Current status and future perspectives of in vivo small animal imaging using radiolabeled nanoparticles

Abstract:

Small animal molecular imaging is a rapidly expanding efficient tool to study biological processes non-invasively. The use of radiolabeled tracers provides non destructive, imaging information, allowing time related phenomena to be repeatedly studied in a single animal. In the last decade there has been an enormous progress in related technologies and a number of dedicated imaging systems overcome the limitations that the size of small animal possesses. On the other hand, nanoparticles (NPs) gain increased interest, due to their unique properties, which make them perfect candidates for biological applications. Over the past five years the two fields seem to cross more and more often; radiolabeled NPs have been assessed in numerous preclinical studies that range from oncology, till HIV treatment. In this article the current status in the tools, applications and trends of radiolabeled NPs reviewed.

Keywords: drug release, molecular imaging, small animal imaging, SPECT, PET, radiolabeled nanoparticles, angiogenesis.

1. INTRODUCTION

Over the past century imaging became a tool that changed the way medicine thinks and practices, mainly for diagnostic purposes and over the past twenty years for therapeutic as well. X-rays gave for the first time the opportunity to obtain information for body's interior, with non invasive methods. As technology evolved in physics, engineering and computer technology, new physical principles were exploited, resulting to new imaging tools that provided different types of information. Starting from simple planar anatomical information, three dimensional functional information can be now obtained and molecular imaging promises to provide (and in many cases already provides) in vivo information about proteins, genes, molecules, stem cells; thus mechanisms related to biological processes and diseases can be studied, with significant benefits, compared to in vitro and ex-vivo studies [1-3].

Having a quick look in medical imaging progress, we can notice that the more complicated the physical principles and data acquisition techniques are the more valuable the obtained information becomes. To explain this statement a simple example is provided: i) X-ray imaging is based on the idea that X-ray photons pass through the body, they are attenuated and a detector measures the total attenuation in the body; thus providing a two dimensional image of the anatomical attenuation map. ii) Single Photon Emission Computed Tomography (SPECT) is based on the idea that a radiopharmaceutical is injected in the body and concentrates in an organ or structure of interest. Radiopharmaceutical carries an isotope (or even more); the emitted photons pass through a collimator and the detector produces a two dimensional image of radiopharmaceutical's distribution, which depends on target's functionality. It must be noted here that the disadvantage of using a collimator is the significant reduction in sensitivity. iii) Positron Emission Tomography (PET) is based on a similar principle, but the radiopharmaceuticals used emit positrons; A positron is annihilated in a distance of 1-4mm from each emission point and two opposite travelling photons are produced. The detector needs to be able to simultaneously detect those two photons, and a reconstruction algorithm is necessary to obtain the three dimensional distribution for radiopharmaceuticals concentration. Very recently the introduction of fast electronics [4] allows time

information of the two photons to be obtained, thus the annihilation point can be estimated with better accuracy; theoretically, further improvement in electronics timing would make reconstruction unnecessary. It is very possible that other physical processes can lead to more exciting imaging options in the future.

The authors provide a contemporary review of current research in radiolabeled nanoparticles for targeted in-vivo imaging focusing on modern technology and well-established applications. In addition, an up to date literature search was performed to identify novel applications of radiolabeled nanoparticles in various fields of medicine. Exciting and innovative prospects are highlighted to offer an intriguing glimpse of the future.

1.1. Molecular imaging with radioisotopes

Many definitions can be given to molecular imaging (MI). According to Weissleder and Mahmood [3] "*MI can be broadly defined as the in vivo characterization and measurement of biologic processes at the cellular and molecular level. In contradistinction to "classical" diagnostic imaging, it sets forth to probe the molecular abnormalities that are the basis of disease rather than to image the end effects of these molecular alterations*". A key parameter in MI is the use of biomarkers; ''Biomarkers are defined as objectively measured, quantitative parameters of normal and abnormal biological processes that serve as indicative endpoints guiding safety and efficacy of an experimental compound for potential drug development [5]''. Biomarkers can be generically described as the molecular signature of biological systems. The challenge of molecular imaging lies in identifying a target suitable for highly specific and sensitive imaging. By delivering a target-specific probe that provides a signal, several imaging modalities can be used to transform this signal into 2D or 3D images (Figure 1).

Nuclear medicine [6] is one of the most widely spread molecular imaging techniques, where radioisotope probes are used. The main steps in molecular imaging with radioisotopes are: i) design of molecules that can target specific receptors ii) attachment of radioisotopes that emit photons (for SPECT) or positrons (or PET) without changing the biological properties of target molecules, iii) injection and in

vivo imaging, by using high resolution and high sensitivity devices. Molecular imaging is not limited to SPECT and PET. The introduction of contrast agents allow functional information to be obtained even by using Computerized Tomography (CT), Ultrasound (US), Magnetic Resonance Imaging (MRI) and the variations of Optical Imaging. More details about these modalities can be found in a number of references [7-9].

1.2. SPECT and PET molecular imaging applications

SPECT has specific advantages that make it a challenging tool for many molecular imaging applications [10]. SPECT can image endogenous ligands such as peptides, antibodies, hormones and selectins, which are relatively easy labelled with Technetium (Tc-99m) or other isotopes. Because of their size those molecules diffuse slowly into tissue and have slow clearance from blood, which can be of the order of hours or even days. The long half-life of the commonly used SPECT isotopes, allows their imaging and makes possible imaging of slow processes such as cell division, infection and inflammatory processes and therapeutic radiopharmaceuticals. It should not be ignored that the use of long life isotopes make SPECT “affordable” for a number of research institutions. Finally, SPECT has the unique ability to probe two or more molecular pathways simultaneously by detecting isotopes with different emission energies; thus different organs or functions can be monitored in the same time [11]. This is for example important in order to ensure a constant physiological state during the experimental observations. Possible applications would be blood flow and receptor binding for assessment of treatment efficacy in cancer, neurodegenerative diseases and psychiatric disorders, two different but related receptors or receptor sub-types, gene expression and functional activity of the protein for which the gene of interest transcribes.

Initially, small animal imaging applications were focused in tumor imaging for the development of new diagnostic or therapeutic radiopharmaceuticals as well as imaging of angiogenesis [12-18]. However, a number of recent publications have already provided several exciting results, which prove the feasibility of the abovementioned research directions. In brain ultra-high resolution SPECT has been used to measure the occupancy of dopamine D2 receptors by a competing drug [19].

Radiopharmaceuticals for inflammation imaging are available [11]. The gene transfer using a reporter receptor has been imaged non-invasively using Tc-99m and Re-188 [20]. Results from simultaneous imaging of Tc-99m and I-123 [21], Ga-67 and Tc-99m [22] have already been published.

PET offers significantly improved resolution when compared with SPECT, and allows the performance of tomographic dynamic studies, something that is not possible with clinical SPECT equipment (with the exception of a few prototypes [23]). In addition, spatial resolution is 4mm in clinical systems and ~1mm in dedicated preclinical scanners. The combination of high resolution and the ability to carry out dynamic tomographic studies lead to a number of dedicated PET scanners (both prototypes and commercial systems). When compared with the SPECT the main limitation of PET is the use of short life isotopes, something that increases overall cost, as well as the number of applications that can be imaged with PET isotopes.

Initially PET studies were limited to brain and heart imaging; however when fluorine-18-fluorodeoxyglucose (FDG) a glucose analogue was introduced in clinical routine the use of PET for the diagnosis and staging of various malignant tumors covered almost 95% of clinical PET exams [24, 25]. FDG PET can screen the entire patient for local recurrence, lymph node metastases and distant metastases during a single whole body examination using a single injection of activity, with a reported average sensitivity and specificity of 96% and 77%, respectively [26, 27]. However, there is increased demand for more cancer specific tracers. A number of positron emitting isotopes are possible candidates including both isotopes similar to biological compounds (C-11, N-13, O-15, F-18) or not (Cu-64, Ga-68, Br-76) [28-30]. On the one hand preclinical research is focused on the development of more specific tracers for cancer diagnosis. On the other hand these tracers have emerged novel molecular imaging applications.

3’-deoxy-3’-[18F]-fluorothymidine (FLT) FLT [31] appears to be of high value for determining response to therapy because cytotoxic chemotherapeutic agents affect cell division earlier and more prominently than glucose metabolism. The dopaminergic system in the rat brain is being explored using probes that reflect dopamine synthesis (e.g., 18F-fluoro-metatyrosine, 18F-FDOPA), D2 receptor binding (e.g., 11C-raclopride, 18F-fluoroethylspiperone), and dopamine transporter

concentration (e.g., 11C-CFT) [32]. Gene expression in tumor bearing mice has been imaged, namely the Herpes simplex virus 1 thymidine kinase (HSV-Tk) gene and the dopamine type 2 receptor (D2R) gene. Cu-ATSM [33] and the [18F]-EF [34] compounds appear to be an effective agent for imaging tumor hypoxia. PET has also been applied to study multidrug resistance and apoptosis (programmed cell death) [35]. A synoptic comparison of SPECT vs. PET major characteristics is outlined in Table 1.

1.3. Small animal imaging equipment

Before emphasizing on radiolabeled nanoparticles (RNPs) and their applications it is necessary to provide some additional information about the considerations that need to be taken in small animal molecular imaging using radioisotopes and the options that are available nowadays [36, 37]. First of all small mice are only ~20-50gr, which means that they are 3000-5000 times smaller than an average human. Their organs are subsequently much smaller and thus high resolution systems are necessary, to ensure images with quality that is comparable to that of human studies. The blood volume of a mouse is only 5ml, which is about 1000 times smaller than that of a human. This means that the injected dose should be minimized down to the order of a few μl. 'In a normal human study 10-30ml are injected with total activity up to few tenths of mCi; Since in small animals only few hundreds of μCi are used, it is evident that a preclinical imaging system must have high sensitivity, in order to be able to produce statistically acceptable images in a reasonable time. Finally, the heart rate and the respiratory rate of a mouse are about ten times faster than that of a human. This difference can introduce motion artifacts that have to be considered and where possible corrected.

The main difference between a small animal imaging system and a clinical system has to do with its size. Usually, small field of view cameras minimize the distance between the object to be imaged and the detector, thus providing optimal resolution and sensitivity. A second difference has to do with the use of more efficient detector components. Since their cost is usually higher it cannot be justified for application in general purpose clinical systems but is affordable for small cameras; however, technological improvements from small animal imaging have inspired the

construction and applications of prototypes for dedicated organs imaging [38, 39]. The breakthrough in small animal SPECT and PET cameras was the development of Position Sensitive Photomultiplier Tubes (PSPMTs) [40], were a single Photomultiplier (PMT) provided position information. Thus, small cameras, based on one PMT were developed. Research in crystals provided new materials such as LaBr [41] with improved energy resolution for SPECT and Cerium-doped lutetium oxyorthosilicate (LSO) and Lutetium-yttrium oxyorthosilicate (LYSO) with improved sensitivity for PET. Moreover phoswich [42] detectors that are based on of two or more crystal layers, provides depth of interaction (DOI) information that improves spatial resolution in PET. New collimator materials and designs have also significantly improved resolution and sensitivity in SPECT. The progress in electronics is also a key factor mainly for sensitivity improvement, while continuous research work in the field of compact and programmable electronics is reducing systems cost.

Nowadays, the trend in small animal imaging is the combination of imaging modalities, in order to simultaneously have functional information (SPECT or PET) and anatomical information (CT or MRI) [43, 44]. A number of such systems are now commercially available by big industries and smaller companies, while a number of research groups can design and develop low cost prototypes [45, 46]. It appears that as small animal imaging systems become more and more affordable by small research groups, new and promising applications will benefit from those tools, leading to impressive results.

2. RADIOLABELED NANOPARTICLES

2.1. What do nanoparticles offer

Nanotechnology, takes advantage of the special properties of various materials when they are in the scale of a few nanometers (usually 1-100nm). It is a rapidly growing research domain with many applications that range from construction materials to medicine. In the latter case, NP drug delivery is a challenging domain, which is expected to improve the therapeutic response to anticancer drugs. Since most of potential therapeutics have poor pharmacokinetics and biopharmaceutical properties, there is a need to develop suitable drug delivery systems that distribute the therapeutically active drug molecule only to the site of action, without affecting

healthy organs and tissues [47]. This possibility will allow reduction of administered doses, since concentration in target will be optimal. The lower the doses and the better the targeting will subsequently minimize side effects [48].

Since nanoparticles have a rather small size range they can be injected without occluding needles and capillaries. This is an obvious benefit in clinical applications; however, it is more important in small animal imaging studies, where repeated injections are not always possible due to the small size of mice tail veins that increase the possibility of bad administration, which will destroy an in vivo study. This can be rather undesirable, especially when one mouse has to be used for studies that require repeated injections. Another advantage is the possibility of local administration in other organs, if injection can be avoided, which is desirable in some applications.

2.2. Methods for labelling nanoparticles

NPs are synthesized from inorganic or organic material and have desirable characteristics so that they can accomplish successfully the role of targeted delivery of drugs which are incorporated into their moieties. In order to visualize the route of the NPs in vivo, an efficient technique is to label them with a detectable radioactive probe. Currently, the more frequently used radionuclides for that purpose are ^{99m}Tc, ^{111}In, ^{125}I and ^{64}Cu [49-51].

Tc-99m and In-111 are radionuclides, which emit gamma radiation and have been widely used up to now due to their availability and suitable half lives for research purposes. Radiolabelling of NPs with those radionuclides can be performed with or without slight modifications of their original structure. Ligands bearing proper groups in order to bind effectively each radiometal can be conjugated directly on the surface of an already formed NP, with or without a spacer or can be attached to it during its synthesis procedure [52, 53]. Common molecules which are suitable for ^{99m}Tc labelling are Histidine (His) residues and 6-Hydrazinopyridine-3-carboxylic acid (HYNIC) [54, 55] and for ^{111}In labelling, diethylene triamine pentaacetic acid (DTPA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) [56, 57]. Beside the abovementioned, various direct labelling methodologies have been applied. The direct approach is very easy to perform via the direct reduction of the eluate of a

commercially available ^{99}Mo-^{99m}Tc generator with a widely used reducing agent such as $SnCl_2$ [58-61]. One very common problem in the NP radiolabelling procedure is the interference of colloidal tin oxide particles when the $^{99m}TcO_4^-$ reduction is performed via $SnCl_2$. Therefore either an alternative reducing agent such as $NaBH_4$ must be applied or special attention must be taken during the reduction reaction with $SnCl_2$ [62, 63]. Finally there is an approach which is less common and it is based on the encapsulation of already formed radiolabeled complexes within the NPs during their manufacture [64]. It is prominent to clarify that it is of great importance to use mild conditions when labelling NPs, such as no great pH range alterations, no constant and extreme heating, etc, in order to avoid harming the original NP structure and maintain the initial NP functional properties.

The incorporation of radioiodine to NPs is usually performed through Tyrosine (Tyr) residues that are somehow present in the NP structure. This is possible if Tyr: a) is a component of the NP polymer, b) is conjugated into proper NP polymer functions and c) is present on a protein which is attached on the surface of the NP [65, 66].

Generally in the PET field the most frequently used radionuclides are ^{18}F and ^{64}Cu. Copper-64 is a PET and β emitter so it can be potentially used in PET imaging and also in therapy [67, 68]. The incorporation of the radiometal in the NP moiety can be performed through the DTPA and DOTA molecules which have groups that can efficiently complex ^{64}Cu. Introduction of ^{18}F to has been problematic because of the complex and insufficient methods required. Nevertheless feasible ^{18}F labelling approaches have been described [69]. Recently, “click” chemistry has started being applied for the attachment of groups to various biomolecules including NPs, because of its simplicity, adequacy and mildness [70, 71].

The commonly used method for quality control of the produced RNPs is the Instant Thin Layer Chromatography (ITLC), which has the advantage of being very quick and easy to use. A more accurate method that can be utilized is the High Pressure Liquid Chromatography (HPLC) using either a reverse phase (RP-HPLC) or a size exclusion column (SE-HPLC) depending on the NPs size and characteristics. The eluate systems used in the above methods may vary depending on the radionuclide and the methodology used for labelling and often a combination of the two methods or of different eluate systems of the same method may be needed in order to determine

effectively the radiochemical purity [49, 54, 56, 57, 69]. A graphical representation of the basic structural components of NPs is depicted in Figure 2.

3. APPLICATIONS

Till recently few imaging studies with RNPs were reported. However, their number is rapidly increasing and is expected to increase even more, since more and more groups select small animal in-vivo imaging for pre-clinical investigations. radiolabeled In the following paragraphs a number of in vivo studies where RNPs have been used are summarized. The paragraphs are grouped according to the type of application or NPs used. To identify relevant studies literature databases including PubMed, Embase and Scopus were searched for combinations of the following terms: ''radiolabeled'', ''nanoparticles'', ''nanomedicine'', ''molecular imaging'', ''tracers'' and ''in vivo''. Only articles that contain in vivo imaging results and not just biodistribution studies were considered. In each case the type of NPs, radioisotope, imaging modality and conditions, and finally application, are reported.

3.1. Nanoparticles in oncology

Bartlett et al. [72], employed PET/CT to monitor whole-body biodistribution kinetics and tumor localization of siRNA NPs. Tf-targeted and nontargeted siRNA NPs were formed by using cyclodextrin-containing polycations. NPs were labelled using Cu-64 and the 64Cu-DOTA-siRNA, Tf-targeted NPs systems were injected in mice. Rapid blood clearance through liver accumulation and kidney filtration into the bladder was observed. The tissue distribution of the 64Cu-DOTA-siRNA delivered by Tf-targeted and nontargeted NPs was very similar for the first hour after injection, with similar blood clearance and tumor accumulation.

Schluep et al. [73] investigated IT-101, a cyclodextrin polymer-based NP containing camptothecin, for cancer treatment using PET. The NPs comprise of a CDP conjugate of the drug camptothecin (CPT), their diameter is 30–40 nm and they have been labelled with Cu-64. MicroPET (Focus 220 PET scanner, Siemens) imaging was carried out at 4 h and 24 h post injection. Data were acquired for 60min. Time concentration curves show that in most organs radiation decreases in parallel with plasma;

however, tumor concentration increases over time and crosses plasma concentration level at 24 h after injection. At that time percentage in tumor is 10±1.1%, while compared to the 4.6±0.7% in the first 6h post injection. An important feature of this work the modelling of tumor uptake of 64Cu-labeled IT-101 using a 3-compartment model incorporating low molecular weight and nanoparticulate label.

Early PET imaging of pancreas cancer and more specifically KRAS protein was assessed [74]. A Mosaic MicroPET was used and images were obtained 24 h post injection. Most of the radioactivity appears in the central region of the xenograft, which indicates that overexpression of the activated KRAS mRNA was concentrated in the center of the tumors.

3.2. Nanoparticles in lungs imaging

Kennel et al. [75], studied mouse lung endothelium using CdTe/ZnS RNPs. In vivo SPECT/CT imaging experiments were carried out in order to visualize the biodistribution of the targeted and control antibody conjugates (NP–mAb). Two mice groups, one (control) injected with NP coupled to control antibody and the second injected with ZnS/Cd125mTe NP targeted with mAb 201B were imaged with a Siemens dual-modality SPECT/MicroCAT II scanner at day one post injection. Results were confirmed by autoradiography studies. In the control group images showed significant accumulation of NPs in the spleen and liver and limited accumulation in the lung cavity. However, in the second group, NPs targeted with mAb 201B were mainly concentrated in the lung and much lower activity was observed in liver and spleen. The authors suggested that those systems can find possible applications in radioimmunotherapy, as well as in medical diagnostic imaging.

Another area of interest is the use of inhaled NPs. Semmler-Behnke et al. [76], studied the disappearance of NPs from the epithelium by sequential lung retention and clearance and bronchoalveolar lavage (BAL) measurements in rats. Following the intratracheal inhalation of iridium-192 (192Ir)–RNPs, SPECT imaging was carried out; however rats were not imaged in vivo, but lungs were removed and placed under the camera. The studies shown that NPs are much less phagocytized by alveolar macrophages (AMs) than large particles but are effectively removed from the lung surface into the interstitium. A clinical gamma camera equipped with a pinhole

collimator was used in this study, which has limitations in small animal imaging. The use of a small camera optimized for the 310keV of Ir-192, would make more feasible the repetition of studies in the same animal, since its long half life (73.83 days) make it an ideal candidate for imaging even for a one year period.

3.3. Nanoparticles in HIV

The application of RNPs in antiretroviral therapy (ART), has been suggested by Dou et al. [77, 78]. The goal of this group's work was to design a novel bone marrow–derived macrophage (BMM) pharmacologic NP delivery system for effective antiretroviral delivery. Because of the small size of the NPs and their highly stable nature, NPs could be packaged within macrophages for subsequent systemic trafficking and sustained drug distribution. As a result, this system has the potential to improve drug distribution to areas of active viral replication, and extend dosing intervals. Indinavir (IDV) nanosuspension was loaded into BMMs and then administered intravenously into naive mice. BMMs were labelled with In-111 and then cell tissue distribution was tracked with SPECT. Pharmacokinetic behaviour and immune and antiretroviral activities were monitored after HIV-1ADA infection and a single dose of NP indinavir–loaded BMMs (NP-IDV-BMMs). Sustained antiretroviral therapeutic responses with concomitant immune reconstitution were seen up to 14 days. Mice were tomographically imaged using a high resolution gamma camera, with a 1-mm pinhole collimator; 64 projections from 0^{o} to 360^{o} were obtained in 1min intervals. Quantitative analysis of BMM density from tomographic images showed significant accumulation of radiolabeled BMMs in lung at six hours after adoptive transfer compared with other tissues (spleen, liver, and kidney). By day 1, radiollabeled BMMs were significantly diminished from lung with concomitant increases in liver and spleen. BMM levels in liver and spleen remained relatively constant, but not significantly different from days 1 through 7. This work showed an excellent combination of nanotechnology, radiobiology and molecular imaging for application in a peak research topic, such as HIV therapy.

3.4. Hydroxyapatite nanoparticles (HNPs)

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) is biocompatible and has been explored in a number of biomedical applications, such as orthopaedic implants [79], sustained drug release systems [80], radiation therapy of arthritis [81], drug or DNA delivery systems [82] and others. Labelling hydroxyapatite NPs (HNPs) with Tc-99m-methylene-diphosphonate, allows to non-invasively follow HNPs, by using SPECT imaging. Ong et al. [83] synthesized HNPs by wet chemical precipitation, using calcium nitrate tetrahydrate and ammonium dihydrogen phosphate. The size of HNPs was 40nm, 100nm and 200nm. Then HNPs were radiolabeled with HNPMDP-Tc99m and imaged 2h post injection with a hybrid SPECT/CT system. Images and analysis of imaging data showed that HNPMDP-Tc99m was rapidly cleared from the blood circulation regardless of particle size. About 10% of injected dose was still in circulation at 10 min post-injection but only 0.6–0.7% remained after 2 h. The largest concentration was observed in liver and presence in the kidney, lung, and heart were negligible. The authors suggest that several clinically available bisphosphonate drugs or phosphonate labelled and loaded HNPs could be useful for targeted delivery of radiation and/or drugs to the liver or cancer cells at other locations.

3.5. Nanoparticles in reticuloendothelial system imaging

Radiolabeled quantum dots were used by Kennel et al. [75] to study the competition between efficient vascular targeting and interaction of the NP with the reticuloendothelial (RE) system, which is the major source of particulate uptake and clearance in the body. CdTe NPs were prepared with natural cold Te (cold) or with a mixture of Te-125m/Te-124 (stable). The CdTe NPs were capped with ZnS to reduce leaching and with mercaptoacetic to provide a functional group for attaching a targeting monoclonal antibody MAb 201B (MAb). MAb 201B binds to murine thrombomodulin expressed in the lumen of lung blood vessels. As control MAb 33 was used. Whole-body micro-SPECT/CT images were collected using microCAT II+SPECT dual modality platform (Siemens Preclinical Imaging, Knoxville, TN). Forty-five projections were acquired with an 8° step for a period of 60 sec per projection. Both targeted with MAb 201B and control MAb 33 mice were imaged at day 1 post injection of the targeted NPs. SPECT images show a predominance of 125mTe in the lungs with some in spleen and a trace detected in liver. On the other hand, the

images of the control animal show that almost all the 125mTe is concentrated in liver and spleen.

3.6. Quantum Dots

The quantitative biodistribution of commercially available CdSe quantum dots (QD) in mice was studied by Schipper et al. [84] PET imaging with Cu-64 was selected as a tool to non invasively follow radiolabeled QDs and provide information about their spatiotemporal distribution. Several compounds were prepared and in vivo tested; 64Cu, 64Cu-DOTA, 64Cu-DOTAQD525, 64Cu-DOTA-QD800, 64Cu-DOTA-QD525PEG and 64Cu-DOTA-QD800PEG. Anaesthetized mice were imaged by a microPET R4 scanner (Siemens Medical Solutions USA, Inc.). The imaging protocol included several series of scans; In the first 10 min post injection, dynamic 10 sec time frames were, to monitor fast phenomena. Then 5 min static acquisitions were performed at 10 min, 60 min, 4.5 h, 12 h, and 36 h post injection. Regions of interest were drawn in various organs. An initial peak in heart and lung uptake, was observed in the first moments, something which is expected from blood-pool activity. QD525PEG and QD800PEG were mainly concentrated in liver and less in spleen and bone and other organs showed low activity, till the end of the study. Figure 3 shows typical images of the six tested compounds.

3.7. Epoxypropylmethacrylate (EPMA) nanoparticles

EPMA (poly-glycidylmethacrylate (poly-2,3-epoxypropylmethacrylate) finds applications in the field of artificial organs or implants [85]. EPMA NPs consist of a compact latex core with a water-soluble corona composed of protuberant linear polymethacrylic acid strands [86]. Cartier et al. [87], labelled EPMA NPs with two different tracers; In-111 and Ga-68. EPMA-based NPs are easily synthesized by emulsion copolymerization, allowing the production of NPs that differ in size, polymer hydrophilicity and surface coverage with functional groups. Radiolabelling was done directly, without the necessity of a chelating conjugate. All animal experiments were carried out in anaesthetized rats. Ga-68 labelled NPs were imaged in a MOSAIC animal PET scanner, 15 min post injection. Images showed localization

in the heart and also in the liver. Lower concentration was visible in the spleen. However, no signal was detected in the kidney, something which probably implies that there is no significant renal elimination of the tracer. Scintigraphic studies were carried out with In-111 labelled NPs 15 min and 1h post injections. In the first 15 min, 75% of the injected activity was found in the blood and 21% in the liver. After 1 h 45% remained in the blood and 40% was located in the liver, something that indicates increased clearance over time by the liver. Low radioactivity is observed in the kidney. EPMA NPs can be labelled with other tracers such as, Tc-99m, Ga-67 and I-23 for SPECT and Cu isotopes for PET.

3.8. Nanoparticles in cardiovascular diseases

Nahrendorf et al. [67], labeled Dextranated and DTPA-modified magnetofluorescent NPs with Cu-64, for Macrophages in Inflammatory Atherosclerosis. MION NPs (monocrystalline iron oxide nanoparticle), labelled with Cu-64 resulted to Cu-64 DTPA NPs. Using FLEX X-PET/X-O micro PET-CT (Gamma Medica Ideas, Inc, Northridge, Calif) apoE$^{-/-}$ mice were scanned in two steps; Initially 1 hour after injection of 18-FDG. On the following day 64Cu-TNP IV were administered and imaged at 24 hours post injection. A strong PET signal was obtained from the aortic root and arch with a 5.1±0.9 target to background ratio in the aortic root. The anatomical information provided by the PET/CT and the administration of an iodinated CT contrast agent, allowed to identify those structures in functional images. PET signal was obtained only by apoE$^{-/-}$ mouse but not by wild-type mice. 64Cu-TNP provided 50% higher uptake values (SUV) when compared to the standard 18-FDG. An additional benefit of 64Cu-TNP is its trimodal character, which allows complementary MRI imaging and probe validation by fluorescence-based techniques on the cellular and molecular level.

3.9 Nanoparticles in angiogenesis

Angiogenesis refers to the formation of new blood vessels and is implicated in a variety of normal and pathologic inflammatory and cancerous conditions. Advances in nanotechnology are transforming our knowledge of angiogenesis and may permit therapeutic manipulation of the phenomenon on a nanoscale in the future.

Molecular imaging of integrin expression represents a recent scientific breakthrough in the detection and therapeutic targeting of cancer [88-91]. Integrins are expressed both on tumor cells and activated proliferating endothelial cells and facilitate tumor cell invasion, angiogenesis and metastasis. Radiolabeled tracers targeting $\alpha_v\beta_3$ integrin expression were recently introduced for SPECT imaging including ^{18}F-galacto-RGD [92], ^{111}In-RP747 [93] and ^{99}mTc-NC100692.

Of special interest, dual-function imaging techniques are emerging. Contrast-agents used for targeted imaging of surface cell molecules may be further modified and employed for direct selective drug delivery [94]. Appropriately designed NPs could combine contrast agents and drug therapy permitting simultaneous cancer imaging and treatment. For example, liposomal NPs that bind $\alpha_v\beta_3$ integrin have been successfully used to deliver a mutant *Raf* gene and induce endothelial cell apoptosis in a preclinical tumor model [95]. Nanotechnology and engineering of novel NPs like biodegradable micelles, semiconducting nanodots and iron-oxide nanocrystals may revolutionize the study and therapeutic manipulation of angiogenesis. Biological camouflage of the NPs with appropriate coatings may help them evade immunological attack and early destruction [96]. For example, external lipid pegylation of the NPs prevents their early removal by the reticuloendothelial system and enhances their circulation half-life [97].

Gadolinium-labelled NPs engineered from lipids, such as liposomes or micelles, or carbon nanotubes, are excellent contrast agents for MR imaging because of gadolinium's paramagnetic properties [95]. Paramagnetic NPs have been applied for the detection of tumoral neovascularization and inflammatory angiogenesis of atheromatous plaque producing a high signal-to-noise ratio [98-100].

4. PROSPECTIVE

Although the number of papers related to RNPs continuously increases, at the moment most of the published studies do not use in vivo small animal imaging. One reason for this could be the resources necessary to obtain and maintain imaging facilities. At the moment even more conventional studies that do not use NPs, do not proceed to in vivo imaging. However, as stated in this article, low cost prototypes

can provide accurate dynamic scintigraphic data [101] and in some cases even tomographic data. As they become more and more popular and economically affordable, it is expected that in the next years a number of studies that have been carried out using radioisotopes and post mortem biodistributions will be repeated in vivo.

A very nice summary of studies with RNPs [102] has shown that many groups using RNPs in various fields could extend their work and benefit from the use of imaging tools. We believe that imaging RNPs will emerge as one of the major directions in molecular imaging due to the additional capability to use NPs for bi- or multimodal imaging. One of the most promising fields related to cancer diagnosis and therapy is angiogenesis, where it is expected that RNPs will play a critical role for early diagnosis and/or targeted therapy and treatment monitoring. It appears that imaging RNPs is in the very beginning and in the next years several impressive results are to be expected with a strong and significant impact on preclinical and clinical research.

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Captions to figures:

Figure 1: Graph illustrating the major contributing role of molecular imaging, along with genomics, proteomics, metabolomics, and radiogenomics in the evolution of modern biology and medicine towards the convergence diagnostics and therapeutics i.e. theragnostics [103-105].

Figure 2: Graph illustrating an example structure of a targeted nanoparticle. Surface ligands are engineered to probe specific cell membrane receptors with a high specificity. Contrast payloads may include, but not limited to, a radiolabeled tracer (i.e. Tc^{99m}), an iodinated contrast compound (Iodine), a Gadolinium chelate (Gd^{+3}) for concurrent high resolution anatomical imaging with either Computed Tomography or Magnetic Resonance Imaging apparata, or a combination thereof. Finally, nanoparticles may carry agents (i.e. drug or DNA/RNA) for targeted pharmaceutical or gene therapy on the cellular level.

Figure 3: In vivo PET images of mice injected with 64Cu (first column), 64Cu-DOTA (second column), QD525 (third column), QD525PEG (fourth column), QD800 (fifth column), or QD800PEG (sixth column). During dynamic image acquisition, 5.55 MBq of the respective agent were injected into tail vein of nude mice. Images were acquired dynamically in 10-s frames for the first 10 min and one 5-min frame thereafter. Coronal (upper row), sagittal (middle row), and transverse (lower row) slices of a 5-min frame from 10 to 15 min after injection are shown (reproduced with permission from [84]).

Table 1: SPECT and PET major properties

Table 1.	Single Photon Emission Tomography (SPECT)	Positron Emission Tomography (PET)
Principle of function	Detection of a single photon after tracer uptake. SPECT tracers emit photons of different energies, ranging from ~70keV up to ~300keV	Detection of photon pair after tracer uptake. PET tracers emit positrons of different kinetic energies, which annihilate with a free electron and produce two antiparallel photons with energy 511keV, independent of their kinetic energy
Tracers (Half life in minutes, hours or days; For SPECT isotopes photons energy. For PET isotopes positrons maximum kinetic energy)	Mainly diagnostic: Tc-99m (6.01h, 140keV) I-123 (13.3h , 159keV) In-111 (2.8d , 171keV & 245keV) Th-201 (3.04d , 70-80keV & 167keV) Mainly therapeutic: Ga-67 (3.26d , 93keV & 185keV & 300keV) Re-188 (16.9h, 159keV) Sm-153 (1.94d , 103keV)	O-15 (2.05m, 1.7MeV) N-13 (9.9m, 1.19MeV) F-18 (109.8m, 0.64MeV) C-11 (20.4m, 0.96MeV) Ga-68 (68.4m, 1.9MeV) Cu-64 (12.7h, 0.58MeV)
Spatial Resolution	5-8mm in clinical systems 1-2mm in preclinical systems Lower limit determined by collimator dimensions, penetration and scattering	4-5mm in clinical systems ~1mm in preclinical systems Lower limit determined by positron range, photons non colinearity and scattering
Sensitivity (Percentage of photons detected over photons emitted)	~0.01% Sensitivity mainly determined by collimator shape and number of heads	Up to 10% Sensitivity mainly determined by number of detector modules, acceptance angle and 2D or 3D acquisition
Types of acquisition and	Planar imaging with static head(s). Few seconds for dynamic studies up to few minutes	Planar coincidence imaging with static opposite heads (dedicated PET systems)

indicative acquisition time	for static scintigraphic studies Tomographic imaging with rotating head(s). Few seconds per projection and up to 30 minutes for an entire SPECT, depending on the number of heads. 2D or 3D reconstruction.	Few seconds in dynamic studies up to few minutes for static coincidence studies Tomographic with no heads rotation and limited angle reconstruction (dedicated PET systems) Few minutes or even few seconds (but with low statistics) Tomographic imaging with rotating heads (dedicated PET systems) Few minutes depending on number of heads and projection angles Tomographic imaging with ring scanner Few minutes or even few seconds (dedicated PET systems). Up to 30min for whole body clinical studies with more than one bed positions
Cost	Relatively low cost instrumentation. Availability of long lived radioisotopes.	More expensive instrumentation. Need of dedicated cyclotron for short lived radioisotopes, which raises overall cost.
Clinical applications	Myocardial ischemia, thyroid imaging, oncology,	Oncology, brain imaging
Pre-clinical in vivo imaging applications using nanoparticles	Oncology, lungs endothelium, HIV study, orthopaedic implants, drug release, reticuloendothelial system, angiogenesis study, implants	Oncology, implants, cardiovascular diseases
Advantages	Use of more than one isotopes for simultaneous imaging of more than one tracers/mechanisms Easily accessible (in equipment and radiopharmaceuticals) Long lived isotopes that allow in vivo monitoring of slow processes	Possibility to carry our dynamic tomographic studies Relatively high sensitivity Whole body imaging Radiopharmaceuticals that are structural elements of animal/human
Disadvantages	Low sensitivity and limited spatial resolution	High cost equipment and radiopharmaceuticals

Only planar dynamic studies are possible

Short lived isotopes that allow monitoring of relatively fast mechanisms