GOLD NANOPARTICLES-BASED RADIOPHARMACEUTICALS FOR NUCLEAR MOLECULAR IMAGING AND THERAPY APPLICATIONS

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Gold nanoparticles (AuNPs) have attracted great interest in a variety of medical applications such as computed tomography (CT), nuclear imaging, therapy, photoacoustic, ultrasound, and magnetic resonance imaging (MRI). Due to their surface chemistry, structure properties, and biocompatibility, AuNPs are a key feature for targeted drug delivery and enhanced imaging as a contrast agent. This paper describes a new method for AuNPs labelling with ^{68}Ga through chelator method and covers recent advances in positron emission tomography (PET), single-photon emission tomography (SPECT), and radiotherapy using gold nanoparticles-based radiopharmaceuticals.

Keywords: radiolabelled nanoparticles, gold nanoparticles, radiolabelling, nuclear imaging, PET, SPECT, radiotherapy

1. Introduction

Gold nanoparticles (AuNPs) have emerged as highly promising in nanomedicine as their unique physical, and optical properties have rapidly promoted them for drug delivery, multimodal imaging applications, and targeted treatment [1]. One of the most important fields in which AuNPs have been applied is nuclear medicine. The possibility to use a single nanotracer for functional imaging, like positron emission tomography (PET), single photon emission

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tomography (SPECT), also for cancer therapy, conferred an advantage to AuNPs against conventional radiopharmaceuticals approved by the European Medicines Agency (EMA) and U.S. Food, and Drug Administration (FDA), like: [^{18}F]FDOPA (dihydroxyphenylalanine), ^{68}Ga-DOTA-TOC (Tyr^3-octreotide acetate), [^{11}C]MET ([^{11}C-methyl]methionine), ^{177}Lu-DOTA-TATE (octreotate) or ^{99m}Tc-MDP ([methyl]diphosphonate). The mandatory requirements that nanotracers must fulfil to be applied in nuclear medicine are: high chemical, and radiochemical purity, stability, low toxicity, favourable pharmacokinetic profile with fast renal clearance and prolonged blood circulation, predominant accumulation in the targeted tissue and no uptake in non-targeted organs.

To meet these requirements, nanoparticles have been formulated in terms of size, surface coating, functionalization with various biomolecules and different strategies for labelling with radioisotopes were evaluated [2].

In this paper, the recent progress on the development of radiopharmaceutical agents that uses gold nanoparticles as targeting system for PET and SPECT molecular imaging and radiotherapy is presented, along with a new fast and reproducible labelling method for AuNPs with ^{68}Ga radioisotope.

2. Radioisotope labelling methods

One of the most important features of a radiopharmaceutical is the *in vivo* stability. Due to the variety of compositions and pH of biological environments, nanodrugs can interact with constituents of biological mediums by steric, electrostatic or hydrophobic interactions [3, 4]. Therefore, they can form aggregates, can be degraded, or can be recognized by the reticuloendothelial system (RES). Thus, the stable incorporation of radionuclide is essential, and the synthesized nanocomposite must remain intact in biological environments. So far, four methods have been developed for labelling gold nanoparticles with radioisotopes:

a) Direct chemisorption on the surface of AuNPs: driven by chemical reaction of halide ions with various inorganic and organic compounds on the surface of gold nanoparticles;

b) Complexation of radiometallic ions (e.g. ^{99m}Tc, ^{68}Ga, ^{64}Cu, ^{177}Lu) with chelators via coordination chemistry,

c) Intrinsic radiolabelling by activation of gold nanoparticles through direct bombardment with thermal neutrons, using ^{197}Au(n,γ)^{198}Au nuclear reaction;

d) Intrinsic labelling by radiochemical doping using a mixture of radioactive ("hot") and non-radioactive ("cold") precursors during the synthesis process.

These main labelling methods of AuNPs with radioisotopes are graphically illustrated in Fig. 1. Each of these methods has its advantages and

limitations, as presented in Table 1, the selection of the optimal method depending on the purpose, the incorporated radioisotope, and the structure of the gold nanocomposite.
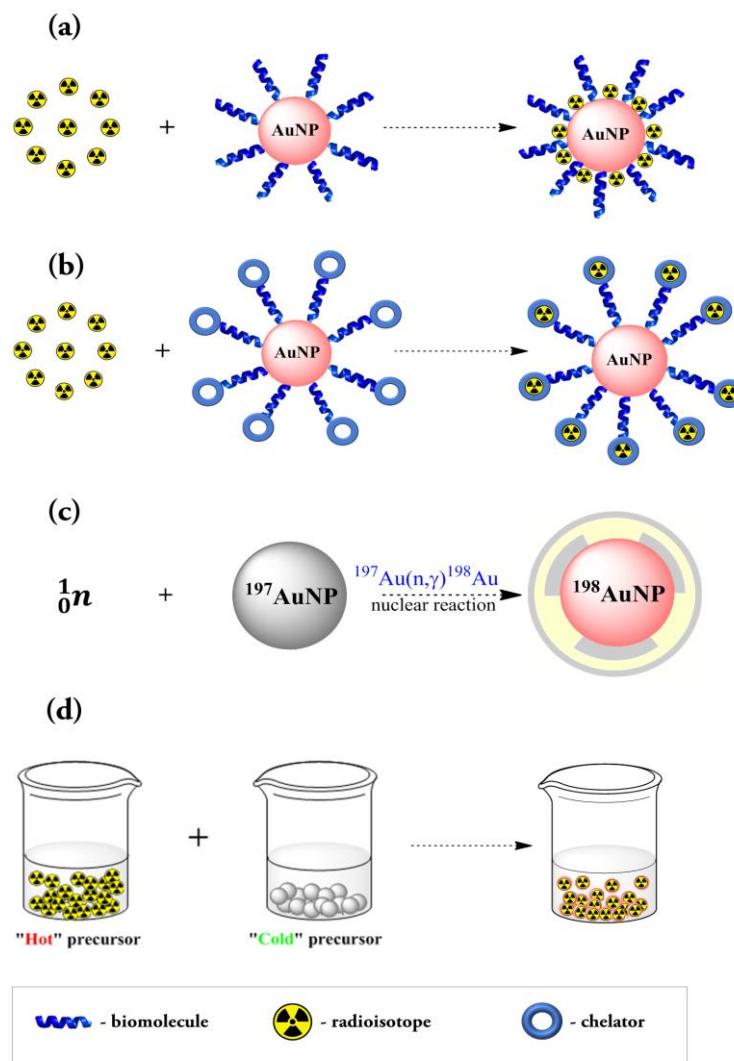

Fig. 1. Methods for labelling of gold nanoparticles with radioisotopes: **(a)** direct chemisorption; **(b)** bifunctional chelator (BFC) method; **(c)** intrinsic labelling by thermal neutron bombardment; **(d)** intrinsic labelling by radioactive and non-radioactive precursors mixing

The complexation method (Fig.1.b) uses small molecules, named bifunctional chelators (BFCs), that trap (by coordinate bonding) the radiometallic ion using several electron-donor atoms and are bound to a biomolecule or nanoparticle [5].

Table 1

Advantages and limitations of gold nanoparticles labelling methods with radioisotopes

Labelling method	Advantages	Limitations
Direct chemisorption	• good stability of radioisotope-nanoparticle complex; • high labelling yield.	• the stability of the complex is dependent on the environmental conditions to which it is exposed, and so far, no *in vivo* stability studies were reported.
Complexation of radiometallic ions	• enables the possibility of labelling with a variety of radioisotopes; • requires short labelling time, which enables the use of short-lived radioisotopes.	• depending on the affinity of the chelator for the radioisotope, there is a possibility of trans-chelation of the radiometallic ion *in vivo* due to competition with other natural chelators within the body (e.g. ferritin, lactoferrin or transferrin), and uptake in non-targeted tissue; • depending on the stability of the biomolecule, there is a probability of blood degradation and rapid elimination of NPs from bloodstream.
Intrinsic radiolabelling by neutron activation	• the most stable labelled AuNPs, as ^{198}Au atoms forms the crystalline structure of the nanoparticles.	• requires special nuclear facilities (reactors); • requires high neutron flux to have a good reaction yield; • requires additional radiation safety measures; • can only be used for labelling with the ^{198}Au radioisotope.
Intrinsic labelling by radiochemical doping	• one-step process, simultaneously with the synthesis of AuNPs; • fast labelling time.	• *in vivo* studies revealed small amounts of dissociated radioisotope from the AuNPs complex and their accumulation in the liver, but extensive research is required.

The main drawback of chelator approach is the trans-chelation of the radiometallic ion that can lead to radioisotope uptake in non-targeted tissues [6]. To avoid this situation, *in vivo* thermodynamic and kinetic stability of different metal-chelator pairs were previously assessed. Several bifunctional chelators have been used for labelling AuNPs with radioisotopes, such as DOTA (1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid), NOTA (1,4,7-triazacyclononane-N,N',N"-triacetic acid) or NODAGA (1,4,7-triazacyclononane,1-glutaric acid-4,7-acetic acid). The radiolabelling of AuNPs using BFC can be achieved by two approaches: binding the chelator on NPs surface followed by the coupling of the radioisotope, or by coordinating the radiometal with BFC and then binding the complex on gold nanoparticles.

3. Gold nanoparticles labelling with ^{68}Ga radioisotope

An example of a short-lived radioisotope often used in nuclear medicine for PET nuclear imaging is ^{68}Ga, that disintegrates 88.9% by β^+ emission, being easily available by irradiation of ^{68}Zn targets with protons at cyclotron or by elution of ^{68}Ge/^{68}Ga radioisotope generator [7]. The chemical form of gallium used for labelling is the Ga^{3+} cation, which forms stable complexes with various chelators, like DOTA, NOTA or NODAGA. Further functionalization of the ^{68}Ga-biomolecule target vector on the AuNPs surface allows it to be used for *in vivo* imaging applications, or for *in vitro* biological studies.

One such nanotracer is ^{68}Ga-NODAGA-NOC-AuNP, where the [1-NaI^3]-octreotide peptide (NOC) was labelled with ^{68}Ga radioisotope and functionalized by chemical absorption on the NPs surface. NOC peptide is a ligand exhibiting high affinity for three somatostatin receptor subtypes (SSTR2, SSTR3, SSTR5), which are overexpressed on the cell membrane of neuroendocrine tumour cells, being a potential *in vivo* vector for targeting this type of tumours.

Materials:

In this study we have optimized the labelling method of gold nanoparticles with ^{68}Ga by chelator approach, using the following materials: ^{68}Ge/^{68}Ga radioisotope generator (ITG Isotope Technologies Garching GmbH, Germany), semi-automated synthesis module Galigand GAL-102 (Veenstra, The Netherlands), suprapur® HCl (Merck KGaA, Germany), ammonium acetate buffer solution (Carl Roth GmbH, Germany), Strata™-X solid phase extraction cartridges (Phenomenex Inc., USA), saline solution (0.9% NaCl, B. Braun Melsungen AG, Germany), ultrapure water prepared in-house on a Millipore Milli-Q Direct 8 (Merck KGaA) system, NODAGA-NOC peptide (piChem, Austria), 20 nm gold nanoparticles stabilized in citrate buffer (Merck KGaA, Germany).

Method and results:

The labelling process begins with the elution of the ^{68}Ge/^{68}Ga generator with 0.05 M HCl. The useful amount of ^{68}Ga eluate, containing the highest activity, consists of 2 ml only, further used in synthesis process. The useful fraction, in form of ^{68}GaCl$_3$, is added to the reaction vessel pre-heated to 95 C (suitable temperature for NODAGA chelator), along with nanomoles of NODAGA-NOC peptide which has been previously buffered with ammonium acetate solution to prevent its degradation (as the pH of the ^{68}GaCl$_3$ eluate is ~1.5-2). After labelling, the solution is passed through a solid phase extraction cartridge to trap the ^{68}Ga-labelled peptide and pass free ^{68}Ga to waste. The peptide is rinsed on the cartridge, then is recovered with ethanol, and transferred directly to a pre-heated evaporation vessel at 95 °C where the ethanol is evaporated, and the peptide reconstituted in saline solution (0.9% NaCl).
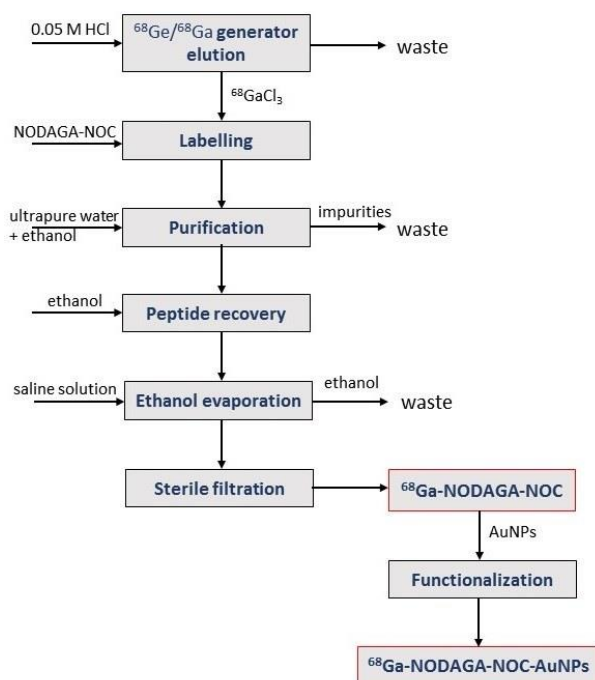
Fig. 2 Schematic representation of labelling gold nanoparticles with ^{68}Ga

Further functionalization of the gold nanoparticles is performed at room temperature, under continuous stirring (~ 450 revolutions/minute) for 15 minutes.
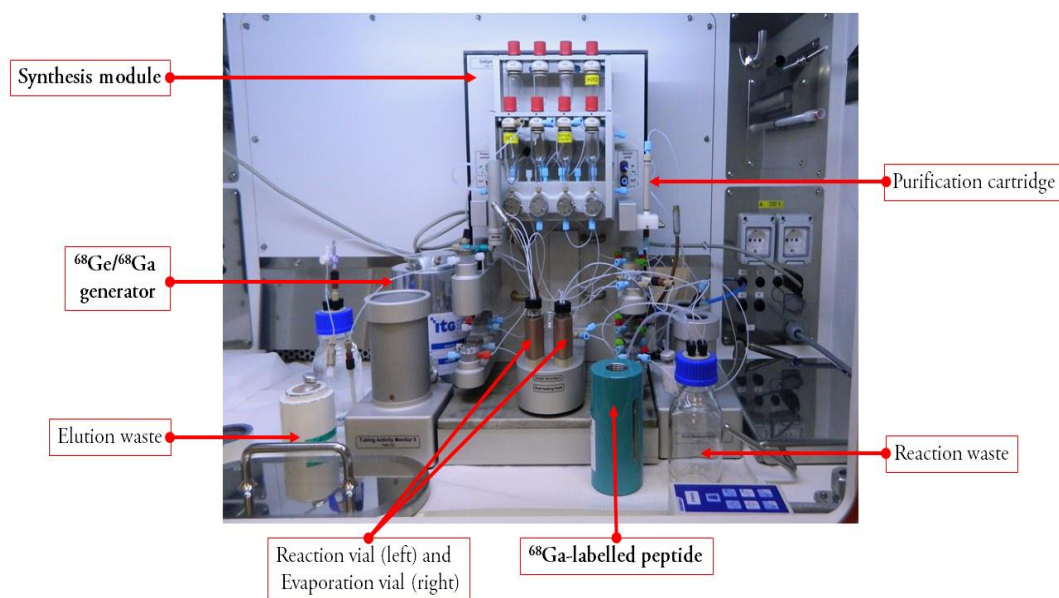

Fig. 3 Semi-automated synthesis module Veenstra Galigand GAL-102 used for labelling molecules with ^{68}Ga radioisotope coupled to an ITG ^{68}Ge/^{68}Ga generator.

The absorption of the peptide at the AuNPs surface allows their functionalization, this process being identified in the UV-Vis spectrum of the nanoparticles by a slight red shift of the absorption-corresponding peak (520 nm).

At the end of this process, a labelling yield greater than 80% can be obtained, within ~ 30 minutes of total preparation time, suitable for short-lived radioisotopes such as ^{68}Ga (with half-life $T_{1/2} = 67.83$ min.).

The radiochemical purity of the radiolabelled peptide evaluated by radio high-performance liquid chromatography (radio-HPLC) is > 95% (Fig. 4).
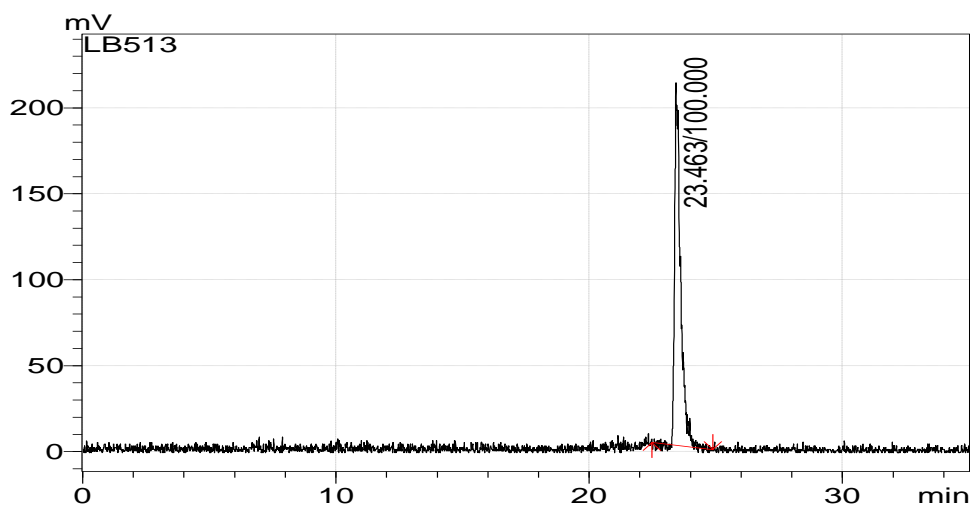
Fig. 4 Radio-HPLC chromatogram of ^{68}Ga-NODAGA-NOC radiotracer.

4. PET imaging applications of AuNPs

The most precise functional imaging techniques used in nuclear medicine is PET, which allows quantitative imaging of radiopharmaceuticals distributed inside the body. In PET imaging, positron emitting radioisotopes are used, which decays by emitting a positron (particle with mass equal to that of the electron, but of opposite charge) and a neutrino (ν). The positron will be further annihilated with an electron from the close tissue during the anti-matter – matter interaction. As a result of the annihilation process, two gamma photons of 511 keV each will be scattered in opposite directions. The PET scanner is based on the coincidence detection of these two gamma photons, using scintillator detectors (e.g. bismuth germanate BGO, thallium-doped sodium iodide NaI(Tl), etc.), that can enable a resolution of 4.4 mm in human PET scanners and 0.5 mm in micro-PET scanners dedicated to small animals (like rats or mice). In order to improve the localization of the signal obtained by PET, the PET scanner is in-line coupled with a computed tomography (CT) or magnetic resonance imaging (MRI) scanner, combining the advantages of both techniques. The obtained fusioned images

provide the functional information with the advantage of a detailed anatomical representation. The commonly used PET radioisotopes are: ^{68}Ga, ^{18}F ($T_{1/2}$ = 1.83 h), ^{11}C ($T_{1/2}$ = 20.36 min.), ^{15}O ($T_{1/2}$ = 2 min.), ^{64}Cu ($T_{1/2}$ = 12.7 h), ^{124}I ($T_{1/2}$ = 4.17 days). They are usually produced in cyclotrons by irradiation of the targets with protons [7], or can be obtained from generators, such as ^{68}Ga, which is obtained from ^{68}Ge/^{68}Ga generator, by elution with HCl [8]. New applications of gold nanoparticles in PET molecular imaging have been developed in recent years, taking advantage of the large specific surface area of AuNPs on which various drugs can be loaded, which offers the possibility of improving the PET/CT dual imaging modality. Some representative examples of the latest applications are presented in Table 2.

The most challenging and sought-after radiopharmaceuticals are those capable of passing through the blood-brain barrier (BBB) that protects the central nervous system (CNS) and facilitate the selective transport of some molecules within the systemic circulation. The development of BBB-permeable radiopharmaceuticals allows the detection of malignant brain tumours such as glioma and neurodegenerative diseases (e.g. Alzheimer's, Parkinson's, or Huntington's). For these types of applications, Frigell et al. [9] developed a novel water-soluble glucose-coated AuNPs (GlcC5Au) functionalized with enkephalin peptide and glycosylated opioid peptides that can access the brain, both peptides being labelled with ^{68}Ga-NOTA-C11/Lip. They assessed the BBB permeation *in vivo* of the synthetized nanotracers and have shown that targeted AuNPs bearing ^{68}Ga-NOTA-Lip-enkephalin can improve the brain uptake by 3-fold of injected dose compared to non-targeted AuNPs. Other gold nanoparticles-based radiopharmaceuticals labelled with ^{68}Ga radioisotope were reported to offer promising results for neuroendocrine cancer cells detection [10].

[^{18}F]-Fluorodeoxyglucose ([^{18}F]FDG) is the most widely used radiopharmaceutical in PET imaging, as it can be distributed in tissues with intense cellular metabolism, that increases the glucose consumption. The use of [^{18}F]FDG along with gold nanoparticles can improve the quality of PET/CT imaging, this combination being tested by Unak et al. [11], who functionalized on the surface of AuNPs both [^{18}F]FDG and the antibody anti-MTDH for the detection of breast cancer cells. The PET/CT images evidenced the high uptake in MCF7 breast cancer cells and increased cells' apoptosis, from 2% to 20%.

Another short-lived emerging radioisotope is ^{64}Cu which has gained increased attention due to its β^+ ($E_{\beta+}$= 0.653 MeV, 17.5%) decay, β^- decay ($E_{\beta-}$= 0.578 MeV, 38.4%), and emitted Auger electrons that make it suitable for both PET imaging and radionuclide therapy. Additionally, copper is involved in several physiological processes and is essential for iron transport, respiration, metabolism, haemostasis, and cell growth [12]. Chen et al. [13] used glutathione (GSH) tripeptide labelled with ^{64}Cu and functionalized on AuNP-PEG

nanoparticles for dynamic PET/CT imaging of both healthy BALB/c mice and mice with acute kidney injury (AKI). Their study emphasized the renal clearance mechanism of nanoparticles with 130 times shorter biological half-life of ^{64}Cu-NOTA-Au-GSH than previously reported nanoparticles, which promotes this radiopharmaceutical for fast diagnosis of kidney diseases in the future.

Lee et al. previously reported a novel synthetic approach for tannic acid-coated gold nanoparticles (TA-AuNPs), labelled with positron emitter [124]I for *in vivo* dendritic cells tracking through PET/CT imaging [14]. Further, they modified the AuNPs coating with polyethylene glycol and conducted *in vivo* targeting studies of breast cancer tumours [15], and *in vivo* photothermal therapy in mice with colon tumours [16]. The results indicate not only effective targeting of tumour lesions through EPR effect, but also the possibility of macrophage-mediated delivery of PEG-[124]I-Au@AuCBs as photothermal therapeutic and imaging nanoplatform.

Table 2

AuNP-based radiopharmaceuticals for PET imaging applications

Radiopharmaceutical	Radio isotope	Molecule	Labelling method	Application
^{68}Ga-DOTA-TOC-*AuNP*	[68]Ga	Octreotide peptide (TOC)	Coordination of ^{68}Ga to bifunctional chelator DOTA	*In vitro* binding assay on AR42J pancreatic cancer cells [10]
^{68}Ga-DOTA-PEG(4)-BBN(7-14)-*AuNP* ^{68}Ga-DOTA-NMB-*AuNP* ^{68}Ga-DOTA-NMN-*AuNP* ^{68}Ga-DOTA-NT-AuNP	[68]Ga	Bombesin (BBN), neuromedin B(NMB), neuromedin N (NMN), and neurotensin (NT) peptides	Coordination of ^{68}Ga to bifunctional chelator DOTA	*In vivo* study on DU-145 prostate and HT-29 colon carcinomas [17]
^{68}Ga-NOTA-C11/Lip-GlcC5Au-Enk ^{68}Ga-NOTA-C11/Lip-GlcC5Au-Glycopep	[68]Ga	Enkephalin (Enk) peptide and glycosylated opioid peptide (Glycopep)	Coordination of ^{68}Ga to bifunctional chelator NOTA	Brain imaging studiesv[9]
[68]Ga-cRGD-Au@DTDTPA	[68]Ga	Integrin peptide cRGD	Coordination of ^{68}Ga to DTDTPA chelator	PET/MRI imaging study of U87MG glioblastoma tumours [18]
C(*AuNP*)LPFFD-C(*AuNP*)K(^{18}F-SFB)	[18]F	Amphipathic peptide CLPFFD and dipeptide CK	Indirect method by covalent binding of ^{18}F-SFB to CK peptide	PET imaging studies [19]
[^{18}F]FDG-AuNP-AntiMTHD	[18]F	Anti-MTHD antibody	Intrinsic labelling	*In vitro* evaluation on MCF7 breast cancer cell line

				[11]
[18]F-*AuNP*	[18]F	N/A	Indirect method by ^{18}F-labelled bicyclononyne reaction with nitrone moieties from the surface of AuNPs	PET imaging studies in C57/B6 mice [20]
[^{18}F]SiFA-maleimide-*AuNP*	[18]F	N/A	Indirect method by maleimide/thiol click chemistry	PET imaging studies in healthy rats [21]
[64]Cu-NODAGA-BBN(7-14)-*AuNP*	[64]Cu	Bombesin peptide (BBN)	BFC/ chemisorption	Prostate cancer PC3 cells and metastatic prostate carcinoma NCaP cells [22]
[64]Cu-NOTA-Au-GSH	[64]Cu	Glutathione (GSH)	Coordination of ^{64}Cu to NOTA chelator	Acute kidney injury diagnosis[13]
PEG-[124]I-Au@AuCBs	[124]I	Tannic acid (TA)	Intrinsic labelling	Breast and colon tumours targeting[15, 16]
[124]I-TA-Au@*AuNPs*	[124]I	Tannic acid (TA)	Intrinsic labelling	*In vivo* dendritic cells tracking [14]

5. SPECT imaging applications of AuNPs

Single photon emission computed tomography (SPECT) imaging is based on gamma photon detection emitted by the radioisotope after electron capture or isomeric transition decay to a lower-energy nuclear state [23]. The SPECT system is composed of a gamma camera (or a set of gamma cameras) mounted on a gantry so that the detectors can record the gamma rays emitted by the radioisotopes. During the analysis, the gamma camera rotates around the patient at regular time intervals. The signal recorded at each location is reconstructed in sets of projections and then in slices of the body. The radioisotopes used in SPECT imaging include: [99m]Tc ($T_{1/2}$ = 6 h), [111]In ($T_{1/2}$ = 67.2 h), [123]I ($T_{1/2}$ = 13.27 h), [131]I ($T_{1/2}$ = 8.02 days), [67]Ga ($T_{1/2}$ = 3.26 days). SPECT imaging technique is commonly used to study cerebral blood flow [24], metabolic changes, bone diseases [25], and oxygen levels in neurodegenerative diseases such as Alzheimer's and Parkinson's [26]. Table 3 lists the gold nanoparticle-based SPECT radiopharmaceuticals and their use in some investigated applications.

Ocampo-Garcia et al. [27] evaluated the biological behaviour of ^{99m}Tc EDDA/HYNIC-GGC-AuNP-mannose for sentinel lymph node (SLN) imaging. The *ex vivo* biodistribution studies and *in vivo* μSPECT/CT images of Wistar rats indicated high lymph node uptake, with 11.58±1.98 % IA (injected activity) after 1 h post-injection (p.i.), and stable binding of the AuNP-based radiopharmaceutical as it was retained during 24 h p.i.

Usually, studies focus on optimizing the surface coating of AuNPs so that the circulatory proteins called opsonin cannot bind to the NPs, and the macrophages of the reticuloendothelial system (RES) do not clean the nanocarrier from the bloodstream. Li et al. [28] relied on RES clearance of ^{99m}Tc-labelled gold nanoparticles for specific targeting of apoptotic macrophages with in pathological process identification of atherosclerosis (AS) plaque. To specifically differentiate and target the apoptotic macrophages from normal ones, they functionalized AuNPs with Annexin V peptide that recognizes the phosphatidyl serine of apoptotic macrophages. The SPECT/CT images of ^{99m}Tc- MAG3-AuNP-Annexin V in C57/B mice showed that during the early period after injection, the nanotracer is located predominantly in the RES organs (liver, spleen, kidneys), covering the imaging signals form AS plaque. The gold nanocarrier is cleared from the body 5 h p.i., through intestinal and renal metabolism, and atherosclerosis plaques can be identified in the SPECT/CT images. These findings highlight the applicability of gold nanoparticles for targeting apoptotic macrophages and further detection of vulnerable atherosclerosis plaques.

Another gold nanoparticles application for SPECT imaging was reported by Ng et al. [29] who directly labelled AuNPs with ^{111}In and further functionalized integrin cRGD peptide on the surface of nanoparticles for melanoma M21 and glioblastoma U87 tumours detection.

Table 3

AuNP-based radiopharmaceuticals for SPECT imaging applications

Radiopharmaceutical	Radio-isotope	Molecule	Labelling method	Application
^{131}I-AuPENPs-CTX	^{131}I	Chlorotoxin (CTX)	Indirect method	SPECT imaging of glioblastoma tumours [30]
$^{123, 131}$I-GNR-PEG-cRGD	^{125}I, ^{131}I	Integrin c(RGDfK) peptide	Indirect by radioiodination method	SPECT imaging and radioimmunotherapy of A549 human lung carcinoma [31]
^{131}I–C225–AuNP–PEG	^{131}I	Cetuximab (C225) antibody	Indirect method (antibody labelling by iodogen method)	μSPECT/CT study on A549 adenocarcinoma bearing mice [32]
^{111}In-AuNP-cRGD	^{111}In	Integrin cRGD	Direct labelling	SPECT imaging

		peptide		of melanoma and glioma tumours [29]
99mTc-DTDTPA-AuNP-BBN	99mTc	Bombesin peptide (BBN)	Coordination of 99mTc to DTDTPA chelator	Prostate cancer PC3 cells [33]
99mTc-AuNP-RGD	99mTc	Integrin peptide: c[RGDfK(C)]	BFC	Tumours [34]
99mTc-EDDA/HYNIC-GGC-AuNP-mannose	99mTc	Gly-Gly-Cis (GGC) peptide	Coordination of 99mTc to HYNIC chelator	Sentinel lymph node detection [27]
99mTc-MAG$_3$-AuNP-Annexin V	99mTc	Annexin V peptide	Coordination of 99mTc to MAG$_3$ chelator	Atherosclerosis plaque detection [28]
67Ga-TDOTA-BBN-AuNP-Gd	67Ga	Bombesin peptide	Coordination of 67Ga to TDOTA chelator	PC3 Prostate cancer detection in xenograft Balb/c mice [35]

6. Radiotherapy application of AuNPs

The treatment of cancer by radiation induced cellular death is achieved through external irradiation of the targeted tissue or systemic delivery of specific radioisotopes, e.g. ^{177}Lu ($T_{1/2}$= 6.73 days), ^{198}Au ($T_{1/2}$= 2.69 days), ^{225}Ac ($T_{1/2}$= 10 days), ^{223}Ra ($T_{1/2}$= 11.43 days), ^{211}At ($T_{1/2}$= 7.21 h), that decay emitting α particle, β$^-$ or Auger electrons. The radiotherapy applications reported so far for functionalized gold nanoparticles are presented in Table 4.

^{177}Lu is one of the most preferred radioisotopes for radiotherapy, as it has a particulate emission of Auger electrons, β$^-$ decay, and low-energy gamma photons (Eγ = 0.208 MeV (11%) and Eγ = 0.113 MeV (6.4%)) which make it suitable for both SPECT imaging and radiotherapy. Several radiopharmaceuticals have so far been reported with ^{177}Lu [36, 37], in current clinical practice ^{177}Lu being successfully applied in the treatment of metastatic bone tumours and the therapy of hepatocellular carcinoma. The applications of gold nanoparticles labelled with ^{177}Lu reported so far are limited to the treatment of breast cancer [38-41], and studies on HeLa adenocarcinoma cells [42]. One of this studies, conducted by Yook et al. [38], describes for the first time the use of a novel dual-receptor targeted system, that bind to both EGFR and HER2 receptors co-expressed on MDA-MB-468 human breast cancer cells. They functionalized the gold nanoparticles with trastuzumab (TmAb) and panitumumab (PmAb) antibodies to target HER2 and EGFR receptors, and they labelled the AuNPs with ^{177}Lu radioisotope via DOTA chelator. The AuNP-based radiopharmaceutical was

injected intratumourally or incorporated into a porous calcium alginate seed (with similar dimensions to the brachytherapy seeds), then implanted intratumourally. ^{177}Lu-DOTA-TmAb-AuNP arrested the growth of EGFR-positive breast cells without causing toxicity to normal tissue. Same results were obtained for ^{177}Lu-DOTA-PmAb-AuNP in breast tumours expressing HER2 receptors. The antitumour effect of the gold nanoparticles-based radiopharmaceuticals and the lack of toxicity to normal tissue are attributed to the anchoring properties of AuNPs that do not allow the migration of the ^{177}Lu radioisotope to normal organs, thus limiting the radiation dose absorbed by the healthy tissue.

Jenitabar-Darzi et al. [43] studied the efficacy of AuNPs in radiotherapy of MCF7 and 4T1 breast cancer. They irradiated [[197]Au]/PAMAMG4 liquid target with thermal neutron flux of 10^{11} neutrons·cm^{-2}s^{-1}. The radioactive [[198]Au]/PAMAMG4 solution was then checked for radionuclide impurities, stability, and morphology. The cytotoxicity study of these nanoparticles against MCF7 cells at 24 h, 48 h and 72 h suggested that the [[198]Au]/PAMAMG4 radiopharmaceutical inhibits the growth of cancerous cells in a dose- and time-dependent manner, the lowest amount of variability determined after statistical analysis being observed at 72 h post-incubation. Moreover, microscopic visualization of the treated cells confirmed morphological alterations after incubation. Comparing the results with those obtained on C2C12 normal cells, it has been observed that at high concentrations it is more toxic to breast cancer cells than normal ones, which encourages further evaluation of this gold nanoparticle-based radiopharmaceutical for *in vivo* assays.

Several other studies were reported [44-47] for intrinsically labelled gold nanoparticles by neutron activation in nuclear reactors, for instance Chakravarty et al. [47] tested [198]AuNP-cRGD *in vivo* on mice bearing melanoma tumours. They evaluated tumour regression after administration of several doses while keeping constant the number of nanoparticles injected. A rapid uptake of 4.9±1.3 %ID/g was observed in the tumour at 1 h p.i., reaching 8.7±2.1 %ID/g after 4 h, this percentage being maintained over the observation period of 168 h p.i. Unfortunately, high uptake and prolonged retention of [198]AuNP-cRGD were also measured in the liver, spleen, and the kidneys. This is a major concern for the radiation-induced detriment to the non-targeted organs and additional studies are required to assess the long-term effects of the radiation absorbed dose in these organs.

The most attractive and efficient radioisotopes used in cancer therapy are α-emitters such as [211]At ($T_{1/2}$ = 7.2 h), [223]Ra ($T_{1/2}$ = 11.43 days), [225]Ac ($T_{1/2}$ = 10 days), that have the advantage of linear energy transfer within a short penetration depth in soft tissue, and high relative effectiveness independent of the cell cycle or hypoxia state. Dziawer et al. [48], published the only study reported so far for AuNPs labelled with an α-emitter radioisotope, who used [211]At-AuNP-

PEG-TmAb as nanotherapeutic agent in HER2-overexpressing human ovarian SKOV-3 cell line. The results showed that the cytotoxic effect of [211]At-AuNP-PEG- TmAb is seven times greater than that reported for ^{177}Lu-DOTA- TmAb [49], despite the 22 times shorter half-life of [211]At compared to[177]Lu β-emitter radioisotope.

Table 4

AuNP-based radiopharmaceuticals for radiotherapy applications

Radiopharmaceutical	Radio-isotope	Molecule	Labelling method	Application
[177]Lu-DenAuNP-folate-bombesin	[177]Lu	Bombesin peptide	Bifunctional chelator method using p-SCN-Bn-DOTA chelator	Human breast cancer (T47D cell line) therapy [41]
^{177}Lu-DOTA-TATE-AuNP	[177]Lu	Peptide Tyr^3-octreotate (TATE)	Bifunctional chelator method	*In vitro* studies on HeLa cells [42]
^{177}Lu-DOTA-GGC-AuNP-c[RGDfK(C)]	[177]Lu	Integrin cRGD peptide	Bifunctional chelator method	*In vitro* studies on MCF7 breast cancer cells [40]
[177]Lu DOTA- TmAb -AuNP	[177]Lu	TmAb antibody	Bifunctional chelator method	*In vivo* studies on SK-BR-3, BT-474 and MDA-MB-361 breast carcinomas [38, 39]
[198]AuNP-GS	[198]Au	Glutathione (GS)	Intrinsic radiolabelling	Biodistribution in balb/c mice [44]
[198]Au/PAMAMG4	[198]Au	N/A	Intrinsic radiolabelling by neutron activation	*In vitro* toxicity studies on MCF7 and 4T1 breast cancer cells [43]
[198]AuNP-RGD	[198]Au	Integrin cRGD peptide	Intrinsic radiolabelling by neutron activation	*In vivo* studies on C57BL/6 mice bearing melanoma tumours [47]
[198]AuNP-EGCg	[198]Au	Epigallocatechin-gallate plant-based compound	Intrinsic radiolabelling	*In vivo* therapeutic efficacy in PC-3 prostate cancer tumours in SCID mice [46]
[198]AuNP-GA	[198]Au	GA glycoprotein	Intrinsic radiolabelling	Therapeutic efficacy studies on prostate tumour–bearing SCID mice [45]
[211]At-AuNP-S-PEG-TmAb [211]At-AuNP-Substance P	[211]At	TmAb	Direct chemisorption	*In vitro* study on SKOV-3 breast cancer cells [48]

7. Conclusions and future perspectives

The nanotechnology, and especially the gold nanoparticles have become an imaging/therapeutic tool in current medical practice, and several gold nanotracers will be proposed for future extensive clinical applications. Several radiolabelling strategies have been reported for now, each of them having its one advantage and limitations, but *in vivo* applications emphasized that the intrinsic labelling method provides the most stable nanocarrier, contrary to the BFC approach, which exposes the radioisotope to natural chelators, such as transferrin, thus increasing the risk for trans-chelation. Despite this, the described new method of labelling AuNP using NODAGA chelator, proves that BFC methods are fast, reliable and can be easily used in the preclinical routine, since they are not as laborious as intrinsic labelling methods, and can be performed with ready to use reagents kits. Despite numerous successful studies, several improvements need to be addressed to translate AuNPs-based radiopharmaceuticals into clinical trials. One of the major challenges is to optimize the tissue-selective targeting to decrease the non-specific uptake into the RES-related organs such as liver, kidneys, spleen, lung, or stomach. In addition, prolonged blood circulation is desired, together with optimal high target-to-background ratio, to increase the contrast of the targeted tissue.

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