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Triptorelin Peptide Conjugated Alginate Coated Gold Nanoparticles as A New Contrast Media for Targeted Computed Tomography Imaging of Cancer Cells

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Abstract

Objective: Increasing research has been focused on the development of various nanocomplexes as targeted contrast media in diagnostic modalities, mainly in computed tomography (CT) scan imaging. Herein, we report a new method that uses Triptorelin [a luteinizing hormone-releasing hormone (LHRH) agonist]-targeted gold nanoparticles (AuNPs) via alginate for early detection of cancer by molecular CT imaging.

Materials and Methods: In the experimental study, the formed multifunctional AuNPs coated with alginate conjugated with Triptorelin peptide (Triptorelin-Alginate-AuNPs) were synthesized and characterized via different techniques, including transmission electron microscopy (TEM), dynamic light scattering (DLS), and fourier transform infrared (FTIR) spectroscopy. The MTT assay was applied to calculate the toxicity of the NPs.

Results: The results indicated that the formed Triptorelin-Alginate-AuNPs with an Au core size of ~18 nm are noncytotoxic at 127-, 254-, 381- and 508-mM concentrations and revealed significant improvement in the attenuation of X-rays intensity and contrast to noise ratio (CNR), compared with non-targeted cells at the highest energies (90, 120, 140 kVp). At 90 kVp, compared to non-targeted cells, targeted cells (Triptorelin-Alginate-AuNPs) enable 1.58, 1.69, 3.7 and 3.43 times greater contrast at a concentration of 127 mM, 254 mM, 381 mM, and 508 mM, respectively.

Conclusion: These results suggest that the developed Triptorelin-Alginate-AuNPs may be considered an effective contrast agent for molecular CT imaging of gonadotropin-releasing hormone (GnRH) receptor-expressing cancer cells.

Keywords: Breast Cancer, Contrast Agents, Triptorelin

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Introduction

Computed tomography (CT) imaging has many advantages due to its availability, good anatomical display, deep tissue penetration, efficiency, as well as better density and spatial resolution compared to other imaging modalities. It has been considered one of the most effective medical imaging tools for diagnosis of disease during recent years (1).

To enhance contrast resolution in CT scan imaging, iodine-based small molecular compounds are most commonly applied as contrast media because of their higher attenuation of X-rays compared to normal cells (2). However, these molecules are not specific to the targeted tissue and are distributed non-specifically in the body, which can lead to toxicity in some organs such as the kidneys. Additionally, these molecules have a short blood circulation time and rapid clearance from the body (3). Therefore, to overcome these disadvantages, it is crucial to target the tissue (tumor) using contrast materials that have a higher atomic number than iodine for better contrast, less toxicity, high biocompatibility, and most importantly, longer blood circulation time in order to provide an adequate opportunity for imaging and accurate diagnosis in the desired texture.

The research for producing optimal contrast materials in CT and eliminating the disadvantages of the existing contrast materials is essential (4). The recent advances in nanotechnology and the role of nanoparticles in diagnostic modalities reveal that nanoparticles (NPs) could be applied as a signal factor in molecular CT imaging probes (5).

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Especially, gold nanoparticles (AuNPs) have received considerable notice as a contrast agent in CT scans due to their characteristics such as high atomic number, small size, ease of synthesis and surface modification, high biocompatibility and long blood circulation time (6). The electron density and the atomic number of AuNPs (19.32 g/cm^3 and 79, respectively) is greater than the currently used iodine nanoparticles (4.9 g/cm^3 and 53, respectively). Therefore, AuNPs can cause a significant improvement in the attenuation of X-rays causing them to be a suitable material for CT contrast agents (1, 7).

To improve imaging properties and contrast-enhancement in CT imaging of tumors, it is necessary to change AuNPs with targeting molecules (8). In the targeted imaging method, the aim is to accumulate targeted contrast only in a particular site because of the binding of ligands to specific receptors, thereby increasing contrast (9, 10). One of the most important causes of human mortality worldwide is cancer, with breast cancer accounting for 25.4% of the most common cancers and the leading cause of death among females worldwide (11).

Today, in receptor-dependent cancers, tumor-targeting methods have been considered due to their high specificity to the target. Some breast cancers occur as a direct result of high hormone receptors on the surface of breast cells. Approximately 70% of hormone-dependent cancers express peptide receptors gonadotropin-releasing hormone (GnRH). GnRH receptors (GnRH-R) are expressed in cancer tissues, including those of the breast (12-14). About 30% of breast cancers are sex hormone-dependent, and estrogen receptors are the most common type of hormone receptor in breast cells. Human breast cancer cells, including the MCF-7 cell line, can be highly specific as estrogen receptors at the cytoplasm level (15, 16). As a result, the peptide may help diagnose estrogen-dependent breast cancer cells in humans (MCF-7). So far in nanotechnology, many ligands have been suggested as targeting agents.

One promising targeting method is with the peptide Triptorelin. The Triptorelin peptide is a 10-amino acid peptide with a protein structure and is an analogue of the hormone GnRH, which is highly specific in cancers with overexpression of the GnRH receptor. Therefore, due to the great desire of human breast cancer cells to absorb Triptorelin, this valuable molecule can be used to target these cells (16, 17). Triptorelin is a decapeptide with the sequence pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH2, a molecular weight of 1311.4 g/mol, and is also a GnRH receptor agonist (18). Studies have revealed that Triptorelin can create an effective targeting agent due to its high specificity in cancers with overexpression of the GnRH receptor and its possible conjugation with AuNPs. Gold nanoparticles are toxic, and the alginate coating on the surface of gold nanoparticles is used to create biocompatibility and hydrophilicity properties and reduce the toxicity of gold on cells (19).

The mechanisms of delivering contrast agents to cancer cells and having an early diagnosis with high sensitivity and good contrast for diagnosis remains a big challenge. Previous research has indicated that targeted imaging can be achieved by linking the ligands onto the surface of the AuNPs (6, 20). The nanostructure's cytocompatibility was assessed using the MTT assay. The feasibility of applying the Triptorelin-Alginate-AuNPs nanostructure as an effective media for imaging of MCF7 cells was investigated at various tube potentials and concentrations.

Materials and Methods

Tetrachloroauric acid (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and Sodium alginate were purchased from Sigma-Aldrich, and synthesized as summarized in the next section. In this experimental study, human breast cancer cell lines (MCF-7) were obtained from the Pasteur Institute of Iran.

Synthesis of alginate-coated gold nanoparticles (AuNPs-Alginate)

First, AuNPs were produced and coated by alginate in aqueous phase under thermal conditions. Auric salt aqueous solution with a concentration of 1 mg was prepared and slowly added to the aqueous alginate solution with a concentration of 0.41 mg/mL to obtain a final concentration of 10^{-2} mg of auric salt. After proper mixing, the solution was gradually heated in an aqueous medium, and the production of AuNPs was apparent by change in the color of the solution from yellow to reddish pink. After that, the AuNPs solution was allowed to cool down to room temperature and diluted with an equal volume of ethanol, then the solution was centrifuged and dispersed in deionized water. It was then dialyzed against deionized water for 1 day (using MWCO 3500 dialysis membrane) and stored in a refrigerator (21).

Surface modification of AuNPs with Triptorelin conjugate

Surface modification and conjugation of Triptorelin at the surface of the AuNPs was performed in two steps. In the first step, 1 ml of the solution of the prepared AuNPs was placed on a magnetic stirrer to reach pH=6.5, then 0.15 mg N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) (8.51007. Sigma-Aldrich, USA) and 0.09 mg N-Hydroxysuccinimide (NHS) (130672. Sigma-Aldrich, USA) were added, and after 15 minutes, 0.5 mg of the peptide was added. In the second step, the complex was stirred for 1 hour at room temperature

to achieve the targeted nanocomplex. At the end, the prepared Triptorelin-Alginate-AuNPs were dialyzed in the refrigerator for 24 hours.

Characterization techniques

The morphology and size of the NPs were analyzed by a LEO 912 AB transmission electron microscope (TEM). In addition, dynamic laser light scattering (DLS) was applied to investigate size distribution and the adequate hydrodynamic size of the NPs by a Vasco 3 (Cordouan Instruments Limited, France). Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to measure the concentrations of AuNPs, and the surface charge was measured using a ZEN 3600 nanosizer (Malvern, UK). The Fourier transform infrared (FTIR) spectra of targeted and non-targeted AuNPs were recorded by using a Thermo Nicolet AVATAR 370 FT-IR (USA) instrument equipped with pressed KB pellets in the wavenumber range of 500 to 4000 cm^{-1} to confirm the binding of Triptorelin peptide on the surface of AuNPs nanoparticles.

In vitro experiments

Cell culture

Human breast cancer cell lines (MCF-7) were supplied by the Pasteur Institute of Iran (Tehran, Iran). These cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS, 16000044. GIBCO, USA) and 1% penicillin-streptomycin solution. Sub-culturing of the MCF-7 cell line was done by separating the adherent cells by 0.025 % trypsin-EDTA. In order to wash the flask and remove the residues and floated dead cells, plates and flasks were washed with phosphate buffered saline (PBS, Gibco, USA). The cells were cultured as a monolayer at a density of 10^4 cells/cm^2 in T-25 tissue culture flasks and were incubated in a humidified atmosphere containing 5% CO_2 and 95% air at 37˚C.

Cytotoxicity assay

The cytotoxicity of AuNPs and Triptorelin-Alginate-AuNPs was determined using an MTT assay (22). This method is based on reducing the ratio of tetrazolium yellow salt crystals by the enzyme succinate dehydrogenase and the formation of insoluble formazan blue crystals, which shows the cytotoxicity level of the material. At a density of 1×10^4, MCF-7 cells per well were seeded in a 96-well plate and incubated at 37˚C in a 5% CO_2-saturated humidified atmosphere. The various concentrations of targeted and non-targeted NPs (127, 254, 381 and 508 mM) were added to each well for 24 hours. After 24-hours incubation, the cell culture medium was removed, and the cells were washed three times with PBS buffer. Then, 20 µl of MTT reagent and 200 µl of DMEM culture medium without FBS were added to each well, and the plates were maintained in the dark and incubated for 4 hours at 37˚C. Then, the MTT solution inside the plates was removed and replaced with 200 ml dimethyl sulfoxide (DMSO, D2650, Sigma Aldrich, USA). At the end, the plates were immediately read on a microplate reader (Stat Fax 2100, USA), and the absorbance was measured at 570 and 630 nm wavelength for each well. This was repeated three times to confirm the test results. Thereby, the Cell viability (%) percentage was calculated using the following formula (Eq.1) (23).

Eq.1

$$\text{Cell viability (\%)} = (OD_{570\,nm} \text{ of sample - OD of control sample}) / (OD_{570\,nm} \text{ of control - OD of control sample}) \times 100\%$$

In vitro computed tomography imaging of cancer cells

MCF-7 cells were seeded at a density of 10^4 cells per well in a 6-well plate and were incubated in a humidified atmosphere containing 5% CO_2 and 95% air at 37˚C for 24 hours. The different concentrations of AuNPs and Triptorelin-Alginate-AuNPs (127, 254, 381 and 508 mM) were maintained and added to each well and incubated for 24 hours. After 24-hours of incubation, to remove unloaded nanoparticles, the cells were washed three times with PBS. Then the cancer cells were trypsinized, centrifuged and suspended with 100 mL PBS and placed in 0.5-ml tubes. Next, the tubes including cancer cell suspensions were put in a phantom made of Plexiglas. After that, the phantom was scanned by a CT (SUPRIA, Hitachi Medical Solutions) with the following parameters: Peak kilovoltage of 90, 120, and 140 kVp; tube current-time of 250 mAs; and slice thickness of 0.625 mm. The images obtained from tubes were analyzed by RadiAnt DICOM Viewer 2020.2.2 (64-bit).

Contrast-to-noise ratio

Contrast-to-noise ratio (CNR) was calculated from the recorded hounsfield unit (HU) by plotting the region of interest (ROI) analysis across the selected images. The CNR was calculated using the following formula (Eq. 2) (24):

Eq.2

$$CNR=(x_S-x_{BG}) / \sigma_{BG}$$

The X_S and X_{BG} are the signal intensity measured in two different structures of interest in the same CT images. Meanwhile, the σ_{BG} is the standard deviation of the background noise of the image. The contrast to noise ratio (CNR) was also calculated using quantitative image analysis (Fig.1).

Statistical analysis

Statistical analysis was done by the SPSS software (version 11, IBM, USA). One-way analysis of variance and Tukey's Supplementary Test statistical method were used to evaluate the significance of the experimental data. A value of $P<0.05$ was considered as statistically significant.

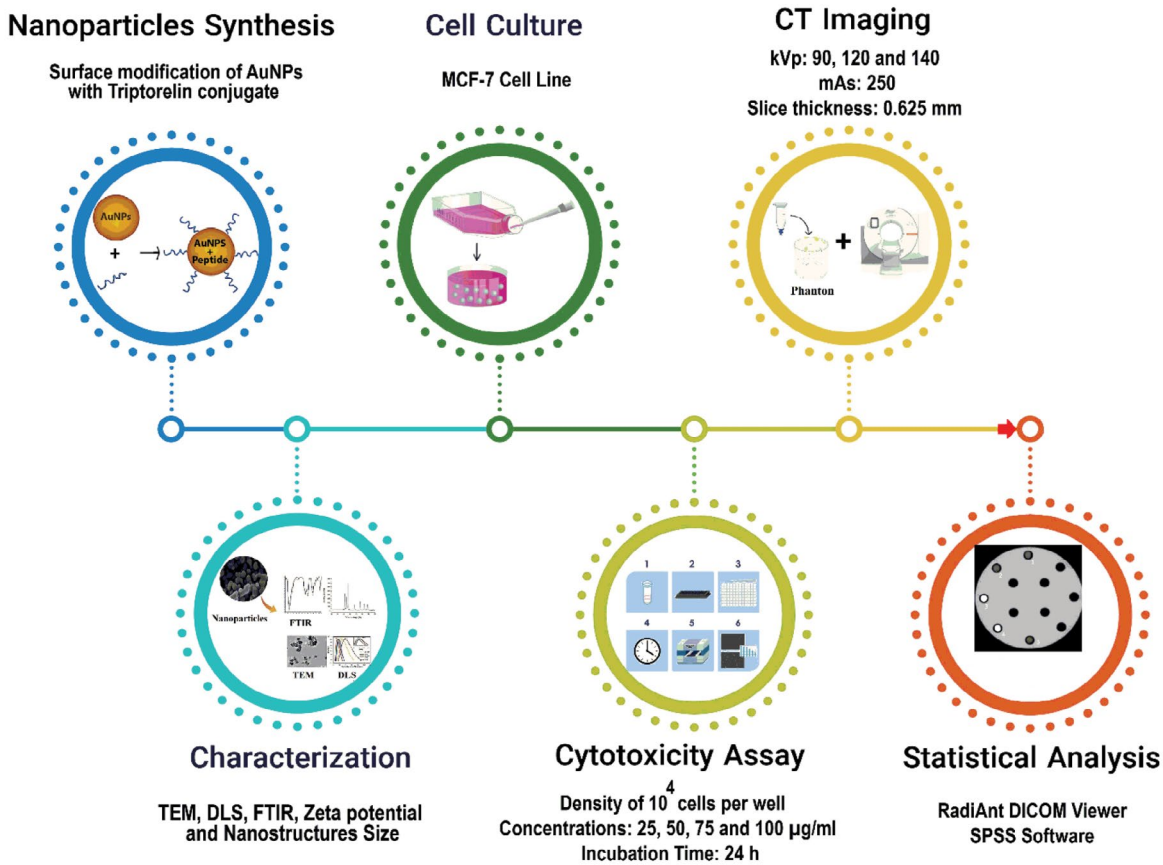

Fig.1: Workflow diagram of the present study. CT; Computed tomography, TEM; Transmission electron microscope, DLS; Dynamic light scattering, and FTIR; Fourier transform infrared.

Results

Synthesis and characterization Triptorelin-Alginate-AuNPs and AuNPs-Alginate

Targeted and non-targeted suspensions were synthesized by the previously delineated protocols. TEM was used to evaluate the morphological characteristics of Triptorelin-Alginate-AuNPs nanostructure. Figure 2A represents a typical TEM micrograph of the synthesized Triptorelin-Alginate-AuNPs, and the findings suggest that the synthesis methods were successful in preparation of a wide range of particle sizes, the NPs are well dispersed and appear to be round in shape with a size distribution of 18 nm (Fig.2B). The hydrodynamic size and size distribution of the NPs, as well as the zeta potential of the NPs, were measured by the zeta potential analyzer and DLS test, respectively. From Figure 2C and D, it was determined that the hydrodynamic size of targeted and non-targeted nanoparticles is 29.5 and 23.45 nm, respectively. The FT-IR spectrum of functionalized Triptorelin to AuNPs and their forming ingredients is shown in Figure 2E. Moreover, the zeta potential of the AuNPs was -13.20 mV, and -31.56 mV for the synthesized Triptorelin-Alginate-AuNPs, as shown in Figure 2F.

Fourier transform infrared analysis

To confirm the conjugation of Triptorelin on the surface of AuNPs, FT-IR spectroscopy was performed. The FT-IR spectrum of functionalized Triptorelin to AuNPs and their forming ingredients is shown in Figure 2E. In order to thoroughly characterize the chemical structure of drug conjugated AuNPs, FTIR spectra of the AuNPs were recorded after each stage of modification. Figure 2E represents the spectra of the AuNPs-Alginate and AuNPs-Alginate-peptide, respectively. The band at 1610 cm^{-1} and 1413 cm^{-1} corresponds to the asymmetric and symmetric stretching vibration of carboxylate in AuNPs-Alginate and at 1663 cm^{-1} relates to stretching vibrations of amide in Triptorelin-Alginate-AuNPs. In the FTIR spectrum of Triptorelin-Alginate-AuNPs, the binding of the peptide to the nanoparticle surface corresponds to amide linkage, which appeared in the new peak at 1663 cm^{-1}, which indicates the displacement of the carboxylate alginate peak from 1610 cm^{-1}. While the small peak at 1028 cm^{-1} is due to the C–O bond stretching vibration in the conjugate, the peak at 1032 cm^{-1} is attributed to the stretching

vibration of C-O functionality in AuNPs-Alginate. The bands between 3393 and 3422 cm^{-1} are assigned to the O–H stretching vibration bands of Triptorelin-Alginate-AuNPs and AuNPs-Alginate, respectively. On the other hand, the presence of a peak at 702 cm^{-1} indicates a bond between aromatic derivatives, which is related to the aromatic ring of the composition of the Triptorelin-Alginate-AuNPs.

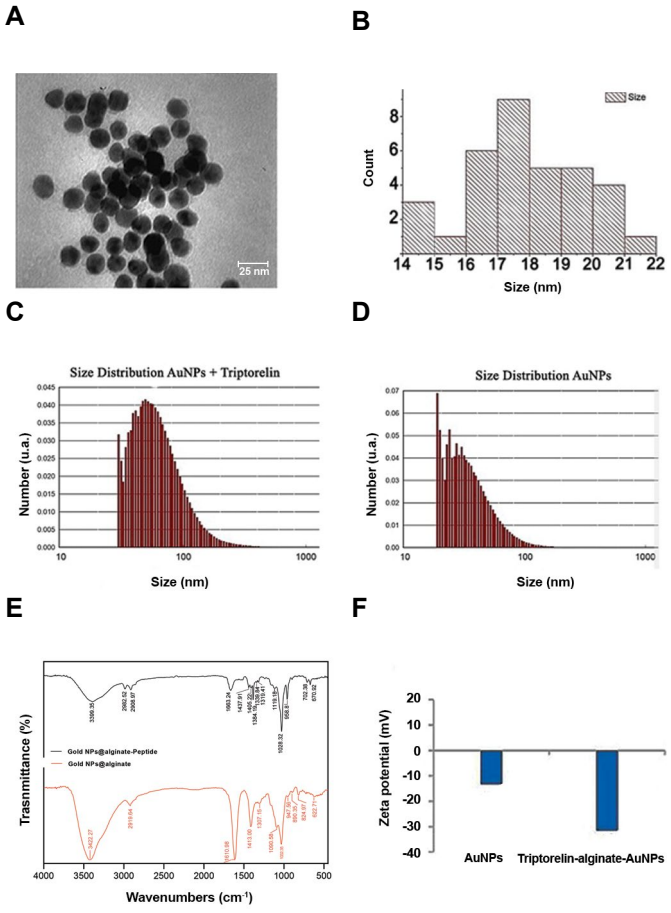

Fig.2: Characterization of targeted and non-targeted gold nanoparticles. **A.** TEM image (inset is the high-resolution TEM image), **B.** Size distribution histogram of TEM image, **C**, **D.** DLS profile of the prepared nanocomplex, **E.** FTIR spectra of Triptorelin-Alginate-AuNPs and AuNPs-Alginate, and **F.** Zeta potentials of various nanoparticles synthesized in this study. TEM; Transmission electron microscope, DLS; Dynamic light scattering, and FTIR; Fourier transform infrared.

Cytocompatibility assay

MTT assay

The cytocompatibility of various concentrations of NPs on the MCF-7 cell line were quantified by the MTT colorimetric assay to reveal the viability of cells. According to the studies, the cell toxicity of gold nanoparticles is always concentration dependent. Figure 3 shows the percentages of viable cells after 24 hours incubation with targeted and non-targeted NPs at concentrations of 127, 254, 381 and 508 mM. According to this figure, the nanoparticles did not show cytotoxicity at any of the expressed concentrations.

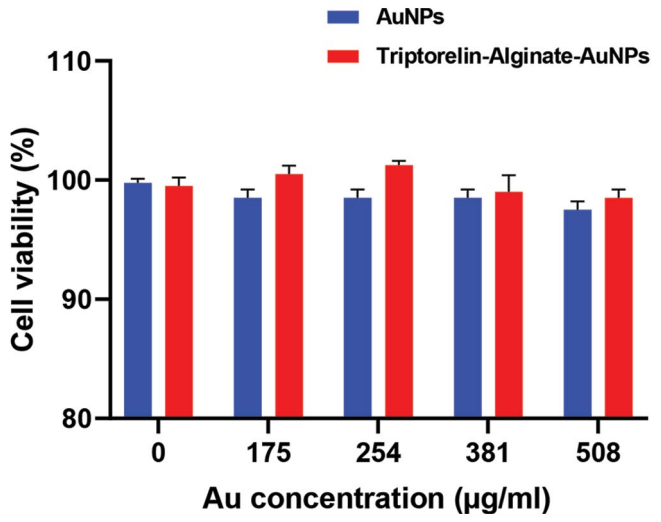

Fig.3: The viability of MCF-7 cells after 24-hours incubation with AuNPs and Triptorelin-Alginate-AuNPs at different concentrations.

The Viability of MCF-7 cells incubated with AuNPs and Triptorelin-Alginate-AuNPs at 508 mM concentration were 102% and 127%, respectively, showing a suitable cytocompatibility range.

Targeted computed tomography scanning of cells

To evaluate the efficacy of the Triptorelin as a targeting molecule of AuNPs on an X-ray attenuation intensity, MCF-7 cells were treated with AuNPs and Triptorelin-Alginate-AuNPs with various concentrations (127, 254, 381 and 508 mM) and were scanned by a CT. The samples showed that the image of the cells which were not treated with NPs as a control were not obtainable, even at the highest kVp and concentration. However, the presence of NPs enhances the CNR ratio of the cancer cells in the images, and the cancer cells that absorbed the NPs became evident as bright spots at the end of the tubes. At the same time, increasing the concentration of AuNPs, leads to an increase in the contrast of images (1, 9). In order to better compare CT images in terms of contrast difference, a quantitative analysis was done. The quantitative analysis of the HU values illustrated that the efficiency of the AuNPs leads to CT contrast-enhancement. Consequently, the HU values of MCF-7 cells incubated with metal (with or without Triptorelin Peptide) were significantly increased in contrast to the cancer cells that were not treated with NPs. The results indicated that increasing the concentration of AuNPs leads to more excellent X-ray attenuation, which can be translated as better contrast

in clinical imaging by increasing the concentration of nanoparticles at the region of interest (6, 25).

Importantly, in the range of the expressed concentrations, the cells treated with Triptorelin-Alginate-AuNPs showed a significant increase in CT contrast compared to cells treated with non-target AuNPs. At the highest energies typically available in CT (90, 120, 140 kVp), experimental findings indicate significant improvement in the HU (contrast enhancement) of targeted cells (Triptorelin-Alginate-AuNPs) compared with non-targeted cells at a 127-, 254-, 381- and 508-mM concentration range. The findings of quantitative analysis of CT values (HU) at concentrations of 127, 254, 381 and 508 mM and different tube current-time products (90, 120, 140, and 250 mAs) are presented in Figure 4. At 90 kVp, targeted cells (Triptorelin-Alginate-AuNPs) at a concentration of 127 mM enable 1.58-times, at 254 mM enable 1.69-times, at 381 mM enable 3.7-times and at 508 mM enable 3.43-times higher contrast per unit mass in contrast with non-targeted cells. At 120 kVp, targeted cells (Triptorelin-Alginate-AuNPs) at a concentration of 127 mM enable 1.7-times, at 254 mM enable 1.66-times, at 381 mM enable 3.8-times and at 508 mM enable 3.7-times higher contrast per unit mass compared with non-targeted cells. At 140 kVp, targeted cells (Triptorelin-Alginate-AuNPs) at a concentration of 127 mM enable 1.55-times, at 254 mM enable 1.57-times, at 381 mM enable 4.1-times and at 508 mM enable 3.7-times higher contrast per unit mass in contrast with non-targeted cells.

Investigation of contrast-to-noise ratio

In order to investigate the increase in the CNR of cells in the presence of targeted and non-targeted nanoparticles, using equation (2), we selected cells without the presence of nanoparticles as the background, and the amount of CNR obtained according to the amount of attenuation and noise of cell images were measured at different tube potentials. The results are presented in Figure 6. At a concentration of 127 mM, targeted cells (Triptorelin-Alginate-AuNPs) at 90 kVp enable 1.64-times, at 120 kVp enable 1.63-times and at 140 kVp enable 1.65-times greater CNR value compared with non-targeted cells. At a concentration of 254 mM, targeted cells (Triptorelin-Alginate-AuNPs) at 90 kVp enable 1.64-times, at 120 kVp enable 1.73-times and at 140 kVp enable 1.76-times greater CNR value compared with non-targeted cells. At a concentration of 381 mM, targeted cells (Triptorelin-Alginate-AuNPs) at 90 kVp enable 4.4-times, at 120 kVp enable 4.06-times and at 140 kVp enable 3.9-times greater CNR value compared with non-targeted cells. At a concentration of 508 mM, targeted cells (Triptorelin-Alginate-AuNPs) at 90 kVp enable 3.9-times, at 120 kVp enable 3.89-times and at 140 kVp enable 3.56-times greater CNR value compared with non-targeted cells. The results show that the targeted nanoparticles (Triptorelin-Alginate-AuNPs) obtained higher CNR at the same tube potentials and concentrations than the non-targeted nanoparticles (AuNPs). Our results showed that the CNR was enhanced for all groups by increasing the concentration and kVp.

Axial CT images of different samples are displayed in Figure 5.

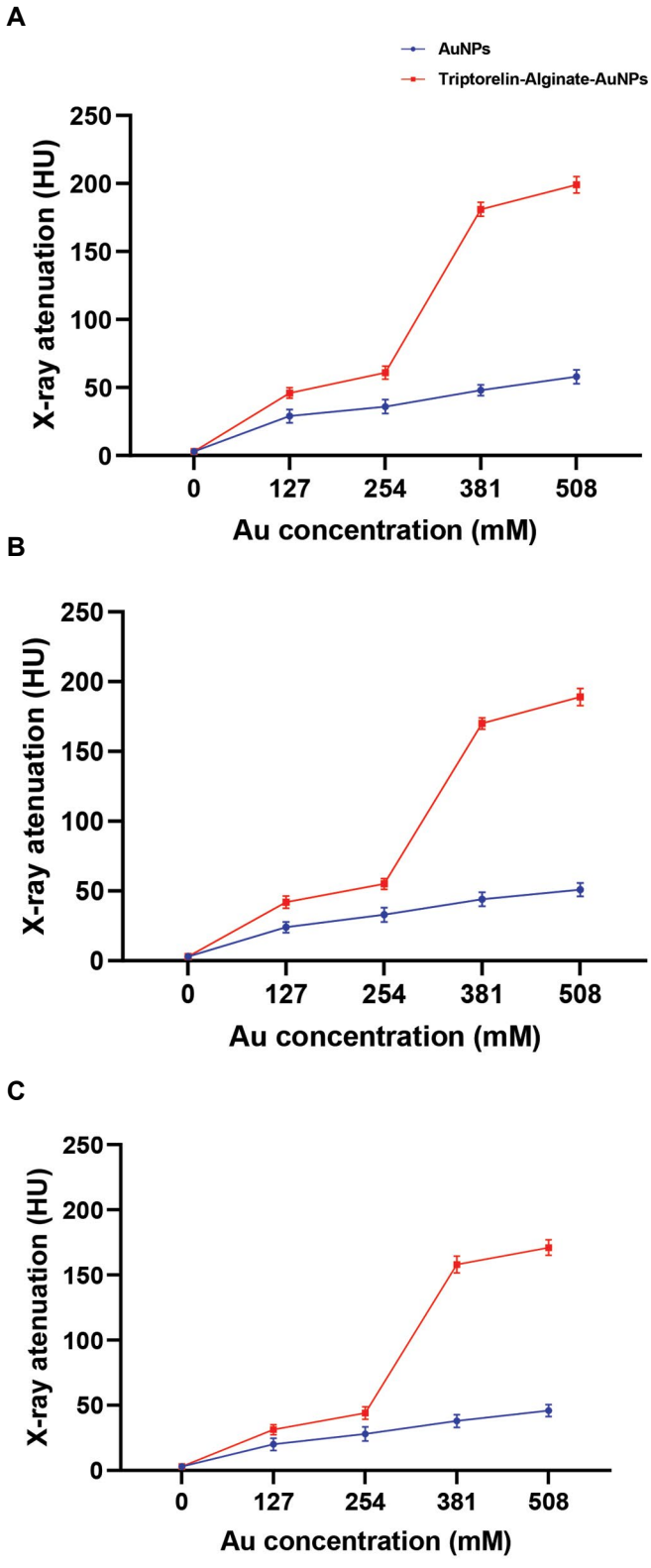

Fig.4: Computed tomography (CT) images and X-ray attenuations intensity of targeted AuNPs and AuNPs at different tube potentials. The contrast per unit of MCF-7 cells with AuNPs and Triptorelin-Alginate-AuNPs at different tube potentials at: **A.** 90 kVp, **B.** 120 kVp and **C.** 140 kVp.

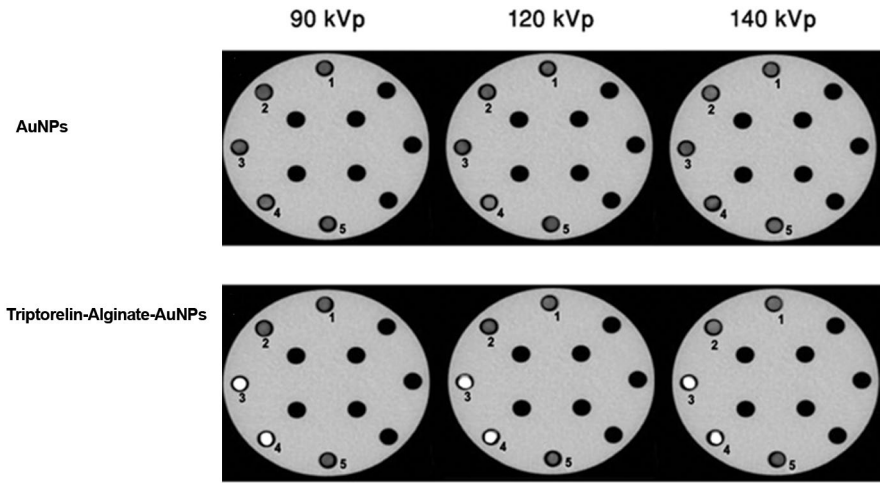

Fig.5: Computed tomography (CT) images and X-ray attenuations intensity of targeted AuNPs and AuNPs at different concentration. **A.** CT images of AuNPs and **B.** Triptorelin-Alginate-AuNPs at different concentration 1; 127 mM, 2; 254 mM, 3; 381 mM, 4; 508 mM, and 5; Control 0 mM and tube potentials.

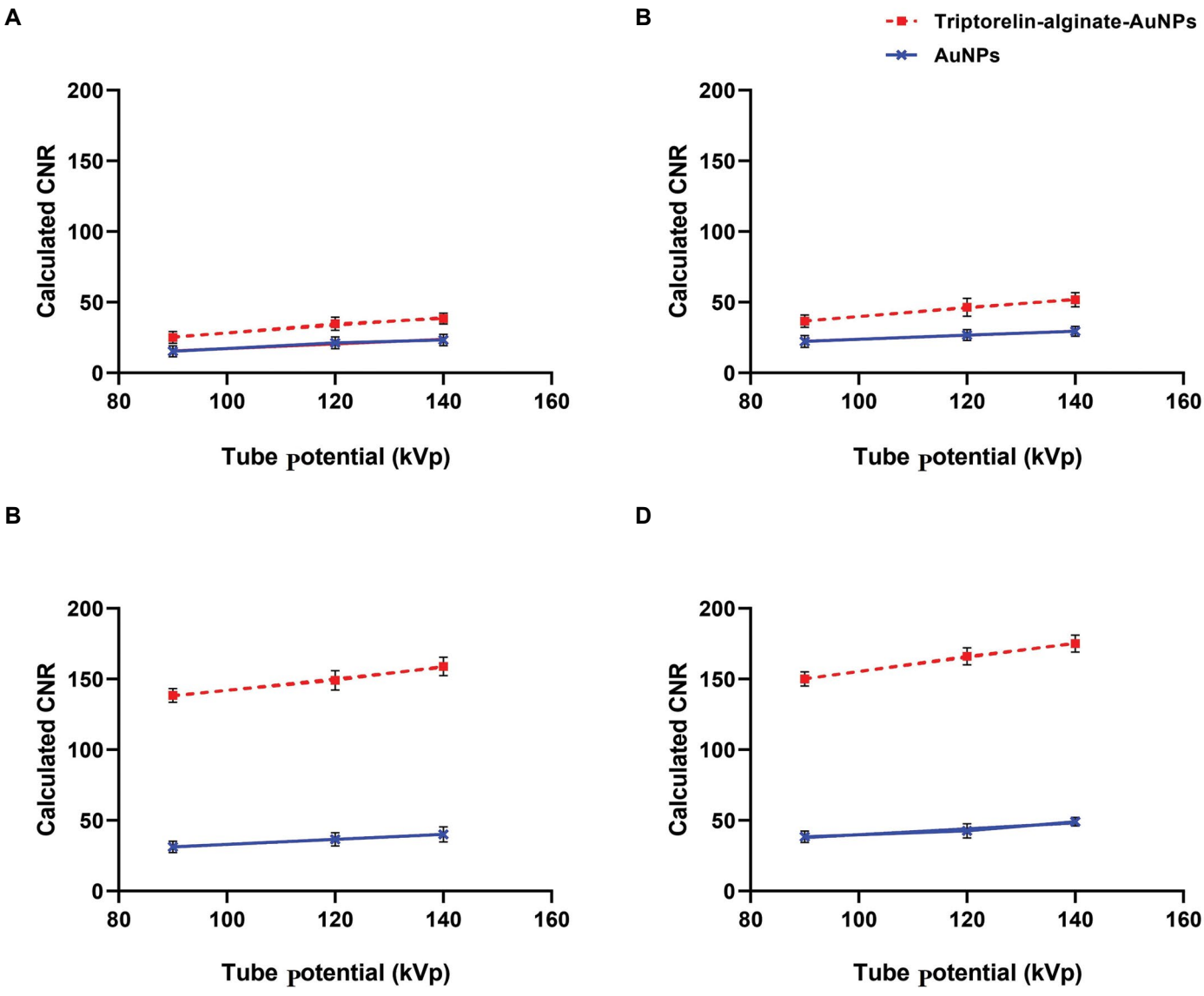

Fig.6: CNR obtained from CT images of cancer cells in the presence of targeted and non-targeted NPs. The CNR variation at different tube potentials and concentrations of: **A.** 127 mM, **B.** 254 mM, **C.** 381 mM, and **D.** 508 mM. CNR; Contrast to noise ratio, CT; Computed tomography, and NPs; Nanoparticles.

Discussion

Recently, several new nanotechnology-based methods using various nanoparticles with specific properties, for cancer diagnosis have been under investigation. Among this, gold nanoparticle (AuNPs), have gained significant interest in developing unique X-ray contrast agents with high potential in cancer diagnosis due to their high atomic number, strong X-ray attenuation, and facile chemical synthesis.

Increasing attention has been focused on the development of various nanostructures as contrast enhancement agents in targeted diagnostic medical imaging, especially in CT. The new generation of molecularly targeted CT contrast agents has changed the concept of CT from anatomical-based diagnosis to diagnosis based on molecular markers (20).

Targeting can be reached by the conjugation of NPs to a different ligand, such as peptides, antibodies, and vitamins that have highly unique cancer- cell detection capability. Hainfeld et al. (26), indicated that targeted AuNPs with anti-Her2 antibodies could increase the visibility of tumors (1.6-fold) rather than non- targeting antibodies. In addition, several studies indicated that targeted AuNPs with folic acid could enhance CT images approximately 2 times more greater than non-targeted cells at the highest concentration examined (4, 27). In another study, Chanda et al. (28) indicated that the AuNPs targeted with bombesin enhanced CT images of prostate and breast cancer cells selectively.

In this study, we have synthesized Triptorelin conjugated Alginate coated AuNPs and investigated their application to be applied as the targeted contrast media in the scanning of MCF-7 cells. The modification of AuNPs with Triptorelin targeting can increase the diagnostic usage of AuNPs in the clinic. This is done by using specific GnRH receptor molecules with Triptorelin conjugated AuNPs on cancer cell membranes (12, 29).

In this study, the formed nanocomplex was evaluated using various characterization techniques. Based on the results, the NPs were monodispersed, and DLS findings confirmed this using a polydispersity index (PdI) of 0.14. It was also that the structure of the NPs were spherical shaped (30, 31). On the other hand, the zeta potential of the AuNPs alternated from -13.20 to -31.56 mV after being modified with alginate, which shows that the alginateylated shells were more permanent than the free shells in water.

The cytotoxicity of the Triptorelin-Alginate-AuNPs nanostructure was done by the viability of MCF-7 cells by a MTT assay. At the concentration range used in this study (127, 254, 381 and 508 mM), MCF-7 cells show great cell viability, suggesting that the contrast media has great biocompatibility. After that, we investigated the feasibility of applying the formed Triptorelin-Alginate-AuNPs for targeted scanning of cells.

To assess the efficacy of the Triptorelin targeting of NPs on a CT number, The MCF-7 cells were incubated for 24 hours with targeted and non-targeted NPs, at concentrations of 127, 254, 381 and 508 mM, then were scanned by a clinical CT at different tube potentials (90, 120, 140 kVp). CT images and the respective X-ray attenuation values for each sample were measured at different concentrations and tube potentials. CT images indicated that X-ray attenuation was dependent on nanoparticle concentration and tube potential (1, 31-33). The MCF-7 cells treated with AuNPs with or without Triptorelin at various incubation times, tube potentials, and concentrations. In CT images, it is hard to visually diagnose the contrast of the cells incubated with AuNPs and Triptorelin-Alginate-AuNPs at various concentrations. The quantitative analysis of the HU should be done with the standard program. At equal concentration and tube potential, the HU show that the MCF-7 cells incubated with Triptorelin-Alginate-AuNPs showed significantly greater X-ray attenuation than other groups treated with the non-targeted AuNPs. It can be visually confirmed using the images of the cells.

Our results indicated that X-ray attenuation enhanced when the concentration of gold nanoparticles increased, and in sum leads to NPs uptake in place of the body at the target area. To explain this, with the increment of concentration, the number of particles in a determined volume increase. So linear attenuation coefficient increases and causes an increase in the CT number. On the other hand, an increase in kVp reduces the number of attenuated photons. Thus, the attenuation coefficient becomes smaller, and it causes a lower CT number for all studied nanoparticles. Nevertheless, as the number of detected photons increases, the noise content of the image is decreased, and consequently, a higher CNR was obtained. This can be attributed to differences in the imaging properties of the x-ray CT scanner, including the beam spectra and detector performances, as well as concentrations and the size of the studied nanoparticles (24, 31, 34).

It is clear that for total NPs a greater concentration causes more cellular accumulation of NPs. Consequently, the contrast will be increased, and valuable data will be clinically provided. However, it is expected that by increasing the concentration, the HU values would increase linearly (9). Our results propose that targeted cells (Triptorelin-Alginate-AuNPs) have a more significant application potential as a positive CT imaging contrast agent than non-targeted cells (AuNPs) at the same concentrations and tube potentials.

Conclusion

In summary, it was demonstrated that the Triptorelin-Alginate-AuNPs contrast agent could be applied as a beneficial nano-molecular probe for cancer diagnosis in CT imaging. Utilizing gold nanoparticles in combination with Triptorelin targeting creates a targeted molecular nanoprobe in CT imaging to diagnose specific cancer cells. The nanocomplex was alginateylated so that it could be biocompatible and have stability. The synthesized nanoprobe was modified with Triptorelin to target the MCF-7 cells that overexpress GnRH receptors. As well as it was revealed that conjugation of Triptorelin to the AuNPs, enhances the accumulation of contrast agents on the tumor surface, thereby increasing the contrast enhancement.

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Authors' Contributions

A.M., S.Kh.; Contributed to conception and design. M.D.-D., F.V.N.; Contributed to all experimental work, data and statistical analysis, and interpretation of data. R.I; Contributed to the synthesis of nanoparticles and interpretation of characterization of the nanoparticles data. A.M.; Drafted the manuscript, which was revised by S.Kh., A.M. All authors read and approved the final manuscript.

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