Nanoparticles : Non-invasive Biomedical Imaging Agents

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Abstract

The necessity for early detection of deadly diseases and to study the anatomical features of internal organs, there is an urge on the development of efficient and reliable method for biomedical imaging. This led to the emergence of nanoparticles as a versatile tool in cellular imaging. The main objective is to summarize the emerging research of nanoparticles for biomedical imaging with increased selectivity and reduced non-specific uptake by improved bioconjugation strategy. Quantum dots (fluorescent NPs), gold NPs and magnetic nanoparticles find several applications in in-vivo imaging techniques due to their exceptional properties. Toxicity of nanoparticles, however, remains a topic of concern which demands further investigation. Broader implication of this study includes improvement on the structural design of nanoparticles for in-vivo imaging and reduction of cytotoxicity. This review mainly emphasizes the properties and types of nanoparticles along with their applications in various imaging techniques.

Keywords Nanoparticles, biomedical imaging, quantum dots, fluorescence, gold NPs.

1. Introduction

Evolution of nanoparticles as in-vivo imaging agents has led to a remarkable advancement of medical science in biomedical imaging. Molecular imaging refers to the development of biocompatible molecular probes for the visualization of cellular function and the measurement of molecular processes in living organisms at cellular level without disturbing them [1]. Generally, a molecular imaging system is composed of an imaging agent/probe, an imaging device and a molecular target /receptor. The diameter of nanoparticles (1 – 100 nm) is comparable to biological functional units, which enables them to act as potential in-vivo imaging agents. Nanoparticles have remarkable characteristics such as diverse surface chemistry, distinct magnetic properties and tunable absorption-emission features, which allows hassle-free investigation of molecular and cellular processes in living organisms.

Development of nanotechnology has led to the detailed classification of NPs and it allowed the visualization of NPs as they function and travel within a living organism. Nano-imaging agents such as quantum dots express bright fluorescence which allows to detect them through optical imaging methods to develop an image or obtain wavelength absorbance data (in nanometres) [2]. Gold nanoparticles (AuNP) due to their high biocompatibility and versatile surface properties act as a safe platform for many biomedical technologies, including biomedical imaging [3].

Recent advanced technologies focuses on the generation of diverse types of nanoparticles which clearly indicates the importance of nanoparticles in imaging of biological systems [4, 5]. Emerging nanoparticle technologies are joined by highly developed imaging modalities like microscopic and scanning techniques to assist in disease detection.

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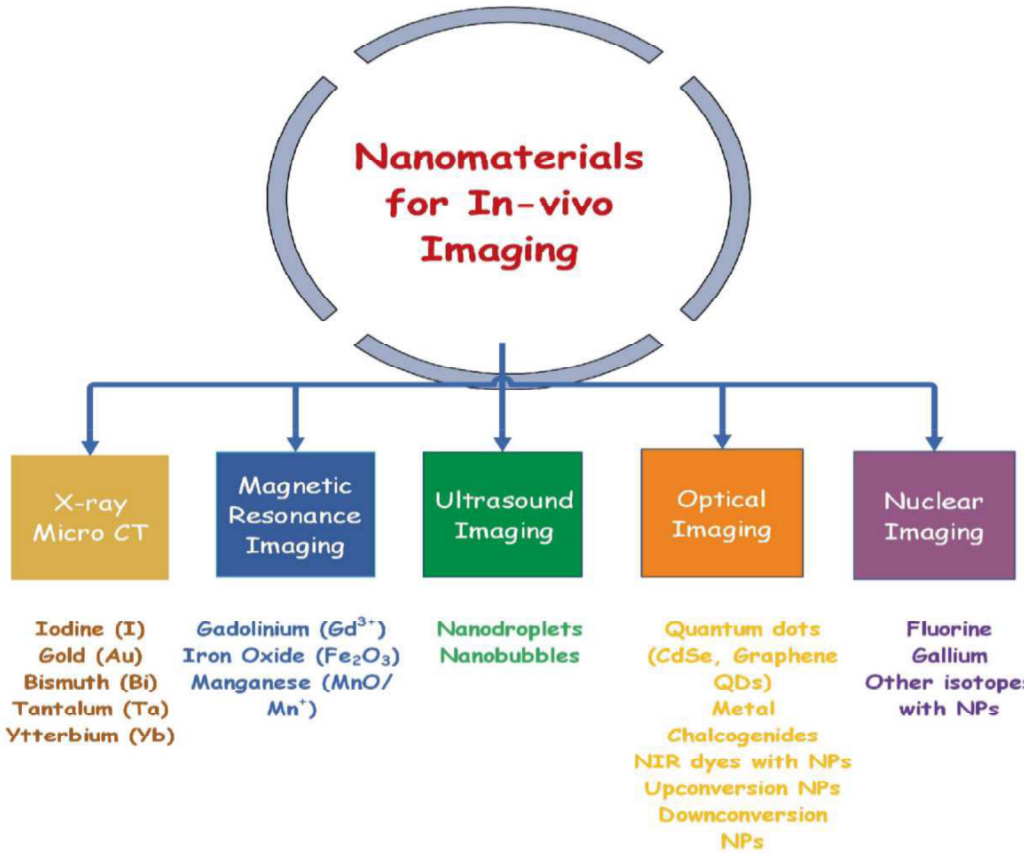

Fig1. Nano materials in in-vivo imaging

2. Types of nanoparticles and their working as in-vivo imaging agents

Imaging applications of nanoparticles have been increasingly developed in last 20 years. In addition to the size of a nanoparticle which is comparable to biomolecules, their high quantum yield and high magnetism also makes them a suitable next generation in-vivo imaging tool. Nowadays, various types of nanoparticle like liposomes, micelles, nanotubes, metallic nanoparticles, quantum dots, dendrimers and polymeric nanoparticles are in the limelight. This review mainly emphasizes the following representative nanoparticles (quantum dots, gold nanoparticles and magnetic nanoparticles) for biomedical imaging applications.

2.1 Quantum dots

Quantum dots are fluorescent semiconductor nanoparticles (~ 1 – 100 nm) with unique optical and electrical properties, most widely used for biomolecular imaging.A quantum dot possesses the following advantages over conventional organic dyes and fluorescent proteins:QDs exhibits near-unity quantum yields and have molar extinction coefficients that are 10–50 times larger than that of organic dyes, making them brighter (about 10 – 100 times) in photon-limited in-vivo conditions[6].Quantum dots have a relatively long fluorescence lifetime (5 to > 100 ns) as compared to that of organic dyes (1 – 5 ns) which increases the probability of absorption at shorter wavelengths, thereby producing a broad absorption spectrum [6].

QD emission wavelengths are size tunable; this property can be utilized in tissue penetration at a greater depth, by extending the emission wavelength into Near Infrared Region (NIR) (which

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ranges from 650 nm to 950 nm), thereby reducing the background autofluorescence [7,8]. This property allows multiplexed imaging applications where one light source is used to excite multicolour QDs concurrently, without the requirement of complex instrumentation [9].QDs have negligible photobleaching (100 – 1000 times less than fluorescent dyes) and the long-term photo stability of QD imaging probes allows the investigation of dynamic cellular processes such as metastasis, cell migration, cell division & differentiation over longer duration [6, 10-18].

These properties have made QDs a topic of intensive interest in biomedical imaging of internal organs and tissues.Use of quantum dots as fluorophores for *in- vivo* fluorescence imaging has increased recently. In-vitro loading of nanoparticles into human cancer cells has been shown successfully (Sage 2004; Li, Wang et al. 2006; Xing, Smith et al. 2006) [19], along with their in-vivo application in mice model (Kim, Jin et al. 2006; DeNardo, DeNardo et al. 2007; Goldberg, Xing et al. 2011) [20]. The division of human cancer cells were visualized and their reforming of tumour was being tracked by fluorescence. Better targeting and sensitivity has been achieved by multiplexing of quantum dots. Surface receptors present on cancer cells can be targeted by the multiplexed nanoparticles. Nowadays, targeting tumours are based on an approach that functionalizes quantum dots with molecules specific to the target [21]. Imaging of lymphatic and cardiovascular system is another example of in-vivo imaging using fluorescent nanoparticle probes [21].

Zimmer *et al.* employed NIR emitting QDs to act as an in-vivo reporter due to their ability to perform at a wavelength minimally absorbed by biological species. He along with his co-workers prepared a series of InAs/ZnSe core/shell QDs having small-sized core, with variation in shell thickness and composition, which offered a range of size tunable emission wavelengths, between 750 and 920 nm [22]. Smith *et al.* visualized the development of blood vessel over time using QDs. QDs conjugated with biotinylated fibrinogen showed specificity towards the membrane of blood vessels during angiogenesis [23]. Recently, Kim *et al.* explored the targeting and imaging of lymphatic vessels using quantum dots conjugated to hyaluronic acid (QD-HA conjugates) [24].

In spite of multiple applications in biomedical imaging, quantum dots have several disadvantages as well. Efforts have been made to enhance the fluorescent signal in the deep tissues by using a two-photon microscope, but it is important to have sufficient number of nanoparticles per cell to clearly visualize the target/receptor. Here, the main problem is that, increased number of nanoparticles will increase the toxicity of the cells. Cadmium or serenide are the main components of binary quantum dot, which are detrimental to cells. Due to the intrinsic toxicity of binary quantum dots, very thick superficial coating is required. As a result, the final core size of quantum dot becomes almost twice as thick as the initial size, which hinders the mobility of quantum dot inside a cell. "Blinking behaviour" is again a major drawback of binary quantum dots (Durisic, Bachir et al. 2007; Lee and Osborne 2009; Peterson and Nesbitt 2009) [25, 26, 27]. Due to this blinking behaviour, tracking of quantum dot becomes difficult inside a bio system.

To avoid the shortcomings of binary quantum dots, silicon nanocrystals can be used instead. Silicon nanocrystals' specializes as a fluorophore. Moreover, silicon does not require a thick surface coating due to its non-toxic nature, so that its average size remains close to its core size. Hence, the intrinsic role of the nanoparticle remains intact [21].

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2.2 *Gold nanoparticles (AuNP)*

Inorganic nanoparticles are springing up as versatile tools in molecular imaging owing to their unique chemical, physical and optical properties [28]. Recently, gold nanoparticles have been used in multimodal imaging technologies due to their unique surface chemistry, biocompatibility, relatively low short-term toxicity, high atomic number and high X-ray absorption coefficient [29, 30]. For biomedical implementation, considering the upcoming concerns about nanomaterial safety and toxicity is important, in agreement to which gold nanoparticles can be synthesized under relatively benign conditions [29, 31, 32]. Recently, gold nanoparticles (AuNP) have gained significant role as CT (computer tomography) scan contrasting agents (CA) on account of their simple surface chemistry, high X-ray attenuation and projected biocompatibility. An imaging contrast agent (CA) may be utilized to highlight disease-specific anatomical features or to enhance distinction between two types of tissues [33, 34]. Contrast agents play an important role by introducing high atomic number media into the body, thereby allowing safer scans with high image contrast.

Gold nanoparticles due to their higher biocompatibility and easy synthesis, serves as a popular choice for near IR emitting nano fluorophores. (Lee, Cha et al. 2008; Shang, Yin et al. 2009) [35, 36]. In order to use gold nanoparticles and their derivatives in bioimaging, several imaging methodologies were developed. Optical Coherence Tomography (OCT) employs the scattering property of gold nanoshells for in-vivo imaging (Agrawal, Huang et al. 2006; Adler, Huang et al. 2008; Skrabalak, Chen et al. 2008) [37, 38, 39]. At the site of the tumour, gold nanoshells accumulates, which increases scattering at that location, thus providing the contrast. Photoacoustic imaging is another tool for imaging gold nanomaterials. A pulse of near IR radiation is developed in photoacoustic imaging, that causes thermal expansion nearby and sound wave is detectable at the surface. Two-photon fluorescence spectroscopy is another in-vivo imaging technique where gold nanoparticles are employed. In this technique, gold nanomaterials can increase the occurrence rate of two-photon excitation and relaxation of energy through fluorescence, since it possesses strong surface plasmon resonance [21]. Additionally, Raman spectroscopy can be used to enhance Raman effect at the surface of gold NPs [21].

Hainfeld *et al.* first demonstrated the use of gold nanoparticles (1.9 nm) as an X-ray CT contrast agent to detect tumours in mice [40]. Gold nanoparticle stabilized by Arabic gum, was successfully demonstrated as a biocompatible X-ray CT contrast agent by Kattumuri *et al.* [41]. Utilizing glutamic acid functionalized AuNPs as CT scan contrast agent, Zhang sought to quantify the micro damage caused to bone [42]. Wang et al. obtained a high resolution CT scan image of the kidney, by using self-created AuNP clusters (<2 nm) coated with bovine serum albumin (BSA) [43]

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2.3 Magnetic nanoparticles

Magnetic nanoparticles have gained considerable attention due to their potential use in optical, magnetic and electronic devices [40, 44, 45, 46]. Similar to gold nanoparticles and QDs, iron oxide nanoparticles are expected to show acceptable biocompatibility at low concentration. Functionalization of iron oxide NPs with antibodies, nucleosides, proteins and enzymes enables us to direct them to diseased tissues such as tumours [47, 48]. Further, magnetic nanocrystals serve as an excellent probe for Magnetic Resonance Imaging (MRI) [21].

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Unique surface property and dimension of iron oxide NPs nanoparticles are responsible for their superparamagnetism and high field irreversibility [45, 49]. Superparamagnetic iron oxide nanoparticles abbreviated as SPIONs, have large magnetic moments and are well acceptable as T2 contrast agents in MRI. Depending on the surface chemistry and particle size, SPIONs are addressed to the Reticuloendothelial System (RES) [50, 51]. Furthermore, the toxicity of SPIONs are perceived to be lesser than optical imaging agents, which is evident from the administration of Feridex(a type of SPION) as a MRI contrast agent [21].

MRI has been extensively used to visualize cellular trafficking with magnetic nanoparticle probes [52, 53]. Well-defined iron oxide NPs conjugated with Herceptin was developed by Huh and co-workers, to image breast cancers using MRI. Herceptin specifically binds to the HER2/neu receptor, which is overexpressed in some breast cancer cells [54, 55]. Engineered nanoparticles having high and tunable magnetism offers improved sensitivity at their lower concentration as compared to conventional iron oxide contrast agents. Recently, Lee *et al.* employed Mn-doped iron oxide nanoparticles for ultrasensitive molecular imaging [54].

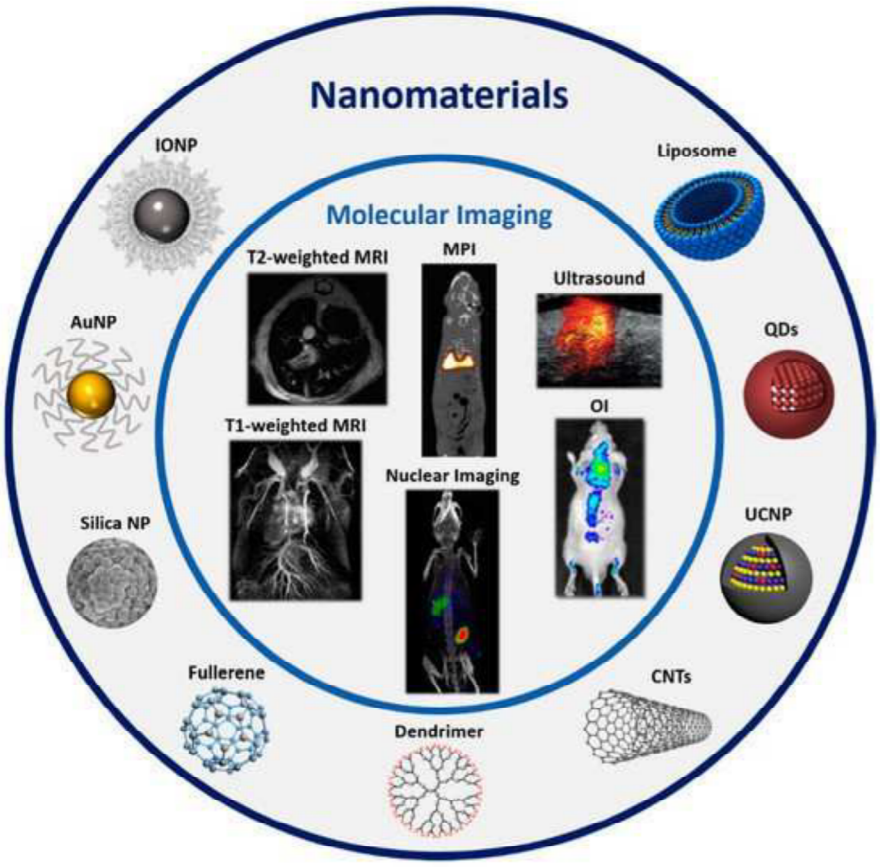

Fig 2. Utilization of different nanomaterials in various molecular imaging modalities

3. Instrumentation: Nanoparticle-based imaging techniques

Imaging techniques play a pivotal role in governing the exact position of nanoparticles inside

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living beings. Imaging instrumentation includes MRI, CT scan, SPECT and PET scanning where nanoparticles are used as contrasting agents and microscopic(optical) imaging techniques like fluorescence, confocal or electron microscopy are employed for in-vivo visualization of nanoparticles.

3.1 Microscopic techniques

3.1.1 Confocal Microscopy

Confocal microscopy uses conventional immunofluorescence methods for imaging of NPs. It has the ability to provide high quality images from within a localized region of the cell/tissue which makes it suitable for the investigation of biological tissue. Secondary (indirect) immunofluorescence technique is most frequently used for the visualization and detection of NPs. The complex structure of NP enables binding of a fluorescent probe or different fluorescent probes ("doped" NPs) to it & subsequently visualization of NPs can be achieved through confocal fluorescence microscopic techniques. Luminescent nanoparticles serve the main purpose of confocal microscopy, especially silica-based nanoparticles doped with fluorescence molecules [56, 57], gold nanoparticles [58], quantum dots and nanophosphors (ceramic nanoparticles containing luminescent lanthanoid ions). Organically modified silica (ORMOSIL) and nanophosphors are the two most promising luminescent NPs, which exhibits aggregation-enhanced fluorescence [59-62].The most important confocal microscopy techniques are summarized below:

Table1. Confocal microscopy techniques

Nanoparticle(s)	Technique	Application	Ref.
Coumarin-6 labelled nanoparticles	Confocal Laser Scanning Microscopy	Study of cellular uptake/transport mechanisms on Madin-Darby Canine Kidney (MDCK) cells	[63]
Zwitterionic quantum dots	Confocal fluorescence microscopy	Study of cellular uptake/transport mechanisms on red blood cells	[64]
60 nm gold nanospheres	Confocal reflection microscopy	Improvement of image contrast of tissue structures	[65]

3.1.2 Fluorescence Microscopy

Fluorescence microscopy is used to visualize the cellular and tissue-level distribution of biologically significant fluorescent nanoparticles. Semiconductor quantum dots are used as fluorescent probes for biomolecular and cellular imaging [66, 67, 68, 69]. Newly developed brain-targeted fluorescent nanocarriers have been utilized as a molecular probe in neuroimaging by fluorescent microscopy. Fluorescent nanocarriers ensure target-specific binding to provide better imaging facility as compared to conventional contrast agents [70].
Advantages of fluorescence imaging over other imaging modalities includes enhanced sensitivity with significantly higher quality of images and its non-invasive nature, using relatively

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inexpensive instruments. But being an optical technique, it is limited in terms of tissue penetration depth. As NPs are able to affect autofluorescence of cells, therefore NPs may be detected by autofluorescence method [71]. Fluorescence microscopy enables the combination of differently labelled secondary antibodies, in addition to multifunctional cell/tissue labelling.

3.1.3 Electron Microscopy

Electron microscopy is an outstanding tool to determine the size and structure of NPs. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) Electron are generally used for in-vivo visualization of nanoparticles. TEM is preferably used to study nanoparticle uptake, cellular compartmentalization, transport processes and their accumulation in different tissues and organs. SEM is an indispensable tool in determining the structural characterization of NPs such as shape and size, area, presence of pores or eccentricity. Moreover, SEM is highly required to control NP quality [72, 73, 74]. Another essential tool for investigating microstructural details of nanomaterials is Analytical Electron Microscopy (AEM) which combines imaging with diffraction and spectroscopy [75].

3.2 Magnetic Resonance Imaging (MRI)

Being non-invasive in nature, MRI is generally used to visualize the structural and functional details of internal organs. Generally, it provides enhanced contrast between soft tissues as compared to computed tomography (X-ray CT) [76, 77, 78]. In order to improve contrast in MRI imaging, several kinds of nanoparticles have been synthesized. Kircher *et al.* employed magnetofluorescent (CLIO-Cy5.5) nanoparticles as a pre-operative MRI contrast agent and intra-operative optical probe for detection of brain tumours using a xenograft model of gliosarcoma [79]. For example, magnetofluorescent nanoparticles labelled with Bombesin(BN) are used for targeting bombesin receptors present on acinar cells of the pancreas, thereby enhancing the tumour visualizing ability by MRI [80].

Superparamagnetic nanoparticles (SPIONs) are useful for magnetic drug targeting [81], cell tracking [82] and medical imaging [83, 84] on account of their switchable magnetic properties. The ongoing development of magnetic NPs as MRI contrast agents (CAs) further enhances image contrast. To serve the purpose of surface functionalization, a paramagnetic CA, usually a gadolinium-based compound is most commonly used [85]. Gadolinium-doped tissues and fluids appear extremely bright in MRI, which is why paramagnetic CAs are called positive CAs. To ensure prior diagnosis of lymph node metastases, Masoudi *et al.* synthesized PEG (Polyethylene Glycol) coated iron oxide NPs to be used as potential MRI contrast agents [86]. Summarily, the contrast of MRI has been significantly enhanced, which enables potential diseases detection at an earlier stage.

3.3 PET and SPECT scan

With the advent of Positron Emission Tomography (**PET**)/Single-Photon Emission Computerized Tomography (**SPECT**), a new pathway has opened for cancer diagnosis and treatment. These techniques help to track different biological pathways and discover typical tumoral features using nanoparticle-based contrast agents (CAs). The combination of a structural imaging modality (CT, MRI) with a highly functional sensitive imaging modality (PET/SPECT) resulted in **multimodal imaging or hybrid imaging**, which can offer multiple advantages over any single modality [87], overcoming its drawbacks and strengthening the peculiarities.

NPs were extensively studied as imaging probes for dual MRI/PET tumour imaging at a preclinical level. Nanoparticles can be categorized into inorganic and organic NPs, depending

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on the chemical composition of their core [88]. On account of unique physical and chemical properties, inorganic NPs have gained significant attention. Particularly, chemical inertness and the ease of surface functionalization make inorganic NPs suitable for imaging of malignant tumours. For example, Chakravarty et al. developed a probe for dual MRI/PET imaging by ^{69}Ge radiolabelling of SPIONs [89]. Similarly, a number of organic NPs such as liposomes, polymeric micelles and proteins are used for cancer diagnosis over the last decade. These organic NPs carrying imaging moieties such as radionuclides, shows potential for tumour detection [88]. For instance, liposomes were radiolabelled through a ^{68}Ga-based radiotracer, allowing a dual-modality tracking of in -vivo distribution of the NPs through MRI and PET imaging. Liposomes radiolabelled with ^{99m}Tc and ^{64}Cu were also used for SPECT and PET imaging. However, their toxicity remains a matter of concern; it was observed that iron-oxide NPs entering into the cells through endocytosis show higher levels of toxicity due to their accumulation in endo-lysosomal compartments [90].

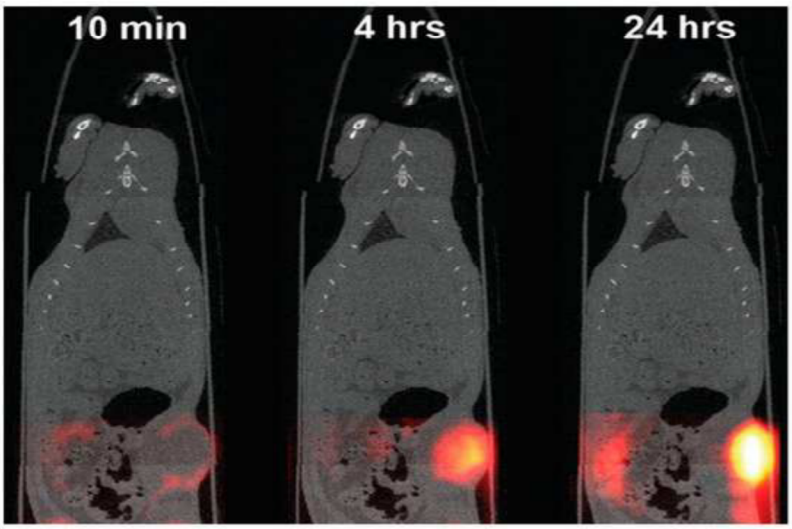

Fig3. In vivo MRI/CT image of a human breast tumour in a murine model, using MRI-based SPIONs. SPIONs can be observed to accumulate in tumour having a contrast peak at 24 hrs post injection [91].

4. Conclusion

This review summarizes the emerging research on several types of nanoparticle-based imaging techniques utilized for biomedical imaging. Quantum dots are recognised as the most promising agents for fluorescent imaging. Gold nanoparticles are used as contrast agents in X-ray CT imaging due to their versatile surface chemistry and biocompatibility. Engineered iron oxide nanoparticles with precise control over particle size and composition along with new Gd-nanoparticle conjugates are being explored as contrast agents for MRI. Imaging techniques are progressing rapidly due to the development of new types of contrast agents and improved instrumentation modalities. Microscopic techniques (Confocal microscopy, Fluorescence microscopy, etc) and several scanning methods like MRI, X-ray, PET & SPECT scan are being employed to explore the in-vivo functioning of nanoparticle based imaging probes. In in-vivo imaging, the potential of NP is remarkable, which opens new possibilities for faster and better diagnostics along with improved treatment strategies.

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However, the toxicity of nanoparticle is a critically important topic for researchers both in material science and biomedical fields. Despite the synthesis of a multitude of functionalized nanoparticles, detailed in-vivo toxicity studies have lagged behind. Therefore, an extra attention should be given to predict the potential toxicity of nanoparticles before their actual in-vivo implementation.

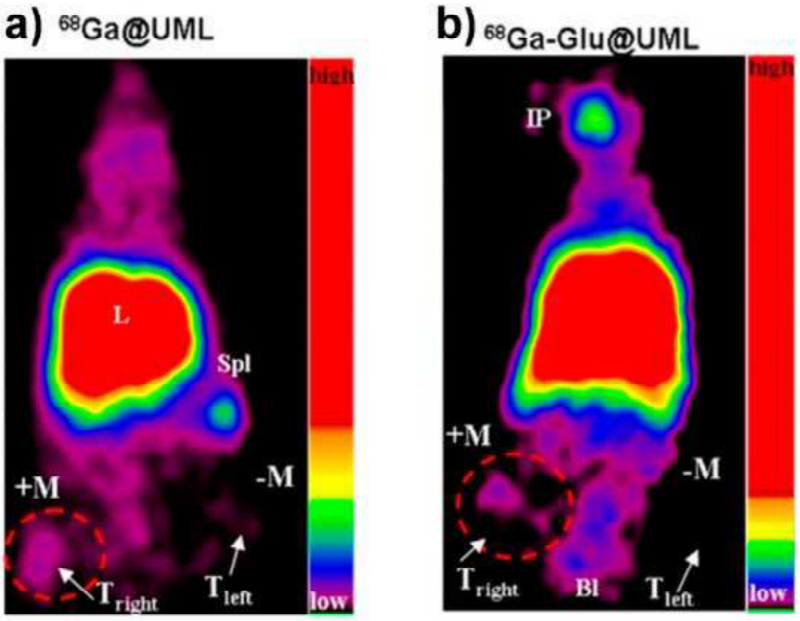

Fig 4 .(**a**) PET image 30 min post ^{68}Ga radiolabelled liposome injection (without glucose in the formulation); (**b**) PET image 30 min post ^{68}Ga radiolabelled glucose liposome injection. The arrows show tumours. IP: injection point; L:liver; Spl:spleen; Bl:bladder; M: magnet; T_{right} and T_{left}, tumours [92].

References

1. Weissleder R. Molecular imaging in cancer. *Science.* 2006;312(5777):1168–71. [PubMed] [Google Scholar]
2. Anthony Singer, Zein Barakat ,Shyam S. Mohapatra, in Nanocarriers for Drug Delivery, 2019
3. Matthew M. Mahan[1] and Amber L. Doiron , in Gold Nanoparticles as X-Ray, CT, and Multimodal Imaging Contrast Agents: Formulation, Targeting, and Methodology**.**
4. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer.* 2005;5(3):161–71. [PubMed] [Google Scholar]
5. Forrest ML, Kwon GS. Clinical developments in drug delivery nanotechnology. *Adv Drug Deliv Rev.* 2008;60(8):861–2. [PubMed] [Google Scholar]
6. Resch-Genger U, Grabolle M, Cavaliere-Jaricot S, et al. Quantum dots versus organic dyes as fluorescent labels. *Nat Methods.* 2008;5(9):763–75. [PubMed] [Google Scholar]
7. Drbohlavova J., Adam V., Kizek R., IIubalek J. Quantum dots—Characterization, preparation and usage in biological systems. Int. J. Mol. Sci. 2009;**10**:656–673. [PMC free article] [PubMed] [Google Scholar]
8. Yu W.W., Qu L.H., Guo W.Z., Peng X.G. Experimental determination of the extinction coefficient of CdTe, CdSe, and CdS nanocrystals. *Chem. Mat.* 2003;**15**:2854–2860. [Google Scholar]
9. Gao X.H., Yang L.L., Petros J.A., Marshal Γ.Γ., Simons J.W., Nie S.M. *In vivo* molecular and cellular imaging with quantum dots. *Curr. Opin. Biotechnol.* 2005;**16**:63–72. [PubMed] [Google Scholar]
10. Baruah S., Ortinero C., Shipin O.V., Dutta J. Manganese doped zinc sulfide quantum dots for detection of Escherichia coli. *J. Fluoresc.* 2012;**22**:403–408. [PubMed] [Google Scholar]
11. Drummen G. Quantum dots-from synthesis to applications in biomedicine and life sciences. *Int. J. Mol. Sci.* 2010;**11**:154–163. [PMC free article] [PubMed] [Google Scholar]
12. Jin Z.W., Hildebrandt N. Semiconductor quantum dots for *in vitro* diagnostics and cellular imaging. *Trends Biotechnol.* 2012;**30**:394–403. [PubMed] [Google Scholar]

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13. Liu Q.H., Deng R.P., Ji X.L., Pan D.C. Alloyed Mn-Cu-In-S nanocrystals: A new type of diluted magnetic semiconductor quantum dots. *Nanotechnology.* 2012; **23**:2–6. [PubMed] [Google Scholar]
14. Mattoussi H., Palui G., Na H.B. Luminescent quantum dots as platforms for probing *in vitro* and *in vivo* biological processes. *Adv. Drug Deliv. Rev.* 2012;**64**:138–166. [PubMed] [Google Scholar]
15. Mukerjee A., Ranjan A.P., Vishwanatha J.K. Combinatorial nanoparticles for cancer diagnosis and therapy. *Curr. Med. Chem.* 2012;**19**:3714–3721. [PubMed] [Google Scholar]
16. Nie S.M., Xing Y., Kim G.J., Simons J.W. *Annual Review of Biomedical Engineering.* Vol. 9. Annual Reviews; Palo Alto, CA, USA: 2007. Nanotechnology applications in cancer; pp. 257–288. [PubMed] [Google Scholar]
17. Pericleous P., Gazouli M., Lyberopoulou A., Rizos S., Nikiteas N., Efstathopoulos E.P. Quantum dots hold promise for early cancer imaging and detection. *Int. J. Cancer.* 2012;**131**:519528. [PubMed] [Google Scholar]
18. Singhal M., Sharma J.K., Kumar S. Effect of biocompatible glutathione capping on core-shell ZnS quantum dots. *J. Mater. Sci. Mater. Electron.* 2012;**23**:1387–1392. [Google Scholar]
19. SageL.2004Finding cancer cells with quantum dots." Anal Chem 76(23): 453A 453 ; LiZ.WangK.et al.2006Immunofluorescent labelling of cancer cells with quantum dots synthesized in aqueous solution." Anal Biochem 3542169174 ; XingY.SmithA. M.et al.2006Molecular profiling of single cancer cells and clinical tissue specimens with semiconductor quantum dots. Int J Nanomedicine 14473481. [PubMed] [Google Scholar]
20. KimT. II.JinII.et al.2006Mannosylated chitosan nanoparticle-based cytokine gene therapy suppressed cancer growth in BALB/c mice bearing CT-26 carcinoma cells." Mol Cancer Ther 5717231732 ; GoldbergM. S.XingD.et al.2011Nanoparticle-mediated delivery of siRNA targeting Parp1 extends survival of mice bearing tumours derived from Brca1-deficient ovarian cancer cells." Proc Natl Acad Sci U S A 1082745750. [PubMed] [Google Scholar]
21. Choi, Jonghoon & Wang, Nam Sun. (2011). Nanoparticles in Biomedical Applications and Their Safety Concerns. 10.5772/18452.
22. Zimmer J.P., Kim S.W., Ohnishi S., Tanaka E., Frangioni J.V., Bawendi M.G. Size series of small indium arsenide-zinc selenide core-shell nanocrystals and their application to *in vivo* imaging. *J. Am. Chem. Soc.* 2006;**128**:2526–2527. [PMC free article] [PubMed] [Google Scholar]
23. Smith J.D., Fisher G.W., Waggoner A.S., Campbell P.G. The use of quantum dots for analysis of chick CAM vasculature. *Microvasc. Res.* 2007;**73**:75–83. [PubMed] [Google Scholar]
24. Bhang S.H., Won N., Lee T.J., Jin H., Nam J., Park J., Chung H., Park H.S., Sung Y.E., Hahn S.K., et al. Hyaluronic acid-quantum dot conjugates for *in vivo* lymphatic vessel imaging. *ACS Nano.* 2009;**3**:1389–1398. [PubMed] [Google Scholar]
25. DurisicN.BachirA. I.et al.2007Detection and correction of blinking bias in image correlation transport measurements of quantum dot tagged macromolecules." Biophys J 93413381346. [PubMed] [Google Scholar]
26. LeeS. F.OsborneM. A.2009Brightening, blinking, bluing and bleaching in the life of a quantum dot: friend or foe?" Chemphyschem 101321742191. [PubMed] [Google Scholar]
27. PetersonJ. J.NesbittD. J.2009Modified power law behavior in quantum dot blinking: a novel role for biexcitons and auger ionization." Nano Lett 91338345. [PubMed] [Google Scholar]
28. Cai WB, Chen XY. Nanoplatforms for targeted molecular imaging in living subjects. *Small.* 2007;3(11):1840–54. [PubMed] [Google Scholar]
29. Murphy CJ, Gole AM, Stone JW, et al. Gold Nanoparticles in biology: beyond toxicity to cellular imaging. *Acc Chem Res.* 2008;41(12):1721–30. [PubMed] [Google Scholar]
30. Sperling RA, Gil PR, Zhang F, et al. Biological applications of gold nanoparticles. *Chem Soc Rev.* 2008;37(9):1896 908. [PubMed] [Google Scholar]
31. Lewinski N, Colvin V, Drezek R. Cytotoxicity of nanoparticles. *Small.* 2008;4(1):26–49. [PubMed] [Google Scholar]
32. Daniel MC, Astruc D. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem Rev.* 2004;104(1):293–346. [PubMed] [Google Scholar]
33. R. Cheheltani, R. M. Ezzibdeh, P. Chhour et al., "Tunable, biodegradable gold nanoparticles as contrast agents for computed tomography and photoacoustic imaging," *Biomaterials*, vol. 102, pp. 87–97, 2016.View at: Publisher Site | Google Scholar
34. N. Teraphongphom, P. Chhour, J. R. Eisenbrey et al., "Nanoparticle loaded polymeric microbubbles as contrast agents for multimodal imaging," *Langmuir*, vol. 31, no. 43, pp. 11858–11867, 2015.View at: Publisher Site | Google Scholar

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