing partial hydrolysis followed by ring opening and oxidation by Au^{3+}.

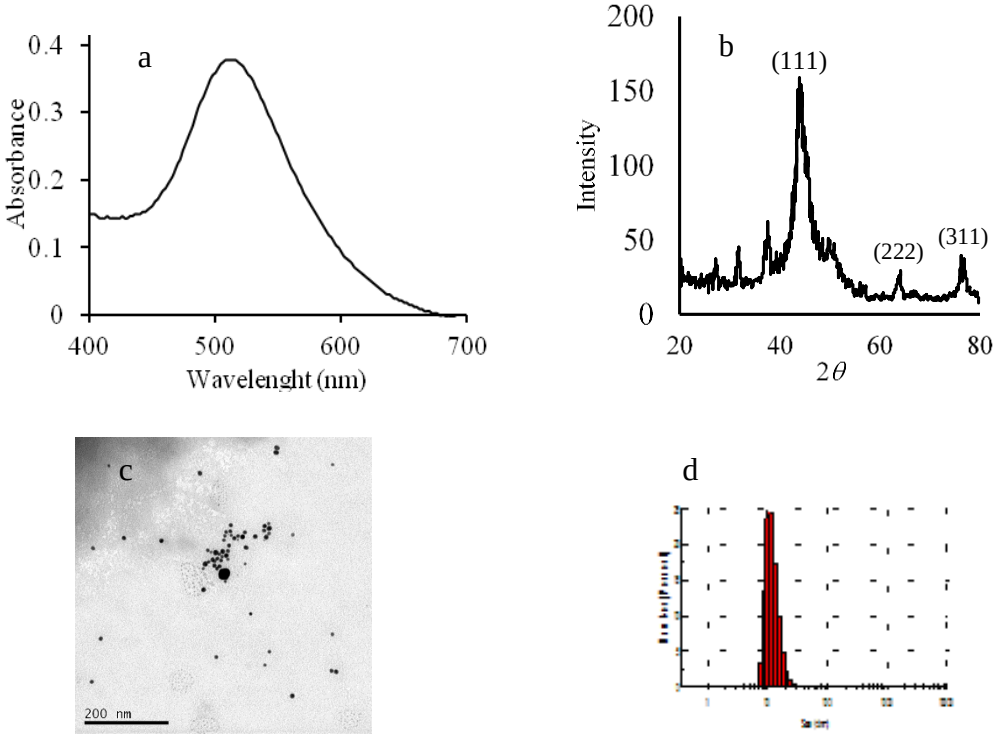


Fig. 1 Characterization of ^{198}AuNPs in AX (a) SPR spectrum (b) Powder XRD pattern of ^{198}AuNPs (c) TEM images (d). Histogram of size distribution ^{198}AuNPs measured by DLS technique.

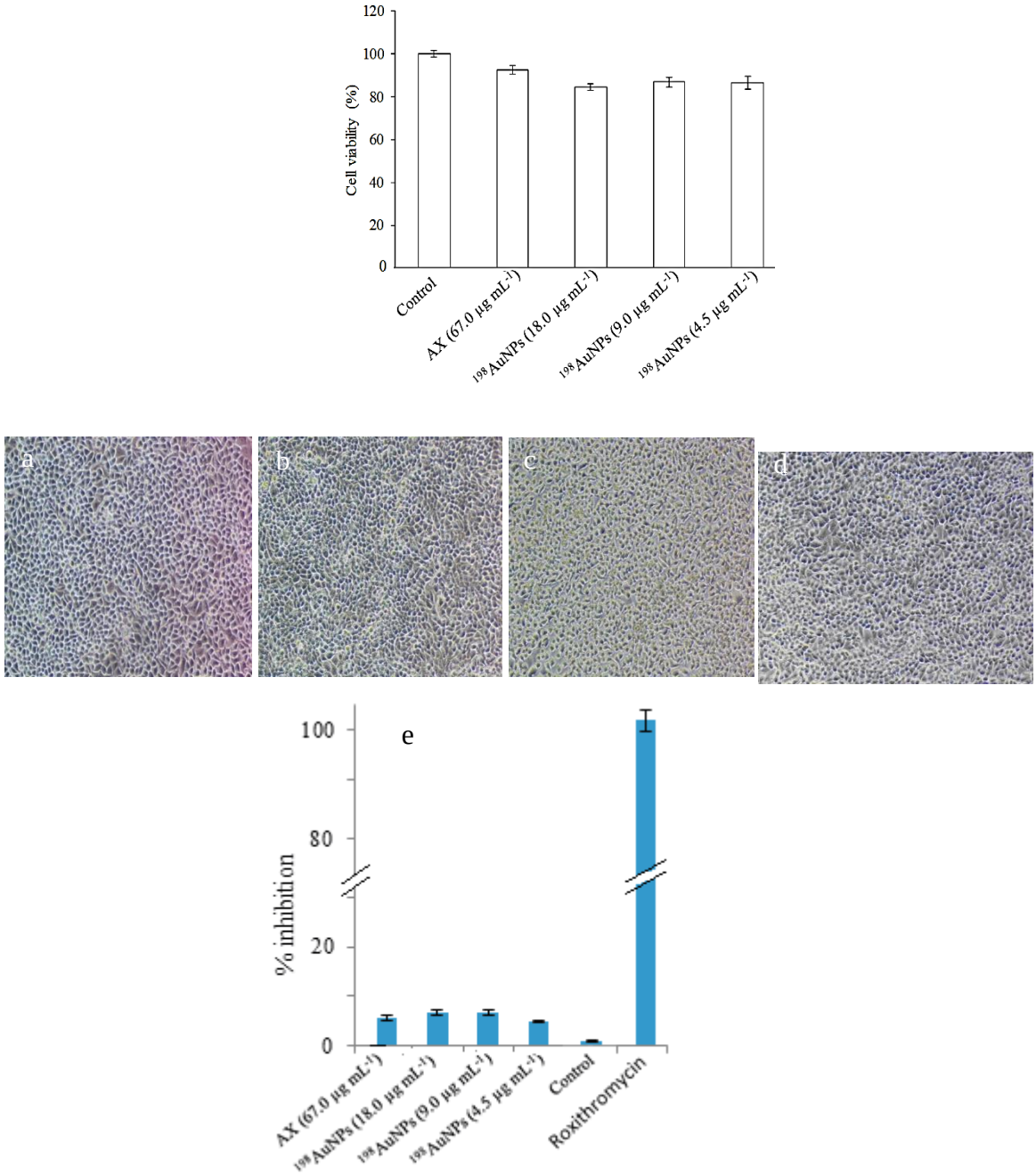
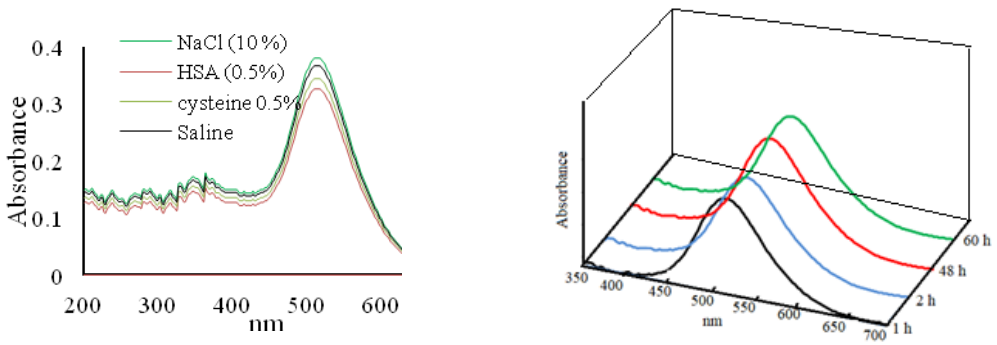
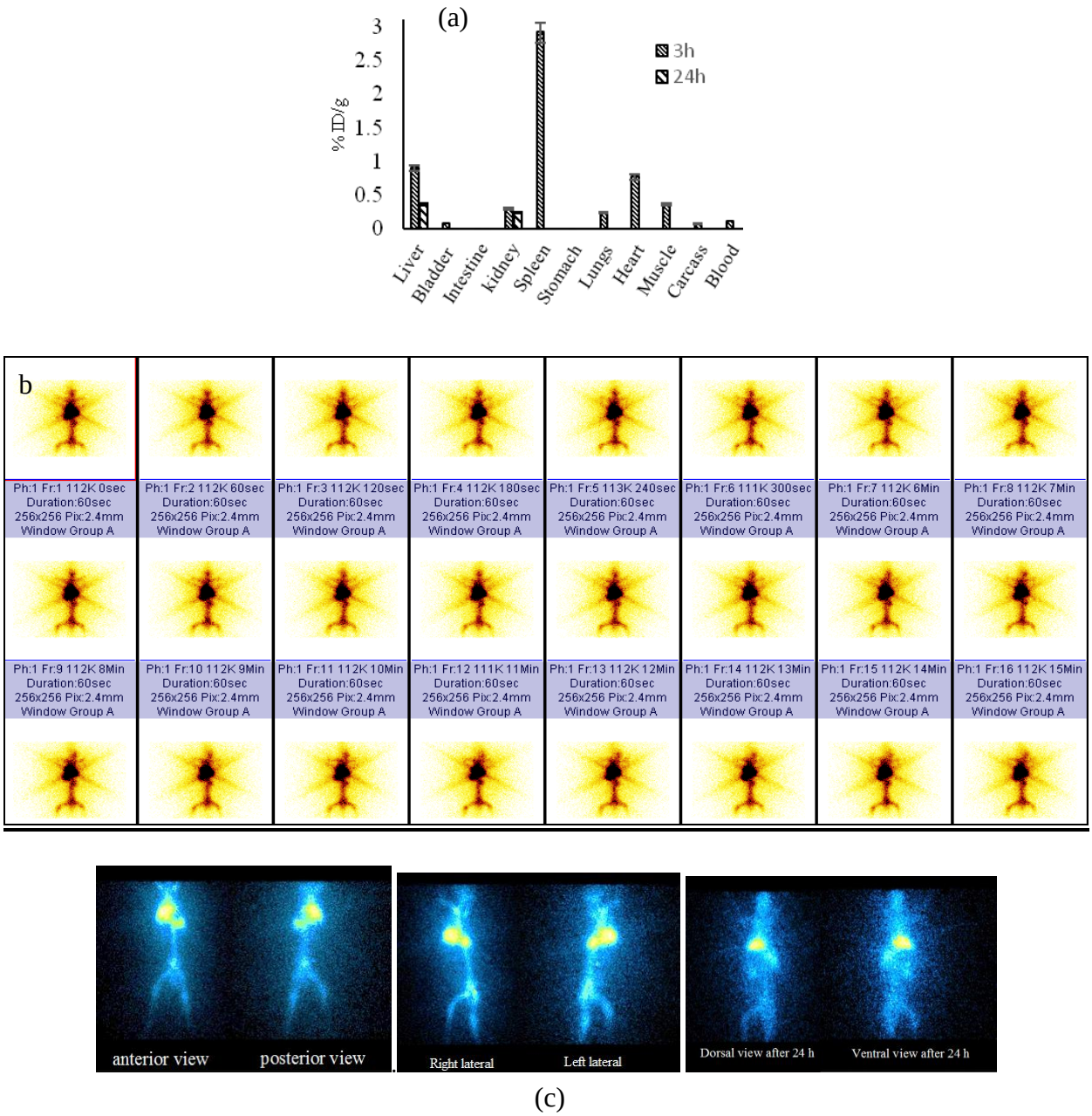


Fig 2 Top: Histogram showing % cell viability against HeLa cell line after incubation with AX suspension; Bottom: (a) microscopic images of the control cells (b) AX-treated cells and (c) AX-encapsulated^{198}AuNPs-treated cells (d) ^{198}AuNPs-treated cells showing no morphological changes, (e) Histogram showing % inhibition of *A. tumefaciens* by AX-encapsulated ^{198}AuNPs and roxithromycin used as positive control in potato disc assay. The results are expressed as the mean ± SD ($n = 6$, $p < 0.05$, the error bars represent SD)



(a) (b)

Fig 3 (a) Spectra showing stability (absence of 320-nm band) of AX-encapsulated ^{198}AuNPs in different media, (b) The SPR spectra of AX-encapsulated^{198}AuNPs in 0.2 M HSA showing no significant shift in wavelength and absorbance with time.



(c)

Fig 4 (a) Biodistribution of ^{198}AuNPs (equivalent to 14.0 µg mL^{-1} Au, activity ~1000 µCi mL^{-1}. The rabbits were anesthetized by 50 mg kg^{-1} intramuscular ketamine injection) before administration of the dose) in various organs at 3 h and 24 h after i.v. administration; the amount of gold is expressed as a percentage of injected dose per gram (% ID g^{-1}) of organ/tissue. The results are expressed as the mean ± SD ($n = 4$, $p < 0.05$, the error bars represent SD). (b) Dynamic gamma-camera images up to 15 min post i.v. injection (pH 7; 14.0 µg mL^{-1}; 1000 µCi mL^{-1}). (c) Gamma-camera images of whole body (anterior, posterior, right lateral and left lateral views) at 180 min post-injection, dorsal at 24 h and ventral at 24 h, showing accumulation of radioactivity in liver and spleen, with negligible amount in other organs.

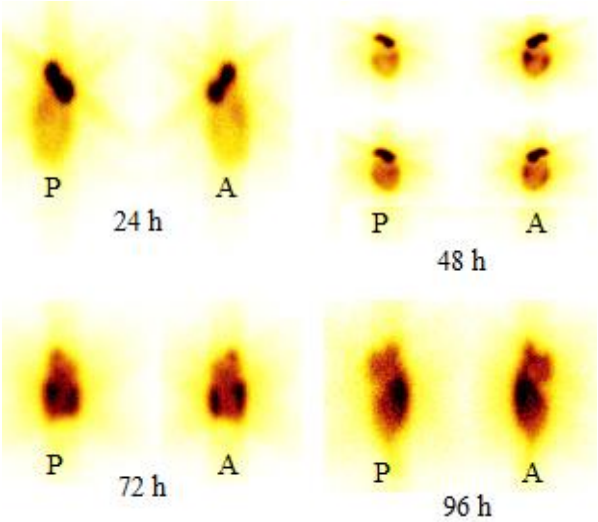


Fig 5 Gamma-camera images exhibiting accumulation of radioactivity at colon after oral administration of AX-^{198}AuNP (equivalent to 20.0 µg mL^{-1} Au, activity ~1200 µCi mL^{-1} in 2.0 mL saline adjusted to pH 7. The animals (n=4) were anesthetized by 50 mg kg^{-1} intramuscular ketamine injection before administration) to rabbit in colon after 24, 48, 72 and 96 h. A: Anterior, P: posterior.

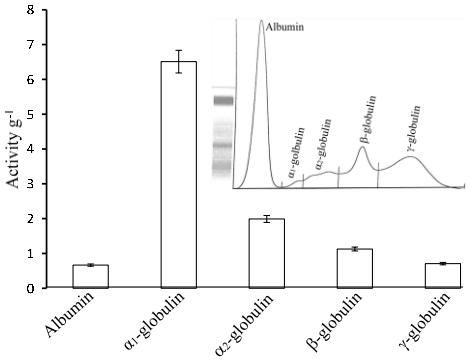


Fig 6 In-vitro distribution of AX-encapsulated^{198}AuNPs (equivalent to 14.0 µg mL^{-1} Au, activity ~1000 mCi mL^{-1}) in serum proteins SDS-PAGE analysis; the results are expressed as the mean ± SD (n=4, $p < 0.05$, error bars represents SD). Inset shows separated protein bands.

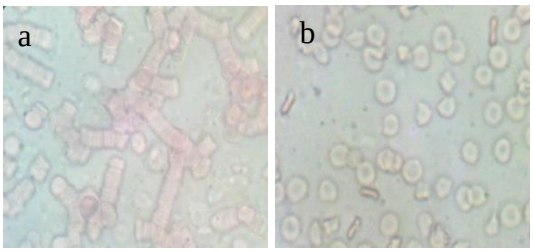


Fig 7 Optical images exhibiting rouleaux effect (a) AX treated-cells and (b) no effect by AX-encapsulated ^{198}AuNPs (200 µCi:500 µL) after 120 min.

Table

Table 1 Analytical data of AX, Experimental (Literature [34, 43] for FT-IR).

CHN (%)	C 31.61 (31.59), H 4.59 (4.66), N 1.05 (1.20)
Monosaccharides (%)	Ara 23.24 (23.11), Xyl 76.67 (76.89), Uronic acids 1.3 (Not determined)
Protein (%)	Not detectable (Not detectable)
Molar mass (g mol^{-1})	3.17×10^{6} (3.26×10^{6})
Moisture (%)	8.31 (8.24)
FT-IR bands (cm^{-1})	ν(OH) 3417 (3414), ν(CH) 2921 (2925), stretch due to absorbed water merged with carboxylic group 1634 (1630), in-plane δ(OH) 1457 (1462), δ(CH_2) 1423 (1417), δ(CH) 1367 (1375), antisym bridge oxygen δ 1257,1152 (1249, 1162), δ(CO) 1041 (1043), ν_{sym} (COC glycosidic linkage) 899 (896), polymer backbone vibrations 612, 531 (617,534)

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