Emerging applications of nanotechnology for diagnosis and therapy of disease: a review

Authors: Richard Bayford, Tom Rademacher, Ivan Roitt and Scarlet Xiaoyan Wang
Correspondence r.bayford@mdx.ac.uk

Abstract: Nanotechnology is of increasing interest in the fields of medicine and physiology over recent years. Its application could considerably improve disease detection and therapy, and although the potential is considerable, there are still many challenges, which need to be addressed before it is accepted in routine clinical use. This review focuses on emerging applications that nanotechnology could enhance or provide new approaches in diagnoses and therapy. The main focus of recent research centres on targeted therapies and enhancing imaging; however, the introduction of nanomaterial into the human body must be controlled, as there are many issues with possible toxicity and long-term effects. Despite these issues, the potential for nanotechnology to provide new methods of combating cancer and other disease conditions is considerable. There are still key challenges for researchers in this field, including the means of delivery and targetting in the body to provide effective treatment for specific disease conditions. Nanoparticles are difficult to measure due to the size and physical properties; hence there is still a great need to improve physiological measurements method in the field to ascertain how effective their use is in the human subject. This review is a brief snapshot into the fast changing research field of measurement and physiological links to nanoparticle use and its potential in the future.

1 Introduction: In recent years the use of nanotechnology has advanced considerably in the medical field. It is generally defined as the manipulation of matter on an atomic, molecular, or supramolecular scale in the order of one dimension less than 100nm. It can also be considered as the understanding and control of the processes within these structures sized between 1 – 100 nanometers (nm) (Kumara et al 2014) [1](Figure 1)

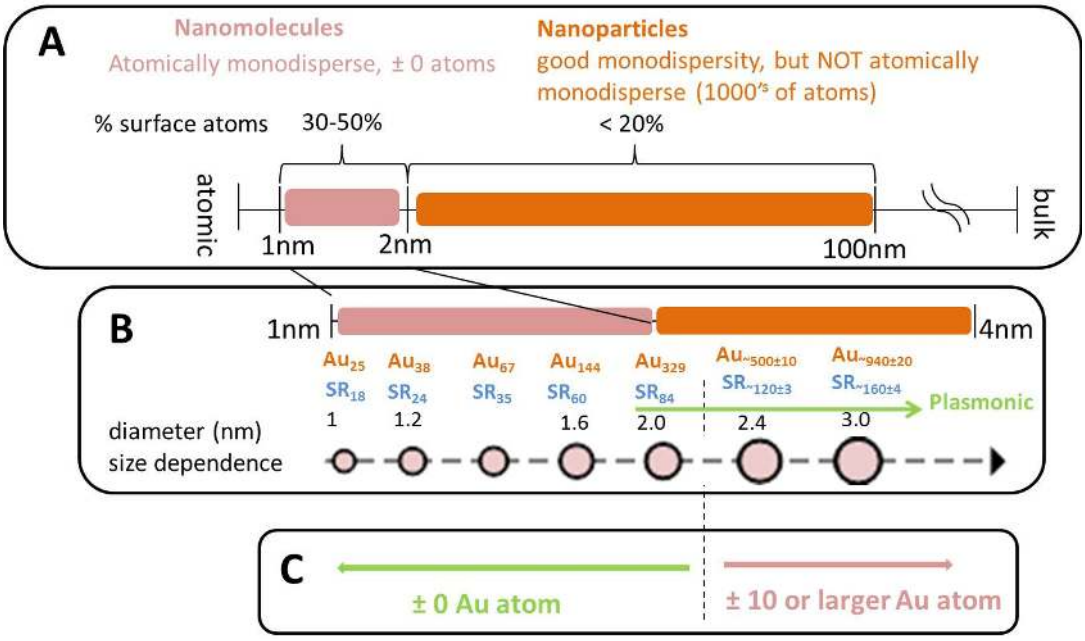


Figure 1. Example of size range of gold nanomolecules, plasmonic Faradaurates and nanoparticles. A. Gold nanomolecules with size range between 1 and 100 nm. B. Thiolated gold nanomolecules with a precise number of metal atoms and organic ligands. C. Indication of the transition between the fixed composition containing nanomolecules with ±0 Au atom variation and polydisperse $Au_{\sim 500\pm 10}(SR)_{\sim 120\pm 3}$ particles with a ±10 Au atom variation.

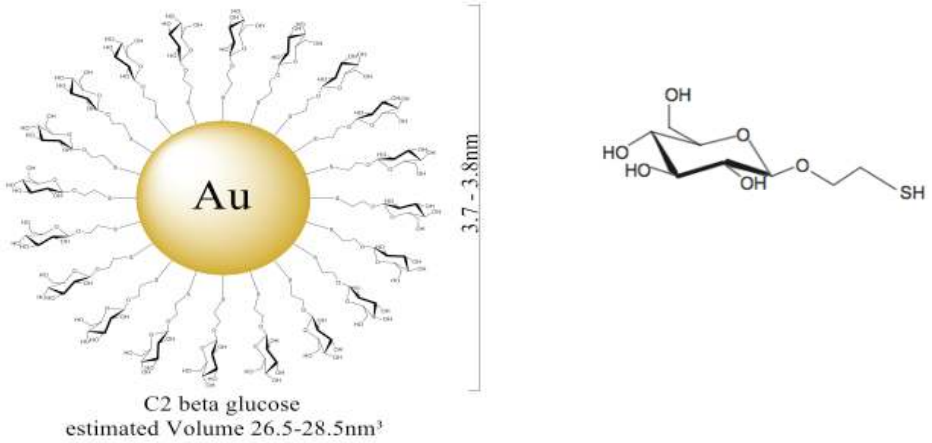
(Lund et. al., 2011) [2](Figure 2). The term nanoparticle (NP) is often used to describe these structures, but this does not necessary give a correct perception of a nanoparticle. These structures can also be considered as scaffolds formed of atoms used to build advance structures; for example 57 atoms of Au can be used as a scaffold on which further atoms can be attached followed by the addition of biological ligands. Nanoparticles can also be created using different composite materials at the Nano-scale, changing their physical properties. Importantly these properties do not generally coincide with larger non-nanoscale counterparts.

Size also affects the NP physical properties so it is important to create particles with consistent structures that can be reproduced. The material used in the fabrication of the nanoparticle can include gold, iron, silver, cerium, titanium dioxide, silica, carbon, zinc, copper, nickel, magnesium and composites thereof. Gold is favoured in the construction of nanoparticles as it is hydrophobic although normally gold is

paramagnetic which can cause issues when they are used as contrast agents. The very first report of a water-soluble gold nanoparticle was 2001 by de la Fuente JM et. al., [3]. Composite have been produced for example gold and gold-iron oxide magnetic glyconanoparticles to combine the advantage of gold and magnetics properties. (de la Fuente el al 2006) [4]

The balance between the nanoparticle physical properties, their biocompatibility and the evidence that there are no cytotoxic effects is key to their successful use in clinical applications. Another important feature is the configuration of the ligands and their interaction with the atoms on the particle surface as they play a significant role in determining the physiochemical properties of the NPs and therefore their interaction with the human body and biological material.

It is also worth noting that extensive efforts have been made to synthesize and characterize nanoparticles at sizes smaller than 6nm, which includes the core and attached ligands (Figure 1), as this is the maximum size that will pass through the kidney. This is size, charge and shape-dependent due to the unique structure of the glomerular capillary wall. A globular AuNP with a hydrodynamic diameter (HD) < 6 nm can pass through the glomerular capillary wall easily, while it is difficult for a large one (HD > 8 nm) to cross through due to the kidney filtration threshold (KFT) which is about 5.5 nm. Furthermore, the surface charges of a NP also play an important role in the kidney filtration: a positively charged NP with an HD (6–8 nm) slightly larger than KFT can pass the kidney filtration barrier due to the favourable charge interactions with the glomerular capillary wall which is negatively charged, whereas filtration through the kidney is difficult for the negatively charged or neutral NP with an HD of 6–8 nm. (Kumara et al 2014) [1] provides an illustration demonstrated the size range of nanomolecules to nanoparticles and Faradaurates.



(a) (b)

Figure 2(a). Example of a glucose nanoparticle, with double C2 domain family, containing two calcium and phospholipid binding domains in its C terminus and (b) structure of surface coating ligands. (Lund et al) [2]

The range of uses of different types of NPs are difficult to fully categorise, but this review will mainly focus on two specific areas, therapy and diagnosis. NPs can deliver therapy by a number of methods: by enhancement of radiotherapy or heating to cause cell death, as a scaffold to construct new molecular structures to help target or deliver drugs, and as an image contrast agent to identify disease conditions. This review gives a brief overview of some of these areas.

2 Diagnostics: Imaging and point of care technologies are two specific areas that could benefit from the application of NPs.

2.1 Imaging: In the case of imaging, a contrast agent that could associate with tumours through bonding of the NP surface ligands to the cancer biomarker would be of significant use in tracking the application of diagnoses and therapy. However, identification of appropriate ligands that bind to the cancer biomarker is still a key challenge. Contrast agents are often used to track particular physiological process during imaging and NPs can play a major role in the future of medical diagnostics due to their many advantages over the conventional contrast agents, such as controlled biological clearance pathways, specific molecular targeting capabilities (Shilo et al, 2012)[5] and prolonged blood circulation time, providing a longer time for imaging. This offers an advantage over contrast agents made from particles larger than 1 μm,

which are cleared rapidly by the body's reticuloendothelial system following injection into the bloodstream.

2.2 MRI: The application of nanotechnology to the field of medical imaging offers a number of advantages. In the case of MRI, iron-based NPs causes a change in the magnetic field but gold-based NPs are non-paramagnetic and although they are biological compatible, do not affect the contrast of the tissues or blood. MRI contrast agents require the proton relaxation to be altered and must be able to perturb the local magnetic field around the proton. The perturbing field in MRI of a super paramagnetic particle is effective at up to 50-times its diameter, and therefore influences water protons in several cell layers around its location (Shilo et. al., 2012) [5]. First generation magnetic nanoparticles (MNP) are already in clinical use and second generation agents, with longer blood half lives, are likely to be approved for routine clinical use in the near future. Similar AuNPs are at present not used due to their non-magnetic properties, although there are Faradaic (generated by the reduction or oxidation of some chemical substance at the Au) AuNPs which do possess magnetic properties that have the potential for use in this area (Mohs et. al., (2010)[6]. Superparamagnetic MNRs which normally consist of a central core of iron-oxide surrounded by a carbohydrate or polymer coat are playing an important role in a number of MRI image systems, for example cardiovascular molecular imaging. The size, physical properties and pharmacokinetics of MNPs make them highly suited to cellular and molecular imaging of atherosclerotic plaques and myocardial injury. They have a sensitivity in the nanomolar range and can be detected with T1, T2, T2*, off resonance and steady state free precision sequences that are used in MRI systems. Targeted imaging with MNP is being actively explored and improvements of contrast-enhanced magnetic resonance imaging have been of considerable interest (Xing, et al. 2014) [7].

2.3 **PET:** Radiolabeled positron-emitting isotopic nanoparticles for non-invasive deciphering of biological events, such as tumor receptor levels and tumor enzyme activities have been developed (Pérez-Campaña, et. al., 2013) [8]; for example, metal oxide nanoparticles have been widely used for the construction of PET imaging probes. Perez–Campana used them for the activation of ^{18}O–enriched aluminum oxide (Al_2O_3) nanoparticles by irradiation with protons to yield ^{18}F–labeled NPs via the $^{18}O(p,n)^{18}F$ nuclear reaction .

2.4 Ultrasound: A number of researchers have investigated the use of NPs for

ultrasound imaging. Solid NPs can enhance ultrasonic grey scale images in tissue phantoms and mouse livers *in vivo*. For example, silica nanospheres (100 nm) dispersed in agarose at 1-2.5% mass concentration can be imaged by a high-resolution ultrasound imaging system (transducer centre frequency: 30 MHz). They also investigated polystyrene particles of different sizes (500-3000 nm) and concentrations (0.13-0.75% mass), which were similarly dispersed in agarose and imaged. (Liu et. al., 2006) [9].

The potential use of platinum nanoparticles (Pt-NPs), as a superoxide dismutase (SOD)/catalase mimetic antioxidant, has been studied by Jawaid, P., et. al., [10] The Pt-NPs in combination with 1MHz ultrasound at an intensity of 0.4 W/cm(2), 10% duty factor, 100 Hz PRF, for 2 min promoted apoptosis in human myelomonocytic lymphoma U937 cells assessed by DNA fragmentation assay, cell cycle analysis and Annexin V-FITC/PI staining. Cell counting and microscopic examination confirmed cell death. The mitochondrial and Ca(2+)-dependent pathways were also investigated. These studies addressed the issue of the mechanisms of cell death following therapeutic ultrasound treatment in the presence or absence of Pt-NPs. The important conclusion was that Pt-NPs appeared to interfere with apoptosis and consequently block ultrasound-induced autophagy, this paradoxically led to increased cell killing. The authors concluded that autophagy induced after ultrasound mechanical effects operates "pro-survival pathway" and its blockade by Pt-NPs causes enhancement of cell killing.

2.5 CT: Enhancement of particular regions in the human body would be of great value for diagnosing a range of disease conditions. Considerable research is focusing on the development of nanoparticle CT contrast agents for molecular imaging of blood (pooling which occurs when the walls and valves of veins in human legs do not work effectively, thereby making it difficult for blood to return to the heart). Each image modality uses different physical principles to obtain the image, and require the physical properties of the contrast agent to be compatible with the physics of the specific imaging system. Most CT contrast agents lack this amplification ability and force and since CT imaging requires millimolar contrast agent concentrations to induce sufficient contrast in the desired organ, a much larger amount is needed.

However, nanoparticle contrast agents can amplify the contrast, which would allow a reduction in the relatively high radiation exposure of CT. Thus, these new generation CT contrast agents based on high atomic number materials, such as gold and bismuth, have a great potential not only because of their ability to produce higher contrast than conventional iodine-based contrast agents, but even more importantly, because of the potential to lower the overall radiation exposure to patients. Hainfeld *et al*. (2004)[11] studied molecular imaging of cancer with actively targeted CT contrast agents. They showed that AuNPs can enhance the visibility of mm-sized xenografted human breast tumors in mice, and that active tumor targeting (with anti-Her2 antibodies) is 1.6-fold more efficient than passive targeting. They also demonstrated that the specific uptake of the targeted AuNPs in the tumor periphery was 22-fold higher than in surrounding muscle. Chanda *et al.* (2010)[12] reported enhanced CT attenuation of bombesin-functionalized AuNPs that selectively targeted cancer receptor sites that are overexpressed in prostate, breast and small-cell lung carcinoma while Mottiei et. al. (2016) [13] recently investigated methods to differentiate between cancer and inflammation during functional computed tomography since PET scanning using ^{18}F glucose is unable to distinguish between an inflammatory lesion and a cancer lesion as both have increased glucose metabolic associated uptake. They found that glucose AuNPs can act as a CT agent and which allows for the differentiation between cancer and an inflammatory process.

Although new research papers are continuously being published in this area the key challenge is the acceptance of their use in humans, which requires more studies on the long-term effect of the NPs.

2.6 Point-of-Care: A promising area in diagnostics is the development of point-of-care biosensor devices. Point-of-Care devices for a range of diagnoses is an expanding research field particularly where there is a need for high specificity and sensitivity. AuNPs can be used to enhance this for a range of applications for example in an electrical biosensor they can be applied to the surface to effectively increase the surface area of the sensor and so increase the sensitivity. There are a number of groups developing these with AuNPs. These include detection of human phospholipase A2 in pancreatitis, aggregation and self-association of monoclonal antibodies (Li et. al., 2013) [14], milk contamination using alkaline phosphatase (Yu et. al., 2015) [15] and anti-tumour antibodies for the diagnosis of prostate cancer (Zheng et. al., 2014)[16].

3. Therapy: The use of NPs for therapy is probably the most important area of development in NP research. The ability to enhance or improve the delivery of target therapy is considerable and some of the most promising findings are detailed below:

3.1 Cancer: Cancer is one of the largest areas in which NPs are beginning to show promising developments. Many cancer patients survive treatment only to have a recurrence within a few years. Recurrences and tumor spreading are often due to cancer stem cells that can be resistant to killing by conventional cancer drugs. But now researchers have designed NPs that specifically target these hardy cells to deliver a drug. The hope is the NPs will target the cancer for both imaging and therapy. For example, Poon et. al., 2015 [17] demonstrated passively targeted NPs to melanoma using either cyclic RGD peptide or a peptide from a cancer-seeking myxoma virus. Lai et al., 2015 [18] used AuNPs as a multimodality imaging agent for brain glioma. They present results that not only identify primary glioma but also follow the development of the glioma with time and most importantly reveal full details of the tumour related microvasculature. They observed leaking of AuNPs from the tumour-related vasculature in contrast to no leakage from normal vasculature.

Radiation therapy along with surgery and chemotherapy are the major therapeutic strategies for cancer treatment. Unfortunately the development of resistance to the therapeutic modality is a major reason for the absence of cure and subsequent tumor growth. It requires the delivery of high intensity ionizing radiations with high accuracy to the tumor tissue resulting in the death of tumor cells. Radiation therapy has its disadvantages including the possibility of injury to the surrounding normal tissue. Another disadvantage is that some tumor cells are farther away from the site of radiation and hence might receive a lower intensity of the radiation beam. Moreover, the cells can develop resistance to the radiation. Usually the sensitivity of the mitotically active tumor cells is only slightly higher than that of surrounding healthy tissue so that the minimum dose of radiation that is sufficient to kill tumor tissue may only injure but not kill the normal tissue. However, due to development of resistance of tumor cells to the dosed radiation results in the requirement of elevated doses which eventually leads to death of the healthy tissue.

Multiple approaches have been utilized to limit the radiation resistance while

simultaneously enhancing the efficacy and safety of radiation therapy. The three major approaches for the improvement of radiation therapy have involved (a) enhancing radiosensitization of tumor tissue; (b) reversing of radiation resistance in tumor tissue; and (c) enhancing radio resistance of the healthy tissue. (Kwatra et. al., 2013)[19]. As with all forms of cancer treatment the aim of radiotherapy is to selectively maximise tumour killing while reducing the damage to healthy tissue. The first demonstration of AuNPs was present by Hainfeld et. al., (2004)[20]. AuNP radio enhancement has become an increasing area of investigation as an approach to increase the effectiveness of ionising radiation in biological systems. The biocompatibility is ideal although there is much work still required to improve the targeting of AuNPs at tumor sites to make it an ideal therapeutic contrast agent. Au's radiosensitization is due to its increased photoelectric absorption cross section in comparison to that of the surrounding cells. During irradiation, this results in an enhancement of the energy deposition in the vicinity of the gold particles due to the generation of photoelectrons, Auger electrons, and characteristic X-rays.

Recently, McQuaid et.al., (2016)[21] presented the first experimental results that take into account both the measured biodistribution of gold nanoparticles at the cellular level and the range of the product electrons responsible for energy deposition. Combining synchrotron-generated monoenergetic X-rays, intracellular gold particle imaging and DNA damage assays, enabled a DNA damage model to be generated that includes the production of intermediate electrons. This is an important result as it is the first time good agreement between the predictions of biological outcomes from both the Local Effect Models has been presented using a DNA damage model with experimentally observed cell killing and DNA damage induction via the combination of X-rays and GNPs. Two distinct models as indicated by this mechanistic study, one for short-term DNA damage and another for cell survival, indicates that, at least for nanoparticle enhancement, it is not safe to equate the lethal lesions invoked in the local effect model with DNA damage events. mostly Auger, electrons. The AuNP efficacy in this application is due to the enhanced secondary emission of low-energy electrons (mostly Auger electrons).

Researchers such as Brivio et al.,(2015) [22] investigated the concept of kilovoltage radiosurgery with AuNPs for AMD (Age-related Macular Degeneration). They concluded that a prescribed dose of x-ray radiation could be delivered using almost half of the radiation when compared to a treatment without AuNPs allowing

reduction of the dose delivered to the neighbouring organs such as the retinal/optic nerve by 49%. Dou et. al., (2015) [23] looked at the optimal size for simultaneous enhanced CT imaging and radiotherapy. Using the enhanced permeability and retention EPR effect to get the larger particles trapped in the stroma around the tumour, they studied particles in the range of 3-50nm and concluded that 13nm particles were optimal for the dual application. Irani et. al.,(2015) [24] found that AuNPs can enhance growth arrest in colorectal cancer cells when they are treated with cold plasma (Plasma can be divided into two broad categories: thermal plasma and non-thermal or cold plasma. Cold plasma may also refer to the barely ionized plasma (e.g., 1%)) as indicated in section 3.1 It is of interest that radiotherapy, proton therapy and also cold plasma therapy are all enhanced by AuNPs. This suggests that a single tumour-targeting compound could be used independently of the external therapy applied. Also, as mentioned in Section 3.1 Popovtzer et.. al.,(2016) [25] found that actively targeted gold AuNPs as radio sensitizers for head and neck cancer produced radiation enhancement which was secondary to greater apoptosis, angiogenesis inhibition and diminished DNA repair. No toxicity was observed for the conjugates prior to irradiation.

Cheng et al., 2014 [26] demonstrated that NPs have a synergistic effect with cold plasma. The addition of the NPs decreased cell viability allowing the cold plasma to be more efficient. This is another example of how NPs can be used as synergistic reagents with various cancer therapeutic techniques such as the radio sensitisation for proton therapy and now cold plasma therapy. The fact that radiotherapy, proton therapy and also cold plasma therapy are all enhanced by NPs, suggests that a single tumour-targeting compound could be used independently of the external therapy applied, but targeting is key to the success of this method.

A pioneering study by Simon et. al., 2015 [27] selected four drugs that are normally used for the treatment of acute myeloid leukaemia, lestaurtinib, midostatin, sorafenib and quizartinib. Their study demonstrates superior cytotoxicity of the nano-conjugates compared with the free drugs. It is estimated that the vast majority of drugs are not developed because of physicochemical problems such as solubility and stability. Other researchers have developed a small molecule inhibitor for Rad6, a protein over-expressed in aggressive breast cancers (Haynes et. al., 2016 [28]). The inhibitor induces cytotoxicity in cancerous cells but spares normal breast cancer. The therapeutic effect however is limited by poor solubility. They demonstrate that

attachment to AuNPs provides a soluble delivery vehicle for the drug and is active and induces mitochondrial cell death via mitochondrial dysfunction. They also suggested it could also have synergistic actions with cisplatin. Yang et. al., 2016 [29] found that tumour angiogenesis-targeted radiosensitization therapy using beams of ionizing radiation was enhanced by the cyclic RGDyC (http://www.creative-peptides.com/product/rgdyc-item-r04013-18265.html) peptide conjugated to AuNPs which included gadolinium (Gd) and ^{99m}Tc-exametazime, a radiopharmaceutical sold under the trade name Ceretec and is used by nuclear medicine physicians for the detection of altered regional cerebral perfusion in stroke and other cerebrovascular diseases. It can also be used for the labelling of leukocytes to localise intra-abdominal infections and inflammatory bowel disease. Exametazime (the part without technetium), sometimes referred to by its chemical name of *hexamethylpropyleneamine oxime* (or *HMPAO*), is used to guide the therapy by MRI/SPECT imaging. AuNPs RGD probes are clearly of considerable use as radiosensitizers capable of guiding and enhancing radiotherapy of tumours.

Many cancer treatments rely on combination of CT with radiotherapy. Dou et al., 2016 [23] estimated the optimal size for simultaneously enhanced CT imaging and radiotherapy. They used the Enhanced Permeability and Retention (EPR) effect to get the larger particles trapped in the stroma around the tumour. Particles were investigated in the range of 3-50nm and they concluded that 13nm particles were the optimum for the dual application.

A number of investigators have studied the use of AuNPs for specific cancers. Enhanced growth arrest of colorectal cancer cell occurred when treated with cold plasma (Irani et. al., 2015[24]). Popovtzer et al., 2016 [25] demonstrated actively targeted AuNPs with radio sensitizers for head and neck cancer. As with many previous studies they demonstrated radiation enhancement. The mechanism of enhancement appears to be a consequence of greater apoptosis, angiogenesis inhibition and diminished DNA repair. No toxicity was observed for the conjugates prior to irradiation. Acute myeloid leukaemia is another condition that could benefit from the use of NPs. The main therapeutic strategy uses tyrosine kinase inhibitors and two thirds of patients achieve complete remission but most will ultimately relapse. Petrushev et. al., 2016 [30] demonstrated that when these drugs are

conjugated to AuNPs they are superior to the free drugs with regards to cellular internalization, cell biology, etc. They validated transmembrane delivery and increased efficacy. Song et al., 2016 [31] investigated the use of endothelial growth factor (EGF) conjugated AuNPs synthesized via the citrate reduction method and then conjugated with chelating ligand DTPA-coupled EGF forming EGF-AuNP which enabled targeted delivery of a highly radioactive payload specifically to EGFR-positive cancer cells (e.g. breast cancer). Fitzgerald et al., 2016 [32] investigated anisamide to target AuNPs to prostate cancer. They claim proof of principle in their study that the nanoparticles can be targeted to the sigma receptor which could be an important finding if validated by others. Yook et al., (2016)[33] also performed a very interesting study looking at the stability and bio-distribution of thiol functionalized nanoparticles containing Lu (Lutetium) for radiation treatment of breast cancer. It is the chemistry of the study that is clever. They coated the cell with functionalizing reagents that have one, two or eight thiol groups. With increasing thiol content one would expect less ligand exchange and they confirmed that the eight thiol form is indeed the most stable but interestingly also has the lowest liver uptake (i.e. non-specific uptake). Another approach studied by Gomaa et al., 2015[34] on the cytotoxicity and genotoxicity of AuNP mediated photo-thermal therapy compared to the chemotherapeutic 5-FU produced an interesting result; they found that while both methods resulted in equivalent cytotoxic effects, the 5-FU, resulted in chromosomal aberrations whereas none were detected with the AuNP method.

3.2 Delivery: An important issue for the use of NPs is the delivery of agents for cancer treatment; these include aptamer-modified AuNPs for the controlled release and reversible delivery of daunorubicin (Taghdis, et. al., 2016 [35]). Aptamers are ideal AuNP targeting agents. Platinum drugs are by far the largest class of anti-cancer agents and Johnstone et. al., 2013 [36] produced an interesting review on the next generation of platinum drugs using NP delivery. Monoclonal antibodies make an important contribution to anti-cancer therapy and if AuNP can be shown to generically enhance the performance of the drugs, this could be a major use of NPs. Indeed, Ma et. al., 2016 [37] showed that the activity of a humanized mouse monoclonal that had been developed to treat hepatocellular carcinoma could be enhanced by conjugation to AuNP. Another approach is the use of AuNP nanoseeds for non-resectable solid tumour treatment (Moeendarbari et al., 2016 [38]). These constructs consist of AuNPs passivated with palladium -103. There appear to be many reports highlighting the use of nanoseeds for brachytherapy

Although there is considerable evidence that NPs do have potential for cancer therapy, only very limited human trials of NPs have been reported essentially due to the need for long term studies to ascertain any future toxicity effects on patients and the requirement for FDA approval.

3.3. Wound healing and burns: This area has considerable potential; a primary objective in dermatology is to achieve wound healing without formation of scar or even a faint scar. Naraginti et. al.,(2016) [39] have used green synthesized AuNPs from *Coleus forskohtii* to look at wound closure in rats. Topical formulations of the AuNPs promoted wound closure and reduction in excision wounds would be of great value for acute wound repair. They suggest the effect could be secondary to the now established antimicrobial, antioxidant and anti-inflammatory properties of AuNPs. Other groups (Ju et al., 2015)[40] have taken advantage of the anti-inflammatory action of AuNPs and loaded electro-spun silk with AuNPs. Full thickness dermal wound healing models were used and they demonstrated superior action for the matrices that had incorporated AuNPs. This reduces scar tissue and enhances matrix properties and good wound closure properties. Another application in wound healing concerns the surface of burns using a methylcellulose gel with or without AuNPs (Volkova et al., 2016) [41]. This accelerated the skin synthetic process and helped in recovering Type I and Type II collagen content. They suggest the results are due to the unique structure and antimicrobial properties of AuNPs. An important need in this research field is a means by which the progression of the wound healing can be monitored. NPs could provide a measure of this by labelling them with a fluorescent (Pittet et al., 2006)[42] agent allowing an optical measurement of progress. Methods to measure the biomarkers using NPs in the wound would be of great value.

3.4 Cardiovascular disease: Restenosis is a major problem in cardiovascular disease. Khobchandani et. al., (2016) [43] demonstrated that epigallacatechin-3-gallate conjugated NPs could be an alternative therapeutic approach to drug-coated stents for preventing restenosis. They demonstrate that the AuNP conjugates are internalized by avid, the laminin receptor; on human smooth muscle cell and endothelial cells. If this type of NPs can be imaged, then treatment could be targeted to specific parts of the cardiovascular system reducing the need for surgical intervention. Apolipoprotein AI (ApoAI) and ApoB are risk indicators of cardiovascular disease. Jiang et. al., [44] describe the use of immunoresonance

scattering to measure the ApoAI and ApoB in serum. A trisodium citrate method was used to prepare 9.0-nm gold nanoparticles labeled with goat anti-human ApoAI and ApoB antibodies. The immune reaction between gold-labeled antibodies and antigens took place in Na_2HPO_4-NaH_2PO_4 buffer solution (pH 6.4 for ApoAI and pH 6.0 for ApoB) in the presence of 75 g/L polyethylene glycol (PEG). They used a transmission electron microscope to observe the shape of the gold nanoparticles. The method showed high sensitivity and good selectivity for quantitative determination of ApoAI and ApoB in human serum.

3.5 Neuroimmunology: The development of NPs use in the brain is of considerable interest in advancing the treatment of brain disorders. The limitation of this use has primarily been the difficulty of NPs passing the blood brain barrier (BBB). This is meant to protect the brain from toxic agents, but unfortunately it also significantly hinders the delivery of therapeutics to the brain. A number of strategies have been employed to deliver drugs across this barrier and some of these may do structural damage to the BBB by forcibly opening it to allow the uncontrolled passage of drugs (Jain et. al., 2012 [45]). Nanobiotechnology-based delivery methods provide the best prospects for achieving this. Some strategies require multifunctional NPs combining controlled passage across the BBB with targeted delivery of the therapeutic cargo to the intended site of action in the brain, although there are currently some limitations and concerns for the potential neurotoxicity of NPs. It has been shown that the size, coating and surface charge of nanoparticles have a crucial impact on the passage of NP's across the BBB and subsequent process of intracellular uptake process (Shilo et. al., 2015 [46]). Shilo et al. concluded that GNPs of size 70 nm are optimal for the maximum amount of gold within the brain cells, and that 20 nm AuNPs are the optimal size for maximum free surface area. However, for the NP to pass out of the kidney they need to be 5nm or less. Morris et al. (2016) [47] reported results from AuNPs (15nm diameter) to study pathways for the passage of fluid into and out of the brain. They concluded that cerebral vascular basement membranes form the pathways by which fluid passes into and out of the brain but that different basement membrane layers are involved.

3.6 Antibiotics: Infections associated with medical devices are estimated to be a multi-billion dollar burden for the world-wide healthcare system. Most physiological measurement devices require some patient contact, for example electrodes, which have to be disposable to reduce the risk of infection. The use of a coating could reduce the cost burden on the healthcare system. Durán et. al., (2011) [48] have produced an AuNP coating that incorporates an antimicrobial peptide which demonstrated high antimicrobial activity against Gram positive and negative bacteria as well as multi-drug resistant bacteria. The coating was compatible with plasma and had low toxicity to human cells and also did not induce platelet and complement activation. These coatings can be transferred to glass, titanium, etc. and other substrates used in medical devices. Other coatings include amoxicillin-coated AuNPs (Kalita et. al., 2016)[49]. These conjugates demonstrated broad-spectrum bactericidal activity against both Gram positive and negative bacteria. The conjugates also showed potent anti-MRSA activity suggesting that by attaching the amoxicillin to the AuNPs there was subversion of the antibiotic resistance mechanism. Mu et al. (2014) [50] produced chitosan-streptomycin AuNP conjugates and demonstrated efficacy against Gram-negative bacterial biofilms. The conjugates were shown to inhibit planktonic cell growth of these bacteria showing these conjugates can overcome antibiotic resistance of microbial biofilms.

3.7 Tissue engineering: The development of tissue repair is of considerable interest and its application in the repair of wound and organ or limb has seen many advantages in recent years. The use of AuNP biomaterial patches composed of decellularized tissue conjugated to AuNPs has been shown to integrate well with the host tissue and not fail over the six month implantation time, indicating its potential applications for vascular repair and blood contacting (Moravej et. al., 2011)[51]. NPs involved in cardiac tissue engineering have a potential therapeutic strategy for end-stage heart failure and may provide new solutions to the serious problems of shortage of heart donors. For example, AuNP-blended polycaprolactone (PCL) scaffolds were found to enhance mesenchymal stem cell (MSCs) proliferation and differentiation into cardiac tissue, making it a promising approach for repairing myocardial infarction (Sridhar et. al., 2015) [52]. Application of nanoparticles in neural tissue engineering involves the utilization of a three-dimensional nanoscale nanoscaffold composed of polymer fibres that allows damaged cell adherence and helps rebuild missing tissue (Feng et al 2015)[53]. Carbon nanotubes have been increasingly used as scaffolds to enhance axon regeneration and synaptogenesis for the effective treatment of central

nervous system injuries due to their ability to promote neurone growth, differentiation, and survival, and allow the modification of their electrophysiological properties (Bokara et al., 2014) [54]. For example, in a mammalian visual system model, a designed self-assembling peptide nanofibre scaffold showed the ability to regenerate axons at the site of an acute injury and knit the brain tissue together to promote functional return of vision (Ruttledge et al. 2005) [55].

3.8 Toxicity: It is important to consider the adverse effects such as potential toxicity, which is determined by various factors including particle size, shape and ability to interact with the surrounding tissue. NPs may "overload" phagocytic cells, which causes a defensive fever and reduced body immunity. NPs may be difficult to degrade, leading to accumulation in the organs. NPs may also affect enzymes and protein activities and disturb biological processes in the body due to their high surface area (Fang et. al., 2015)[56]. Several possible mechanisms, such as oxidative stress, autophagy, lysosome dysfunction, and the activation of certain signalling pathways, may be involved. Researchers investigating the toxicity of NPs have carried out an increasing amount of research. Recent studies suggested that NPs induce considerable neurotoxicity in animals. For example, ZnO NPs were demonstrated to attenuate learning and memory ability in rats (Han et. al., 2011)[57]. Another study in mice suggested that TiO_2 nanoparticles accumulate in the mouse hippocampus after intragastric administration, which leads to hippocampal apoptosis and impairment in spatial recognition (Ze et. al., 2014) [58]. Furthermore, fatty degeneration of the hippocampus and brain lesions was observed in mice after the injection of a nanosized TiO_2 suspension via the gastrointestinal tract. *In vivo* studies have indicated that the neurotoxic effects of TiO_2 NPs on mice are likely to be attributed to the disturbed homeostasis of trace elements, enzymes, and neurotransmitter systems (Song et. al., 2015)[59]. In addition, silver NPs have been shown to cause damage to the BBB and astrocytes and induce neuronal degeneration in rats after subcutaneous injection (Xu et. al., 2015)[60]. Polyamidoamine coated AuNPs reduced cell metabolic activity in algal and bacterial cells, but no change of mitochondrial activity in mammalian cells was observed after 24 hours of treatment (Perreault et al., 2014) [61]. AuNPs were also found to increase endothelial paracellular permeability *in vitro* and elevate BBB permeability *in* vivo (Li et. al., 2015)(Gromnicova R, et. al., 2013) [62][63]. Lee U et. al., demonstrated that AuNPs toxicity is dependent on the dose or size of nanoparticles administrated into the brain by injection, and small particle size AuNPs induced more nestin expression compared to large particle size at short-term

implantation according to *in vitro* toxicity and *in vivo* gene expression studies. Similar results were found in the research on 10 nm Fe-NPs, indicating a neurotoxic potential of very small size iron nanoparticles and use of these ferric oxide NPs could result in neurotoxicity (Syed et. al., 2015)[64].

Some studies had investigated the impact of NPs on the reproductive system and offspring. Mn_2O_3NPs were found to significantly reduce luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone levels which results in a significant reduction in testicular cytology (Negahdary et. al., 2015)[65]. Other studies indicated that the inhalation of TiO_2 NPs led to long-term lung inflammation in mating adult mice, and their offspring exhibited abnormal neurological behaviour as a result of the gestational exposure to NPs (Vandebriel et. al., 2012)[66]. Naz et. al.,(2016)[67] examined the bio-kinetics of three different sizes of AuNPs – 2, 5 and 10nm diameter and followed the biokinetics, redistribution and urinary excretion for 90 days. No mortality, changes in behaviour, hair colour, weight and food intake was observed for the 2nm particles. Neither was evidence for any significant changes observed for haematocrit, serum biochemistry and tissue histology. Excretion was predominantly in the urine.

4 The role of NPs in the delivery and intracellular release of drug conjugates

4.1 Drug Vectorization: The intracellular penetration of a drug can enable optimal efficacy of its active ingredients, especially in cases of multidrug resistance. Vectorization, or drug transportation, systems consist of transporting biologically active molecules to their target by crossing the biological barriers to administration for example by the epithelium, endothelium, cell membrane, as well as mechanical, physico-chemical and enzymatic barriers in the body. A number of researchers have devised methods to overcome this problem using polyalkylcyanoacrylate (PACA) NPs (Sulheim et. at., 2016)[68]. The blood brain barrier has been of considerable interest in recent year for application of drug vectorisation and imaging (see above section 3.5). Submicronic systems (nanoparticles, liposomes) for drug transport have been used, in particular to treat hepatic metastasis (Zhang et. al., 2014)[69]. If improved targeting of the drug to increase the efficacy of the treatment could be achieved then the side effects could also be reduced. For example, when doxorubicin is transported by biodegradable nanoparticles, its cardiac toxicity is greatly reduced (Gu et. al., 2012)[70]. Also biodegradable porous silicon NPs (pSiNP) functionalised with cancer cell targeting antibodies can also be loaded with the hydrophobic anti-cancer drug

camptothecin (Secret et. al., 2013)[71]

4.2 Uptake and intracellular fate of NP constructs: One of the key research areas at present is uptake of NPs in cancer cells. Huang et. al., (2010) [72] showed that nutrient deprivation or an increase in the requirement of nutrients in tumour cells can promote the uptake of NP from the microenvironment. Like Yang et. al., (Section 3.1)[29] they also used RGDyC to target tumour cells. The conjugates also contained Pt(IV) as the cytotoxic agent. They found the constructs more active than the free drug and were able to show that the increased cytoxicity was due to cell cycle arrest. This is a very different mechanism of action from that of the free drug. Volsi et. al.,(2016)[73] have published a paper that demonstrates that if AuNPs are passivated with the polymer inulin there is enhanced delivery of the anti-cancer drug doxorubicin leading to preferential accumulation in tumour cells. Experimentally they also use an attractive cancer/non-cancer cell co-culture model.

A number of other researches have investigated the chemotherapeutic agent doxorubicin and its use with AuNPs for treatment of cancer Dhamecha et. al., (2015) [74] use a green method (involving biological and environmental safety of their production. The main methods for nanoparticle production are chemical and physical approaches that are often costly and potentially harmful to the environment) to make the AuNPs loading these particles with doxorubicin. They showed in some cancer cell lines that NP constructs had greater activity than the free doxorubicin. Chaudhary et. al., (2015) [75] demonstrated that they could achieve a single synthesis of doxorubicin loaded NPs. Showing that doxorubicin can act as both the passivating agent and also the reducing agent removing the necessity of borohydride in the synthesis, is a rather interesting approach. Interestingly, Curry et. al., (2015)[76] published almost simultaneously an empirical and theoretical paper proposing that doxorubicin could itself be a passivating agent for gold nanoparticles as was actually demonstrated by Chaudhary et. al., (2015) [75] above.

Another key area in the targeting of nanoparticles for drug delivery is the loading on nanosystems by covalent conjugation or non-covalent interactions (Ulbrich et. al., 2016)[77], either with the coating or directly with the metallic surface.

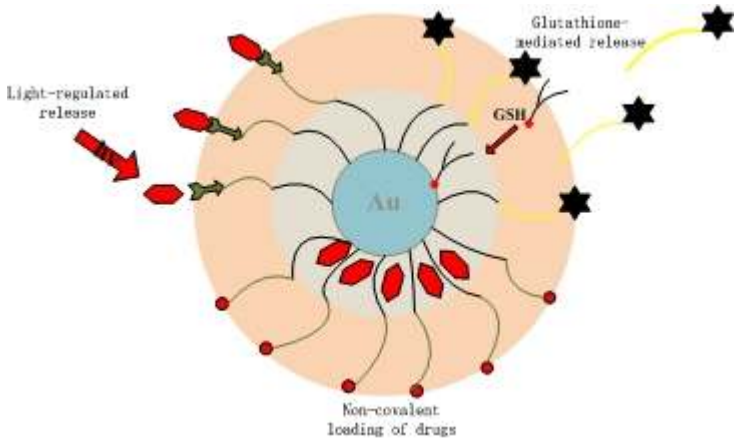


Figure 3. Three different types of interactions and release of payloads for AuNPs

This non-covalent approach employs active drugs while the covalent attachment in general requires intracellular processing of a prodrug (Han et. al., 2011) [78]. The release of the attached payloads can be triggered by internal (endogenous) or external (exogenous) stimuli, such as high concentration of glutathione which would split S-S bond attachment (Ostdiek et. al., 2015)[79] or excitation with light (Sridhar et. al., 2015, Williamson et. al., 2013, Bokara et. al., 2013) [80, 81, 82], respectively (Fig. 3). Silva et al. (2016) [83] investigated the relative importance of receptor-mediated uptake pathway of AuNPs compared to the EPR (Enhanced permeability and retention) effect using bombesin peptide-functionalized gold nanoparticles in a human prostate model. Interestingly, they found the EPR effect far outweighs the receptor-mediated pathway. The above observation was true for i.v. administration but in contrast for i.p. administration, the peptide receptor-mediated mechanism played a significant role in pancreas uptake. This study highlights the difficulties in establishing a validated study of tumour targeting of nanoparticles with peptides. In contrast to the work of Silva et al., Cumis et al., (2016)[84] showed that Au conjugated with a tumour–homing peptide containing NGR can be targeted to CD13 receptors on tumour vasculature, suggesting that NGR-tagged AuNPs can be used as a platform for tumour endothelial cell targeting and therefore for cancer therapy.

6 Heating of nanoparticles: NPs have been under study as a potentially viable mechanism for hyperthermic tumor treatment for some time using two regimes of the electromagnetic spectrum: laser and radio frequency excitation. Aspects of NP chemistry which affect thermal dissipation include the hydrodynamic diameter of the particle, the oxidation state and related magnetism of the core, and the chemical nature of the ligand shell. There are advantages and disadvantages of the different heating NPs and a number of reviews in this area have been published. For example Abaderr et. al.,(2016)[85] reviewed recent progress in cancer therapy using simple AuNPs for thermal and radiation enhancement of cancer.

6.1 Radio frequency therapy: The RF frequency range is linked to the size of the NPs and materials used to construct them. (Moran el. al., 2009)(Figures 4 and 5)[86] Magnetic field hyperthermia can be made more effective by the use of biocompatible super paramagnetic NPs, principally iron. In principle, these particles heat cancerous tissue by creating oscillations that produce heat, although the mechanism is not fully understood. Cancer cells could also be treated with AuNPs, which have the advantage that they are biocompatible and allow a range of ligands to be attached to them. Bulk gold metal is diamagnetic but 2 nm dodecanethiol (thiol) capped gold nanoparticles have been reported to exhibit ferromagnetism. This ferromagnetism is believed to result from spin-orbit coupling between surface-bound thiols and gold surface atoms. As the gold nanoparticle size decreases and the surface area to volume ratio increases, the likelihood of ferromagnetism increases. AuNPs, nanorods and nanoshells have been radiated with visible laser sources that excite the particles at or near their plasmon resonance frequency, and this mechanism has been well studied (Urban et. al., 2013) [87] The physical processes that describe the experimentally observed heating at radio frequencies of 13.56 MHz are not fully understood. Differing results have been reported in semi-solid phantom materials and liquid phase suspensions. This numerical modeling study was undertaken to inspect the relative importance of several candidate physical processes. The use of heating to destroy cancer cells is not the only mechanism that could be involved. It has been suggested that RF excited nanoparticles could destroy cancer cells by disrupting the Brownian motion in the cell. Aspects of RF, which may affect thermal dissipation, include power, frequency and antenna designs that emphasize relative strength of magnetic or electric fields.

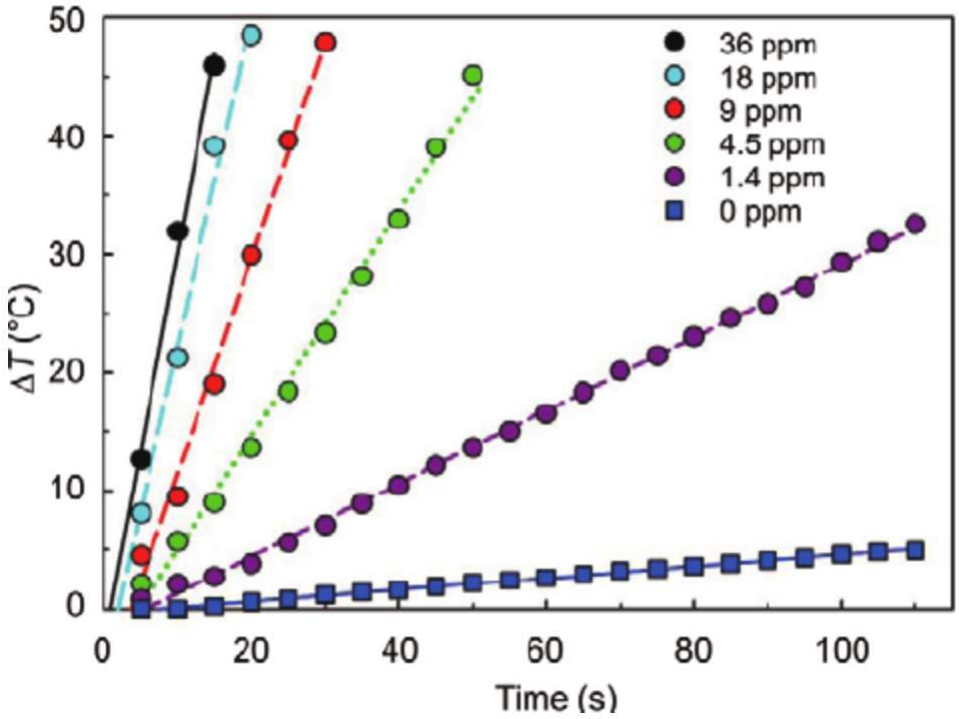


Figure 4 Change in temperature with time of 10 nm diameter gold nanoparticle suspensions (circles) and water alone (squares) exposed to RF fields.

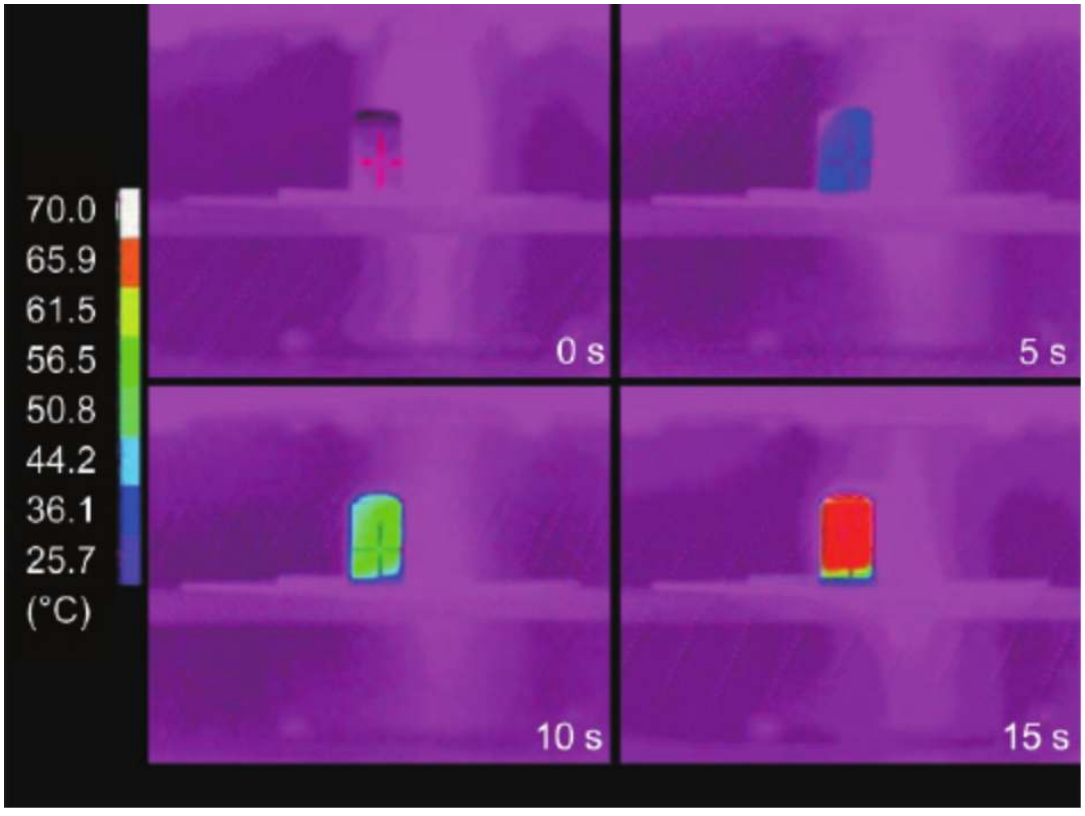


Figure 5. RF energy absorbed by 1.4 ppm of gold nanoparticles exhibited a fi nal Δ T ~35 °C in 2 min, whereas water alone exhibited a Δ T ~5 °C

The problem of heating at lower radio frequencies (RF) has not been well elucidated to this point, and remains somewhat controversial. Curley et. al., 2008 [88] show heating at 13.56MHz up to 60 degrees, but a number of papers have been published disputing these results.

6.2 Photothermal therapy: A popular area in nanomedicine is the implementation of plasmonic AuNPs for cancer diagnosis and photothermal therapy, due to their intriguing optical properties. Surface plasmon resonance, a unique phenomenon of plasmonic (noble metal) nanoparticles, produces a strong electromagnetic field on the particle surface and consequently enhances all the radiative properties such as absorption and scattering. The heating efficiencies and spectral working ranges vary with the type of NP. There have been important results obtained in both *in vivo* and *in vitro* NP assisted photothermal treatments. The results of photothermal radiation of two different liver cell lines exposed to two different concentrations of albumin (Alb)-coated AuNPs. In one case, the Hep G2 cell line exposed to the higher AuNP concentration showed severe changes to intracellular structures; Hep B5 cells were essentially unaffected. (Mocan et. al., 2015)[89]

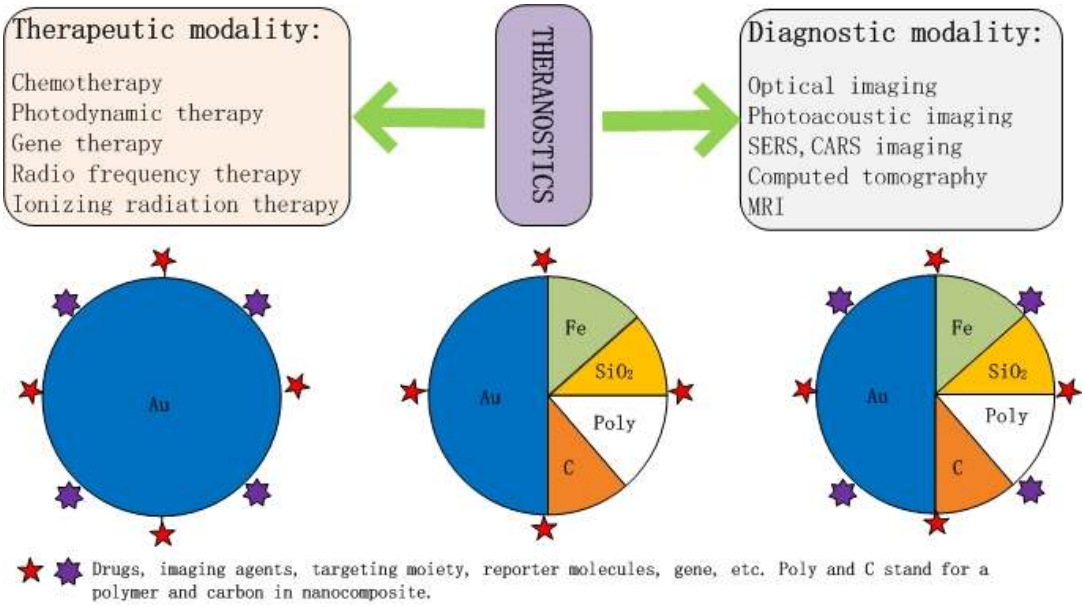


Figure 6. Nanoparticles with different compositions have been utilized in theranostics which refers to therapy and diagnostics.

7 Summary: The table below demonstrates the wide range of NP applications. They have considerable potential to impact many areas of health and will require many new methods to be developed to measure their effects on body functions.. Nonetheless, there are still many challenges to overcome not least the optimal targeting of cells associated with specific disease conditions. Molecular nanotechnology is a rapidly emerging field that will allow for the precise and purposeful arrangement of matter atom by atom and eventually the building of submicron-scale medical sensors and therapeutic devices. These tools could provide the means to analyze, understand, and precisely control the molecular machinery of the human body, allowing the detection and correction of any undesired structural changes (disease or aging) at the finest level of detail and the earliest possible time. Possible rejuvenation followed by the indefinite maintenance of an optimal physiologic state, or molecular homeostasis, may ultimately become possible (Wu et. al., 2016)[90].

Although this review divides diagnostics and therapy combine both sometimes termed Theranostics (A combination of diagnostics and therapy) as illustrated by Figure 6. Theranostics, refers to the development of molecular diagnostic tests and targeted therapeutics in an interdependent, collaborative manner with the goals of individualizing treatment by targeting therapy to an individual's' specific disease subtype and genetic profile. This strategy could enable optimization of drug efficacy and safety could have considerable benefit in the future. A key challenge is measurement at this scale to assess the long-term effects on the human body. New technologies are required to measure cell physiology in vitro and toxicity. Examples of some of the key papers are presented in table 1.

Table 1: Example of key papers on AuNP

Subject	References						
Imaging	Shilo et. al.,	Mohs et al.	Liu et. al,.	Pérez-Campaña, et. al.,	Jawaid, P., et al.	Xing, et al.,	Hainfeld et al., Chanda et al., Mottiei et al.,

							Dou et al.,
Point-of-Care	Li et. al.,	Yu et. al.,	Zheng et. al.,				
Cancer	Amed, M., et al	Mottiei et al.,	Haynes et. al.,	Poon et al.,	Lai et al.,	Cheng et al.,	Simon et al., Irani et al., Popovtzer et al., Song et al. , Fitzgerald et al., Yook et al.,
Delivery	Taghdis, et. al.,	Johnstone et. al.,	Ma et. al.,	Moeendarbari et al.,			
Wound healing and burns	Naraginti et. al.,	Ju et al.,	Volkova et al.,	Pittet et al.,			
Cardiovascular disease	Khobchandani et. al.,	Xing, et al.	Jiang et. al				
Neuroimmunology	Jain et. al.,	Shilo et. al.,	Morris et al.,				
Antibiotics	Durán et. al.,	Kalita et. al.,	Kalita et al.,				
Uptake	Huang et. al.,	Yang et. al.,	Volsi et. al.,				
Tissue engineering	Moravej et. al.,	Sridhar et al.,	Feng et al.,	Bokara et al.,	Ruttledge et al.,		
Toxicity	Fang et. al.,	Han et. al.,	Ze et. al.,	Song et. al.,	Xu et. al.,	Li et. al.,	Perreault et al., Gromnic

							ova R, et al., Syed et al., Negahdary et al., Vandebriel et al., Naz et al.,
Heating	Irani et. al.,	Curley et. al.,	Abaderr et. al.,	Dou et. al.,	Moran et al.,	Urban et al.,	Gomaa et al.,

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