



## Review

# Nanomaterials for Photo-Based Diagnostic and Therapeutic Applications

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## Abstract

Photo-based diagnosis and treatment methods are gaining prominence due to increased spatial imaging resolution, minimally invasive modalities involved as well as localized treatment. Recently, nanoparticles (NPs) have been developed and used in photo-based therapeutic applications. While some nanomaterials have inherent photo-based imaging capabilities, others including polymeric NPs act as nanocarriers to deliver various fluorescent dyes or photosensitizers for photoimaging and therapeutic applications. These applications can vary from Magnetic Resonance Imaging (MRI) and optical imaging to photothermal therapy (PTT) and chemotherapy. Materials commonly used for development of photo-based NPs ranges from metal-based (gold, silver and silica) to polymer-based (chitosan, dextran, poly ethylene glycol (PEG) and poly lactic-co-glycolic acid (PLGA)). Recent research has paved the way for multi-modal 'theranostic' (a combination of therapy and diagnosis) nano-carriers capable of active targeting using cell-specific ligands and carrying multiple therapeutic and imaging agents for accurate diagnosis and controlled drug delivery. This review summarizes the different materials used today to synthesize photo-based NPs, their diagnostic and therapeutic applications as well as the current challenges faced in bringing these novel nano-carriers into clinical practices.

Key words: nanoparticles, theranostics, photothermal, photodynamic.

## Introduction

Although treatment of various diseases using light has been in existence since ancient times, significant research on photo-based surgery and other medical applications began in the late twentieth century [1]. Specifically, pioneers such as Finsen, Raab and Von Tappeiner have played a major role in combining chemotherapy and light for drug administration, resulting in the use of photo-based chemotherapy for tumor treatment in the early 1900s [2]. Today, photodetection and photodynamic therapy are in use worldwide to diagnose and treat various diseases ranging from cancer and inflammation to skin

wounds.

With the increasing incidence of fatal diseases like cancer, there is a growing need for greater efficacy of therapeutic agents and precise methods of diagnosis. As a result, researchers are increasingly turning to nanomedicine for disease imaging as well as controlled and targeted drug delivery. The term 'nanomedicine' implies the use of nanotechnology in medical applications to provide a comparatively accurate diagnosis and treatment for diseases previously considered incurable. This recent surge in nanomedical research has resulted in photo-based na-

nomedicine gaining prominence in the treatment of cancer and other diseases due to spatially and temporally controllable drug release, localized therapy and minimally invasive nature of treatment [3].

Several nanomaterials have been developed and investigated for photo-based nanomedicine. Of all the nanomaterials used in photo-based applications today, nanoparticles (NPs) made of noble metals such as gold and silver are prominent due to their highly desirable and tunable optical properties described in detail in later sections of this review paper. These properties make them invaluable for various biological sensing, imaging and therapeutic applications. Quantum dots (QDs) are another category of metal-based NPs that can be used for hyperthermia in the presence of light [4]. Further, biocompatible polymer-based NPs can be used for photo-based therapeutic and diagnostic applications. As most of these materials might not have imaging capabilities, they can encapsulate the aforementioned metals as well as fluorescent dyes for imaging and/or therapy [3]. The dyes that can be incorporated in these polymeric NPs include the Food Drug and Administration (FDA)-approved Indocyanine green (ICG) [5], Near Infrared (NIR) region fluorescent Cyanine7 (Cy7) [6], and dialkylcarbocyanine fluorophores (DiI, DiR) [7].

The need for efficient and biocompatible treatment methods that can simultaneously deliver imaging and therapeutic agents for combined diagnosis and therapy have resulted in the development of theranostic NPs in recent times. These multi-functional NPs can be used for disease diagnosis and imaging applications via contrast agents with modalities such as Optical Coherence Tomography (OCT), Magnetic Resonance Imaging (MRI) and fluorescence imaging. Further, therapy can be carried out using light-activated hyperthermia and by modulating the drug release rates based on the application and the polymer chosen [3]. The following sections briefly describe the different materials used in the preparation of these NPs, their various photo-based diagnostic and therapeutic applications as well as the hurdles to be overcome to translate them into clinical trials.

### Commonly used nanomaterials for photo-triggered medical applications

The fabrication of different biomaterials and technologies over the last several decades has opened the door for new possibilities of therapy and diagnosis for various diseases. In the field of biomedical research, interest in 'smart' materials that can be controlled using external stimuli such as pH, temperature, and light remain high. Among these external

stimuli, light-based stimulus is rapidly gaining popularity in the development of new biomaterials and drug delivery systems. The attractive qualities of light-responsive materials include the use of near-infrared range of visible light, the high spatial and focus control, minimally invasive modalities used, and the wide range of materials that can be utilized and tested. Further, light-based treatment and diagnostics are more advantageous than existing stimuli-responsive modalities due to its non-contact approach for treatment, the greater suitability of NIR light for biomedical applications, and accurate control of the release rate of encapsulated agents via external light source [8]. The light responsiveness of the materials is due to their specific light absorption properties. Due to this, most of the light-responsive materials come from plasmonic metal compounds that have high optical abilities in the near-infrared region where tissue penetration is possible [9].

**Gold-based NPs.** The chief material that has been used for photothermal therapy (PTT) is gold and/or gold based materials. Gold has very unique optical and physical properties, which come from the surface plasmon resonance (SPR) often observed within noble metal NPs due to their high surface area to volume ratio [10]. Other factors that make gold an ideal material are ease of synthesis, surface and structure modification as well as heat conversion using NIR light [11]. Further, gold nanocarriers are known to be cytocompatible and are also stable as they do not undergo photobleaching or blinking, unlike QDs and other fluorophores [12]. Due to these advantages, a wide range of gold materials, including gold nanorods (GNRs), nanocages, nanospheres, hollow gold nanoshells, and gold-sulfide NPs, have been developed and tested towards photo-triggered treatments [13, 14].

Of the gold-based materials, GNRs have two absorption bands from the SPR oscillations going in both directions along the rod structure, due to their specific shape. This leads to higher absorption and scattering efficiency, thus making GNRs excellent contrasting agents. By tuning the structure of the gold nano-systems, optical properties are also being tuned for better effects. Despite their many advantages, the major concern for the use of GNRs in a medical setting is the possibility of cell cytotoxicity due to the use of cetyltrimethylammonium bromide (CTAB) as a surfactant during their synthesis [15]. In the research study done by Kuo et al. [16], GNRs were coupled with other polymers and photosensitizers to create biocompatible gold-poly(styrene-*alt*-maleic acid) - indocyanine green (gold-(PSMA)-ICG) nanorods that served as a platform for photodynamic therapy and

hyperthermia.

Besides GNRs, gold nanocages also demonstrate many attractive properties for photo-based imaging and therapeutic strategies. The hollow and porous gold nanocages are bio-inert and provide increased spatial and temporal resolution for optical imaging while overcoming the drawbacks of ultrasonic imaging [17]. Yavuz et al. [18] encapsulated these nanocages within a smart polymer poly-N-isopropylacrylamide (PNIPAAm) for temperature-dependent controlled drug release. The incident NIR light was absorbed by the nanocages and converted to heat by the photothermal effect, which resulted in collapse of polymer chains and subsequent release of encapsulated doxorubicin (DOX). *In vitro* studies showed that increasing the time of NIR irradiation resulted in increased cancer cell death due to greater DOX release. Also, a recent study by Chen et al. [10] demonstrated that gold nanocages can be effective light transducers for cancer treatment by showing accumulation of gold nanocages in tumors and causing irreversible damage to tumor cells. Further, thiolated PEG nanocarriers encapsulating gold and Raman reporter molecules can be synthesized for tumor targeting and surface-enhanced Raman scattering. These particles showed greater fluorescence than QDs on exposure to NIR laser and selectively accumulated within tumors *in vivo* when conjugated with single-chain variable fragment (ScFv) antibody specific to epidermal growth factor receptors overexpressed on tumor cells [19]. The first *in vitro* experimental reports on the use of gold nanoparticles for photothermal theranostics was published by Lapotko et al. [20, 21] using gold nanoparticles for both specific targeting of leukemia cells as well as nano-thermolysis of the cells by generation of microbubbles around the nanoparticle clusters on laser irradiation. This concept was later implemented by the same group for use in 'single cell theranostics', where the gold nanoparticle conjugated with anti-epidermal growth factor receptor (EGFR) antibody C225 could actively target EGFR-positive A549 lung carcinoma cells. Following cellular uptake, the gold nanoparticles could be used for providing diagnostic information as well as for generating transient plasmonic nanobubbles (PNB) during laser irradiation, to cause mechanical damage to cell membranes resulting in cell death [22]. Further, the *in vivo* performance of these gold nanoparticles was validated in zebra fish embryos implanted with human prostate cancer cells. These cells were previously labeled with C225-conjugated gold nanoparticles. It was observed that individual cells could be detected and ablated by generation of PNBs by gold nanoparticles when ex-

posed to laser pulses [23]. Recently, the same group has used this concept for both localized delivery of molecular cargo as well as mechanical destruction of cells by generation of a transient PNB around the gold nanoparticles with a single incident laser pulse. Small PNBs can create a transient hole on the cell membrane to 'inject' molecular cargo without damage to the cells. Large PNBs on the other hand can cause mechanical destruction of the cells of interest [24]. Various *in vitro* studies on gold nanocarriers imply that the choice of materials depends on the purpose of the diagnosis and treatment. For example, large surface structures are preferred for imaging applications due to their high light scattering efficiency while smaller structures are used for photothermal effect since most of the light will be locally converted to heat for the destruction of diseased tissues [25].

Despite their multiple benefits, gold-based nanocarriers are limited by their weaker optical signals emitted compared to QDs and other fluorophores. This can be rectified by the use of NIR-absorbing gold nanocarriers, which are capable of enhancing image contrast by absorbing from a different wavelength than that of tissue chromophores [26].

**Carbon nanotubes.** The choice of materials for photo-based therapy and diagnosis also extends to several novel carbon-based nanotubes incorporating inorganic materials, which have shown promise as theranostic agents [27]. The most well-known example of this class is the single-walled nanotubes (SWNTs) that have strong absorption in the NIR range. These carbon nanostructures can be further functionalized for cell selective uptake making them an ideal material for theranostic purposes [13]. In a recent study, it was shown that SWNTs can also be covalently oxygen-doped to provide clearer images due to their stronger absorption of the NIR emission wavelength [28]. Multi-walled carbon nanotubes and metal-filled carbon nanotubes have also shown some better unique physical, chemical and optical properties than SWNTs [29, 30]. A study done by Fisher et al. [29] reveals many positive aspects of multi-walled carbon nanotubes for future cancer therapy applications. In their study, multi-walled carbon nanotubes showed a decrease in viability of human prostate cancer cell line (PC3) and a decline in the heat shock protein (HSP) expression of tumors. These results indicate that these multi-walled carbon nanotubes have properties of becoming an effective drug carrier system. However, despite these positive results, more studies must be done to ensure biocompatibility of this carbon nanomaterial. In addition to SWNTs, metal-filled carbon nanotubes can also serve as a new

hybrid class of carbon-based nanostructures. Due to carbon nanotubes' specific physical and chemical properties, they make ideal templates for metal attachments. The interesting aspects of these metal-filled carbon nanotubes are the unique optical properties they possess as dark field light scattering agents for imaging applications [31].

**Inorganic photosensitizers.** Some examples of inorganic materials used for photo-based applications are titanium oxide nanotubes, calcium phosphosilicate, nanoscintillators, copper selenide NPs and silica-cored gold NPs [32, 33]. Titanium oxide is a highly functional material, and its nanotubes are biocompatible and noted for their drug delivery applications [13]. Recently, Hong et al. [34] have shown the potential of using these titanium oxide nanotubes in PTT to treat various types of cancers such as skin, breast, and colon cancers. In the study, TiO<sub>2</sub> nanotubes were used as a thermal coupling agent with a high photothermal effect to ensure the localized permanent destruction of tumor cells without damaging the normal cells. An important finding from this study was that the rate of *in vitro* cell death was highly dependent on the concentration of TiO<sub>2</sub> nanotubes used. Furthermore, the *in vivo* animal study revealed that the efficiency of treatment using TiO<sub>2</sub> nanotubes and NIR laser can be regulated by modulating the laser intensity, laser exposure, and the amount of TiO<sub>2</sub> nanotubes in the suspension [34].

**Polymeric nanocarriers for photosensitizer/dye encapsulation.** Several biomaterials and their complexes are being researched for encapsulation of light-responsive agents for photo-triggered treatment and imaging [35]. These materials are advantageous as they help to overcome issues with existing photosensitizers such as toxicity, rapid clearance from the body, and low water solubility. These materials are also used to facilitate controlled delivery of encapsulated drugs/dyes for both therapy and diagnosis [36, 37]. Further, limitations of metal-based NPs such as QDs and gold NPs in terms of cellular toxicity [38] and lack of specificity can be corrected by using biocompatible polymer coatings and targeting ligands for targeted and controlled delivery. Liposomes, dendrimers, micelles, conductive materials, polymeric NPs and/or capsules are generally used under this category [13]. An example is the study by Montanari et al. [39], who synthesized ultra-deformable liposomes containing zinc phthalocyanine as a photosensitizer for treating early stages of infections by *Leishmania braziliensis*. *In vitro* studies demonstrated that the leishmanicidal effect is a result of immune reactions rather than the selective effect by photodynamic therapy. Due to these results, more studies must be

performed in order to confirm the therapeutic effects of photodynamic and non-photodynamic properties of ultradeformable liposomes. Further porphyrins can be prepared from porphyrin-lipid conjugates which have intrinsic fluorescence properties for photoacoustic and fluorescence imaging [40]. Within 2 days of intravenous administration of these porphyrins into mice bearing KB tumor xenografts, strong fluorescence was observed due to their accumulation at the tumor site. Intradermal injections also resulted in strong photoacoustic signals from the porphyrins for visualization of the lymphatic system.

Dendrimers are another class of polymeric carriers being used due to their high biocompatibility, small size, ease of synthesis, and simplicity of surface functionalization [41]. A recent paper by Shen et al. [42] showed the preparation of highly photosensitive degradable polymeric nanocapsules from 4, 4'-Azobenzene dibenzoyl chloride (ADC) and B<sub>3</sub>-type monomer triethanolamine (TEA)-based dendrimer grafted nano-silica templates that produced a hollow structure. Photodynamic therapy using dendrimers is possible by adding photosensitizers to the surface of these dendrimer structures. Despite their great potential as drug delivery agents, some toxicity issues remain. Dendrimers with positively charged surface groups can cause cell death, and the toxicity of these structures is highly dependent on their generation period [41].

Besides dendrimers, Moleavin et al. [43], created amphiphilic macromolecular micelles through the bonding of micelles to amphiphilic polysiloxane or poly(chloromethyl) styrene-based azopolymers. By being amphiphilic, these micelles structures' side effects are decreased substantially. There is also the ease of screening drug and long-term stability of the structures inside the patients that allow for both targeted delivery and controlled release of the drug. Further, Zhou et al. [44] synthesized micellar nanocarriers containing pH-sensitive tetramethyl rhodamine dye which gives increased fluorescence emission within 5 minutes of pH activation, due to increased fluorophore release. Interestingly, PEG-based electron-rich micelles containing the photosensitizer 5,10,15,20-tetrakis(*meso*-hydroxyphenyl) porphyrin (mTHPP) synthesized by Ding et al. [45] can generate increasing amounts of O<sub>2</sub><sup>-</sup> by the energy transfer process, thereby competing with <sup>1</sup>O<sub>2</sub> production under hypoxic conditions. This results in increased photoactivation, resulting in greater phototoxicity when exposed to hypoxic cancer cells.

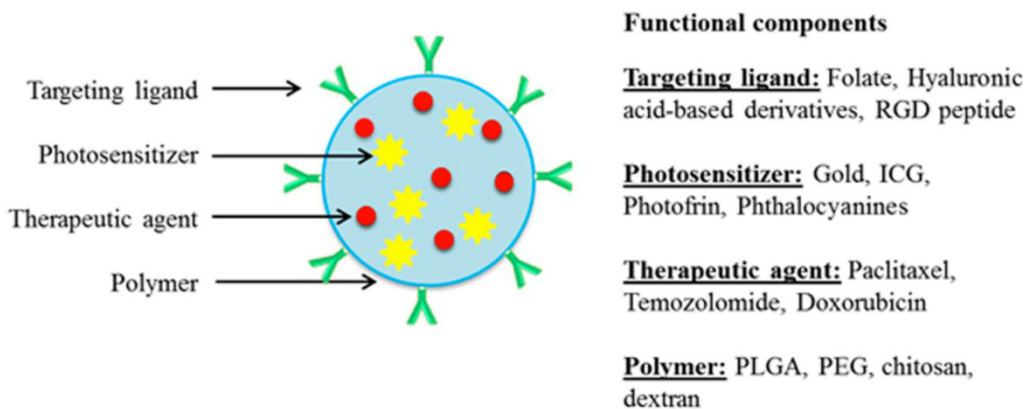
Many other polymer materials such as polyazobenzene, PEG, dextran, and poly(lactic-co-glycolic acid) (PLGA) are also being investigated to synthesize

nanocarriers encapsulating agents that react to the external light stimulus [46, 47]. Among these polymer materials, polyazobenzene has unique physical and chemical light-induced interconversion properties that make them ideal photo-triggered switches for drug delivery systems. An example of this is shown in the study done by Umeyama et al. [48] involving polyazobenzene polymers used as dispersants for the single-walled carbon nanotubes. These nanotubes were highly tunable through intermolecular interactions within the polymer, thereby demonstrating the possibility of carbon nanotube-based polymeric drug delivery systems for therapeutic purposes.

Among commonly used polymers for light-stimulated drug delivery, PEG is frequently implemented for surface modification and formations of complex nanocarrier systems. Controlled surface modification using PEG can overcome issues in terms of stability, water solubility and cell targeting seen in gold NPs [49]. Further, studies have shown that PEGylation of QDs helped in improved biodistribution following administration, thereby preventing rapid clearance of these particles from the system [50]. Umeda *et al.* [51] made use of PEG in the formation of a photothermal system. By developing a PEG attached PAMAM dendrimers encapsulating gold NPs, the photothermal properties were greatly enhanced compared to the previously used gold NPs. Furthermore, the results indicate that these structures can be useful devices for specific cell targeting while maintaining effects of PTT. Another material of interest is *N*-(2-hydroxypropyl)methacrylamide (HPMA) which can be used by itself or as a copolymer for encapsulation or conjugation of therapeutic and/or imaging probes for imaging and drug delivery [52]. HPMA has also been used by Ren et al. [53] in the synthesis of copolymer conjugates labeled with imaging agents such as IR dye 800CW to target and treat parti-

cle-induced inflammation, suggesting aseptic implant loosening. In this study, male Swiss Webster mice models implanted with ultra-high molecular weight polyethylene particles (UHMWPE) were used to generate inflammation *in vivo*. The HPMA copolymers were successfully uptaken by cells at the inflammation site following administration and were observed using NIR optical imaging.

The applications of polymeric materials can vary from imaging to cell targeting. In the recent study by Bogart et al. [54], dextran polymer-coated iron oxide NPs was used for photothermal microscopy. Their results suggest increased sensitivity and resolution in imaging, indicating promising imaging abilities for live samples. PLGA polymer has also been highly utilized in different nanocarrier systems. Recently, Cheng et al. [55] prepared Fe<sub>3</sub>O<sub>4</sub> and QD-conjugated taxol-loaded PLGA NPs onto which poly(styrenesulfonate)-coated GNRs were attached. These multi-functional NPs could be used for chemotherapy and NIR-based photothermal ablation of cancer cells. The results of their *in vitro* and *in vivo* studies indicate that the combination of chemotherapy and photothermal therapies via these NPs provide a more effective treatment against cancer compared to either of these treatments alone. These studies demonstrate the potential of use of different photo-triggered materials in combinational therapy approaches in future cancer treatment. The current research is moving towards the synthesis of multi-functional NPs that can be used for simultaneous imaging and therapy represented in **Figure 1**. Many new types of polymers and systems synthesized could also be used for multiple drug release and photothermal treatments as well as multiple imaging strategies [32, 35].



**Figure 1.** Multi-functional theranostic polymeric NPs for simultaneous targeting, photo-imaging and -drug delivery/therapy.

**Table 1.** Nanomaterials for photo-based diagnostic applications.

CORE	SHELL	MODALITY	APPLICATION	Ref.
Gold/Silver	Silica	Optical Sensing	Labeling cancer cells	[106]
Silica	Gold	Fluorescent Bioimaging	Fluorescence marking of cells/tissues for imaging	[106]
Magnetic NPs	Silica	Dual-imaging	Targeting, Cell sorting, Bioimaging	[107]
Gold/CdSe	PEG	Nanoscope sensing	Bioimaging	[108]
SPIOs [17]	Amphiphilic Polymer	MRI	Cancer Imaging	[109]
Nanocrystals [18]	PLGA	Medical Imaging	In vivo Cancer Imaging	[110]
QDs/Gold NPs [18]	PLGA	CT/Optical Imaging	In vivo Cancer Imaging	[110]

## Photo-triggered diagnosis using nanocarriers

Photo-based diagnostic modalities are used extensively for several applications including *in vivo* imaging, targeted detection of tumors, and various diagnostic and therapeutic purposes. Commonly used photo-based techniques include molecular imaging, optical imaging, fluorescence imaging, OCT, multimodal imaging, positron emission tomography and so on. Molecular imaging is a rapidly developing field wherein optics has played a major role. The spectroscopic nature and high resolution imaging capabilities of light provides a means for characterizing biological morphology and function at molecular and cellular levels [56]. To identify and characterize various fundamental processes at the organ, tissue, cellular and molecular levels, several different NP designs using light-sensitive novel imaging agents have been developed. The modalities used to track these NPs *in vivo* have been summarized in **Table 1**. Optical imaging comprises of detection of light photons that travel through the tissues [57]. After the drug-loaded particle is administered inside the body, optical imaging is a tool that follows or tracks the delivery vehicle to determine whether the drug is effectively delivered to the desired tissue or the organ [58]. Nanomaterials play an important role in optical imaging. Metal-based NPs such as QDs, gold and silica nanoparticles and photosensitizer-containing nanocarriers including liposomes as well as polymeric and ceramic NPs can all be used in optical imaging.

Among the different imaging techniques available, OCT is used to obtain high resolution (around 10-15  $\mu\text{m}$ ) cross sectional images in real time for biological tissues. OCT works on the principle of detecting reflections of a low coherence light source which is targeted into the tissue to determine at what depth the reflection has reached [59]. OCT is thus an optimal technique for examining complex structures such as tissue scaffolds to dimensionally characterize and optimize cell growth [60]. Gold and iron oxide NPs have been frequently used as OCT contrast agents [61]. Besides metal-based NPs, Au et al. [62] was the

first to report a polymer-based contrast agent for OCT using polypyrrole (PPy) NPs that gave a strong contrast at around 1300 nm in an intralipid tissue phantom using OCT.

Besides OCT, quantitative spectroscopy is another light-based technique in use. For example, tracking of NPs delivered to living cancer cells can be done with the aid of fluorescence microspectroscopy. A spectral shift in the wavelength suggests the delivery of NPs in the cancer cells. The cellular uptake and aggregation of the particles can also be determined by spectroscopy. Fluorescent conjugated/encapsulated polymer NPs are often used for this technology. For example, PLGA polymeric NPs are used to encapsulate various fluorescent dyes for cellular imaging [63]. Another example includes fluorescent polydiethylfluorenebenzothiadiazole-bisthiophenyl benzothiadiazole copolymer (PFBTTB) NPs, which were synthesized and conjugated with Herceptin to make a probe suitable for targeting and imaging of HER2-overexpressing cancer cells. This type of system is also used for targeted therapy and imaging of cancer [64].

Imaging of cells can be further done by photoacoustic imaging, which is a noninvasive, nonionizing imaging modality that is a combination of high sensitivity of optical methods and outstanding resolution of the acoustic methodology. Photoacoustic imaging is done by two main techniques: photoacoustic microscopy and photoacoustic computed tomography. Photoacoustic imaging has been used to image blood vessels, tumors, hemoglobin oxygenation, and tumor angiogenesis. For photoacoustic imaging, gold nanoshells with a silica core and GNRs are frequently used. *In vivo* imaging of rat brain cortical blood vessels showed a significant increase in blood vessel absorption with gold nanoshells, which denotes its successful application as an NIR (Near Infrared) photoacoustic contrast agent [65]. Gold nanomaterials are commonly used for photoacoustic computed tomography as well. They have gained attention because of their plasmonic properties that are size and shape dependent and can absorb light in the visible to NIR region. This quality of bioengineered gold NPs also

makes them useful for image guided therapy and photothermal ablation of the tumors. Gold surfaces can also be conjugated with biologically and chemically relevant entities for multimodal capabilities like MRI, PET, CT, and conventional microscopic optical techniques.

Multimodal Imaging is a cutting edge research which has been developed and applied because of the issues faced by biomedical technology today, including heterogeneity of the disease and patients. Personalized therapies might be more effective as the progress of disease is different for each patient, depending on genetic factors, environmental factors and the characteristic of the disease [66]. Thus multimodal imaging has several advantages over single imaging modalities, like high sensitivity, multicolor imaging, no tissue penetrating limits and so on. To overcome the limitations of the single imaging technique, researchers combined two different contrast agents into a single nanoparticle, which can be further imaged by different imaging modalities. For example, combining MRI for anatomical resolution and optical imaging for its sensitivity is a very effective procedure for finding and quantifying the size of the tumor or metastases that cannot be detected by MRI alone. This multimodal imaging is also used for monitoring enzyme activity and imaging brain tumors [67] as well as for detecting apoptosis and atherosclerosis [65]. Multimodal NPs containing up-conversion NPs as the core, layer of iron-oxide particles as the intermediate layer, and the outer-most layer of gold have been developed and used for upconversion luminescence (UCL)/magnetic resonance (MR) multimodal imaging and photothermal ablation of tumors [32]. Dual-modal fluorescent magnetic NPs by co-encapsulation of far-red/near-infrared

(FR/NIR)-emissive conjugated polymer (PFVBT) and lipid coated iron-oxides into folate conjugated PLGA-PEG and PLGA NPs are other examples for nanomaterials used in the dual imaging system, *in vivo* fluorescence and MRI, to detect tumors [68].

## Photo-triggered therapy using nanocarriers

In addition to imaging and diagnostic applications, nanomaterials have also been developed for several light-based therapeutic uses as summarized in **Table 2**. Photodynamic therapy (PDT) is an important approach that uses light-activated drugs for the treatment of various ailments such as cancer [69]. This method of therapy involves three essential components—light, photosensitizers and oxygen [69, 70]. The photosensitizers convert light energy into a type II chemical reaction. In clinical practice, photosensitizers are basically from three families of porphyrins, chlorophylls and dyes. Some of the photosensitizers currently in clinical trials have been summarized in **Table 3**. Among the different photosensitizers in use today, metallo-phthalocyanine has attracted substantial interest due to their photodynamic (PD) properties that can be easily tuned by the type of metal ion involved. For example, zinc-phthalocyanine nanowires synthesized by Moon et al. [71] showed excellent photosensitizing capabilities as well as highly efficient dual PD and photothermal (PT) effects upon irradiation of NIR laser. Studies performed by Jeong et al. [70] also demonstrated the use of photosensitizer-conjugated human serum albumin NPs as impressive photodynamic drug delivery candidates with good biocompatibility and tumor-targeting abilities.

**Table 2.** Nanomaterials for photo-based therapeutic applications.

Materials	Encapsulated agent	Modality	Applications	Therapy	Ref.
Gold NPs	Toluidine Blue O (TBO)	Absorption spectroscopy FTIR spectroscopy	Colon cancer treatment	PDT	[111]
Human serum Albumin (HAS)	Chlorin e6 (ce6)	In vivo fluorescence Imaging	Colon cancer treatment	PDT	[70]
CdTe & CdSe QD / silica shell	-	Fluorescence spectrophotometer	Skin cancer treatment	PTT	[4]
Gold-NPS	ICG	Femtosecond laser imaging system	Lung cancer treatment	PTT, PDT	[74]
Gold-nanorods/PEG	-	Inductively coupled plasma mass spectrophotometry (ICP-MS)	Cancer treatment	PTT	[77]
Pluronic NPs	Pthalocyanine dye	NIR fluorescence imaging	Liver cancer treatment	NIR therapy	[82]
Gold/Silica nanoshells	-	IR thermography	Cancer treatment	Laser -induced hyperthermia	[81]
Silica / Gold nanoshells	ICG	Absorption spectrometry, photoacoustic tomography (OAT/PAT)	Cancer surgery	Laser activate NP for tissue bonding	[112]
Gold NPS	PEG-Si RNA	fluorescence spectrophotometry	Cancer treatment	Photoinduced RNA interference therapy	[84]

**Table 3.** Photosensitizers currently in various stages of clinical trials for photo-based applications

No.	Photosensitizer	Application	Stage completed	Ref
1	Photofrin	PDT of various carcinomas	FDA-approved	[113], [114]
2	Levulan	PDT for actinic keratosis treatment	FDA-approved	[115]
3	Metvix	PDT for actinic keratosis treatment	FDA-approved	[116]
4	Visonac(methyl aminolevulinate)	PDT of moderate acne by killing bacteria and action on sebaceous gland	Phase IIb	[117]
5	Verteporfin	PDT of neovascular age-related macular degeneration	Phase IIIb	[118]
6	$\delta$ -aminolaevulinic acid	PDT for basal cell carcinoma	Phase III	
7	Zinc phthalocyanine tetrasulfonate	PDT for naturally occurring tumors in dogs	Phase I	[119]
8	Radachlorin	PDT of skin cancer	Phase II	[120]
9	HPPH (2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a)	PDT of obstructive esophageal tumors	Phase I/II	[121]
10	Silicon phthalocyanine	PDT of cutaneous neoplasms	Phase I	[122]
11	Hexaminolevulinate (Hexvix)	PDT of intermediate or high-risk urothelial cell	Phase I	[123]
12	BF-200 5-aminolaevulinic acid	PDT of actinic keratosis	Phase III	[124]
13	Hemoporphin	PDT for port-wine stain	Phase IIa	[125]

PTT is another type of photo-based physical treatment which has recently attracted a lot of interest as a minimally invasive treatment modality and an alternative to currently used cancer treatments. This therapy involves irradiating the tumor with electromagnetic radiation (via NIR), which is absorbed by photoabsorbers and is converted to heat, which in turn causes thermal damage. It is very important to choose the appropriate wavelength where the absorption of the diseased tissue is greater than the surrounding tissue. Various photothermal agents like GNRs, nanoshells, nanocages as well as carbon nanotubes have been developed for PTT treatment of cancer [72]. QDs have also been recently gaining prominence chiefly due to their size-dependent fluorescence, narrow emission spectra, stability against photobleaching and stronger fluorescence than commonly used dyes [73]. Chu et al. [4] worked on CdTe and CdSe QDs which converted light energy rapidly into heat when injected into mouse melanoma tumor. Results showed a remarkable decrease in the size of the tumor. In addition to QDs, light-absorbing dyes have also been used to increase the thermal damage in the target tumors. For example, nanostructures consisting of ICG and phospholipid-polyethylene glycol (ICG-PL-PEG) were developed for PTT [74, 75]. The results suggested that ICG-PL-PEG suspension showed a more efficient NIR-temperature dependent increase compared to ICG dyes alone. Moreover, they could be retained inside the tumor when conjugated with integrin  $\alpha(v)\beta(3)$  monoclonal antibody. We have previously encapsulated ICG in PLGA NPs conjugated with targeting ligands for prostate cancer-specific targeting, optical imaging and PTT [76].

Results showed that our NPs were biocompatible and uptaken significantly by PC3 prostate cancer cells. Additionally, these NPs gave significant contrast even at 3 cm depth below in the laboratory tissue phantom and showed significant temperature gains *in vitro* and in phantoms, demonstrating their potential for deep tissue imaging and cancer hyperthermia. Further, Lin et al. [77] developed GNRs which showed enhanced PTT when used on the soft tissue of genetically engineered mice, due to higher absorbance of these nanorods when compared to chromophores and NIR dyes. The absorption cross sections of GNRs are at least five orders larger than commonly used dyes. Further, the scattering of light by these nanorods is much larger than the light emission from highly fluorescent dyes [78].

Besides PDT and PTT, NIR also has various applications in applied biology. Due to the wavelength being longer than the red light, it can penetrate into deeper tissues easily. Moreover, it offers low scattering, energy absorption and inhibits most of the auto-fluorescence emitted from surrounding tissue [79]. Small diameter GNRs, gold-nanocages, gold-nanoshells have been recently gaining a lot of attention in NIR due to their stability and biocompatibility [80]. Recently, a novel near infrared photoimmunotherapy (PIT) using a new type of monoclonal antibody (mAb) has been used to monitor *in vivo* acute necrotic cancer cell death in real-time. This method proved to be advantageous in acutely monitoring the cytotoxicity effects of NIR mediated mAb induced PIT with the help of fluorescence time (FLT) imaging even before the morphological changes are seen in the targeted tumor [81]. Phthalocya-



nine-aggregated Pluronic (FPc) NPs were formulated as another novel type of NIR absorber for PTT by Lim et al. [82]. The FPc NPs were injected into SCC7 tumor-bearing mice and the NIR fluorescent images displayed a marked image contrast which was only seen at the tumor. Moreover, the tumor signal was further increased with the passage of time, while the whole-body signal decreased.

One of the present day tools using phototherapy to cure cancer is the laser, which can be used to induce hyperthermia [79]. A major drawback of this technique is the low spatial selectivity in heating of tumors and surrounding tissues. Terentyuk et al. [83] attempted to resolve this issue using plasmonic silica/gold nanoshells, which were used to produce controllable laser hyperthermia in tissues, hence increasing the photothermal effect in cancer cells. The combination uses of the laser and other minimally invasive techniques have resulted in their increasing popularity in the field of surgical medicine. This is because of their ability to reduce pain and promote faster recovery. One of the innovative approaches implemented today is the combination of NIR, laser and optical absorber for treatment. Indocyanine Green (ICG) dye has attracted attention recently for this combination approach, as it is FDA approved and has proven to be safe for surgical applications in humans [5]. Despite the vast research in this area, only a few designs have reached the clinical trials due to their failure at local heating of tissues.

In addition to drugs, genetic materials can also be incorporated in NPs for photo-based therapies. RNA interference (RNAi) has been majorly used to understand the gene functions. Photoinduced RNAi was developed in 2005, which was then modified with the help of photochemical internalization to RNAi [84]. An example is the development of a polymer using  $\beta$ -cyclodextrin containing polymers based on six methylene units, which entraps short interfering RNA (siRNA) and which could be controlled completely by light [84]. The human S100A4 protein in OHS cells was used as the target gene in this study. The results showed that silencing efficiency was almost 90% compared with the untreated control, and there was no effect on cell viability by the treatment. Moreover, gold NPs have been conjugated with siRNA which helps in achieving deep penetration of the tissues at NIR wavelengths [85]. Another novel design is proposed by Endoh et al. [85] for delivering of short hairpin (shRNA) by photo-stimulation of fluorescently labeled protein carriers.

### Photo-triggered theranostic nanocarriers

Theranostic nanomedicine is a branch of nano-

biotechnology that involves state-of-the-art NPs capable of carrying out simultaneous detection/diagnosis and treatment of the disease following administration. It is derived from a combination of the two words 'therapy' and 'diagnosis'. Theranostic NPs that utilize light-based techniques for monitoring and treating diseases are of interest as they make use of minimally invasive modalities that reduce patient discomfort (**Figure 2**). For example, Khlebtsov et al. [86] synthesized multifunctional NPs consisting of a gold-silver nanocage core surrounded by a silica shell containing NIR photosensitizer Yb-2,4-dimethoxyhematoporphyrin (Yb-HP) for monitoring tumor as well as simultaneous therapy by PDT and plasmonic heating. Significantly higher death of HeLa cervical cancer cells occurred *in vitro* when they were incubated with the NPs and irradiated with light, due to plasmonic PTT by the gold-silver nanocages as well as photodynamic therapy in the presence of Yb-HP. Further, the IR luminescence of Yb-HP can be used for diagnostic purposes within the tissue transparency window. Another example of theranostic NPs are the gold layered-carbon nanotubes prepared by Galanzha et al. [87] and conjugated with folate and CD44-specific antibodies to specifically detect and target circulating breast cancer cells *in vivo*. Non-plasmonic silica NPs containing multiple dyes have been formulated by Singh et al. [88] for theranostic applications. These particles consisted of NIR fluorescent heptamethine cyanine dye and Si-naphthalocyanine heating dyes for NIR fluorescence imaging and increased light absorption respectively. The latter was incorporated for enhanced PTT. By direct tumor injection into mice, the authors were able to observe high fluorescence intensity and about 95% decrease in tumor viability following irradiation with NIR laser source.

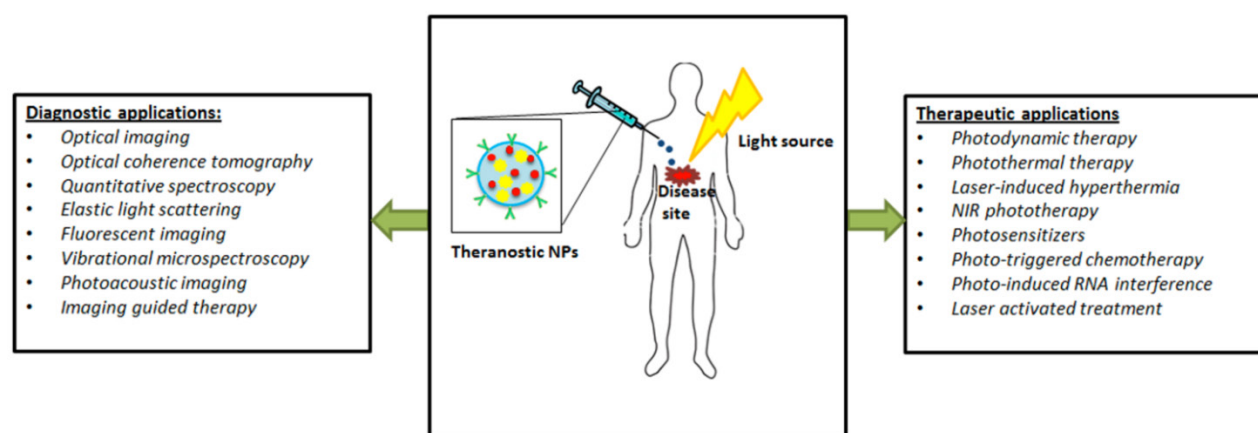
With advances in nanotechnology-based research, NPs with multifunctional capabilities are gaining importance due to the combined advantages of various imaging and therapeutic modalities encapsulated within a single nanoparticle [89]. For example, Santra et al. [7] formulated novel folic acid-conjugated, poly acrylic acid-coated iron oxide NPs that are encapsulated with NIR dyes, 4-Dil and 4-DiR, and with an anticancer drug, Taxol. These NPs could aid in cancer diagnosis using both optical and MR imaging and cancer treatment via targeted drug delivery. Another novel NP formulation for dual-imaging was prepared by Huan et al. [90]. This group synthesized multi-functional folic acid-conjugated and silica-modified gold nanorods (GNR-SiO<sub>2</sub>-FA) which can be simultaneously imaged *in vivo* using X ray or computed tomography (CT)

followed by treatment via radiation therapy (RT) or PTT. These multi-functional nanorods could specifically bind to folate receptors on the surface of MGC803 gastric cancer cells *in vitro* and were imaged *in vivo* using both X-ray and CT imaging.

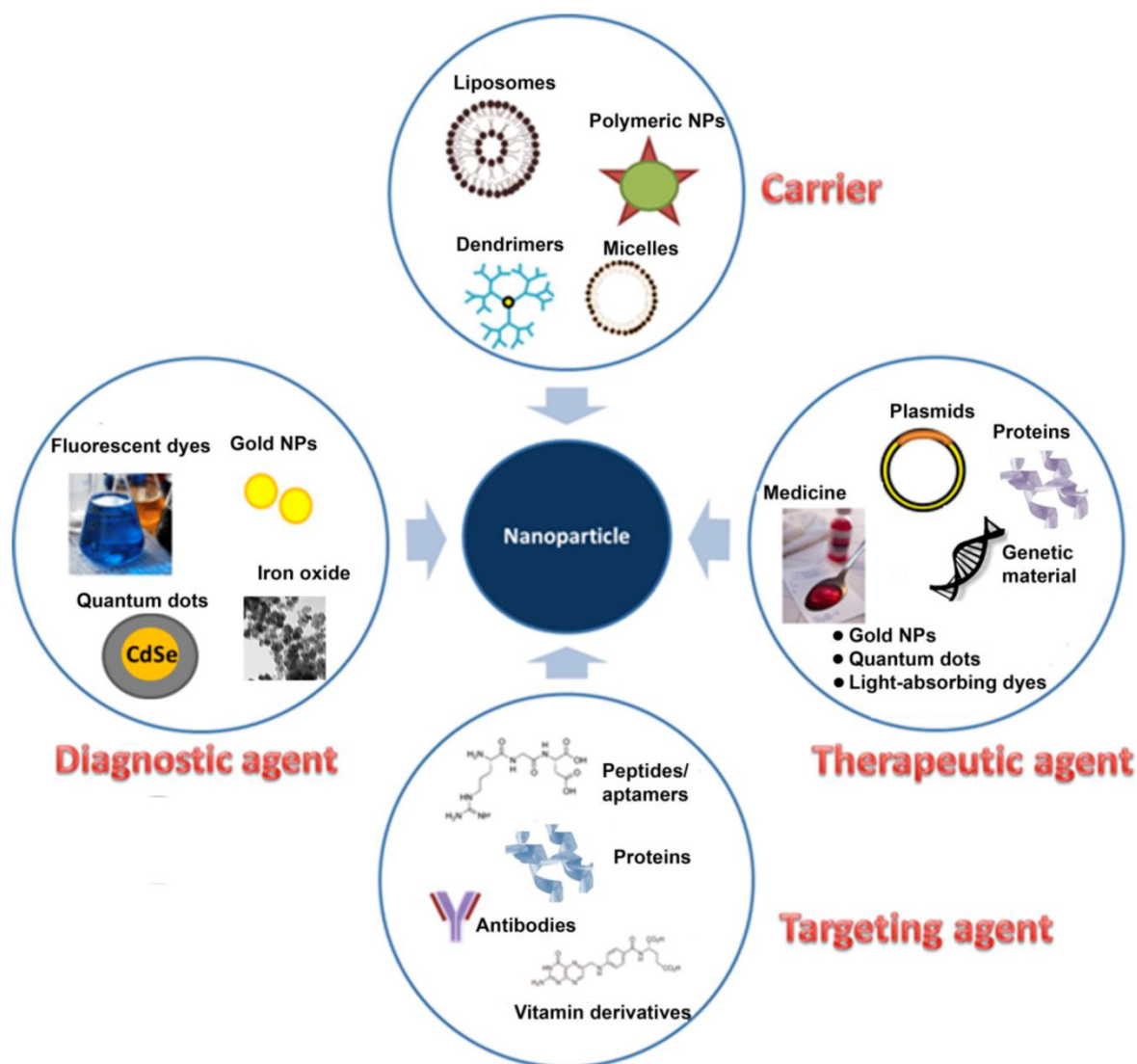
The interest in dual therapy-enabled theranostic NPs has also increased due to their ability to demonstrate a greater therapeutic effect than the individual treatment alone. This is clearly demonstrated by Wu et al. [91], who had synthesized novel core-shell nanogels comprising of a silver-gold bimetallic core and a thermoresponsive poly ethylene glycol (PEG) based shell containing hyaluronic acid-based targeting ligands. These nanogels can be simultaneously used for cancer-specific targeting, fluorescence imaging, optical temperature-sensing as well as both chemo- and photothermal-therapy. The enhanced intensity of photoluminescent emissions from silver-gold core, in response to temperature-dependent shrinking of the shell, can be used for optical temperature sensing as well as cellular imaging. The hyaluronic acid strands on the surface can specifically target cluster determinant 44 (CD44) overexpressed on various cancer cells. Moreover, irradiation of B16F10 skin cancer cells *in vitro* using NIR light following uptake of Temozolomide (TMZ) -loaded NPs showed a photothermal effect due to the bimetallic core. The subsequent temperature increase in the region further stimulated the release of chemotherapeutic TMZ from the thermosensitive shell. Also Lee et al. [92] developed novel PEG-PLGA-gold half-shell NPs (~120-125 nm) encapsulating DOX for chemo-photothermal therapy and imaging following NIR irradiation. Intravenous and intratumoral injection of NPs in nude mice resulted in their accumulation in the tumor region. Further, the combined chemo- and photothermal therapy on NIR irradiation resulted in tumor destruction without causing weight loss in the mice

and/or recurrence of the tumor. This method of cancer treatment was found to be more effective than chemotherapy or PTT alone.

Aside from chemotherapeutic drugs, NPs can also be encapsulated with a select radionuclide for nuclear imaging and/or radiation therapy of tumors. For instance, Rossin et al. [93] synthesized  $^{64}\text{Cu}$ -radiolabeled shell cross-linked (SCK) NPs conjugated with folate for imaging and radiotherapy of tumors. The micelles prepared using poly(acrylic acid-b-methyl-acrylate) diblock copolymer conjugated with folic acid and radiolabeled with  $^{64}\text{Cu}$  were retained in the tumor *in vivo* and could potentially be used for imaging and treatment of early-stage small-sized tumors. Another example is a novel QD-photosensitizer (Photofrin) conjugate synthesized by Yang et al. [94] for enhancing radiation therapy. *In vitro* studies on H460 human lung carcinoma cell lines showed significant cell death in the presence of the QD-photofrin conjugates in combination with radiation, than in the presence of only one of the components [94]. These conjugates are safer than radiosensitizers which tend to have toxicity issues following administration. Besides QDs, gold NPs can also be used in radiation therapy as demonstrated by Hainfield et al. [95]. The gold NPs exposed to high X-ray doses of 42 Gy radiation and 157 kiloelectron volt (keV) beam energy was successful in reducing tumor volume as well as long-term survival in radio-resistant mouse head and neck squamous cell carcinoma by combined radiation therapy and hyperthermia. Thus we can see that theranostic photo-triggered NPs have opened the doors to new and advanced research that can integrate diagnostic and therapeutic components into a single particle for effective detection and treatment of various diseases (Figure 3).



**Figure 2.** Diagnostic and therapeutic applications of photo-based theranostic NPs to treat various malignancies.



**Figure 3.** The various functional components of a theranostic photo-triggered nanoparticle for effective diagnosis and treatment of the disease.

## Medical applications of photo-based theranostic NPs

With the rising incidence of cancer among people in recent years, research is now extensively focused on the development of multifunctional NPs that can simultaneously be used for diagnosis as well as site-specific treatment of cancer. Photo-based theranostic NPs have been used for applications ranging from detection and treatment of circulating tumor cells to photo-based therapy of small-sized and late stage tumors [87]. For example, Bagalkot et al. [96], had developed novel aptamer-QD-DOX conjugates that can selectively target prostate-specific membrane antigen (PSMA)-overexpressing prostate cancer cells, be imaged by dual fluorescence reso-

nance energy transfer (Bi-FRET) due to interaction between DOX-QD and DOX-aptamer, and provide cancer therapy by release of DOX within the cells. Following endocytic uptake, doxorubicin is separated from the nanoconjugates resulting in separate fluorescence from the QDs. This novel formulation thus helps in detection of NPs at a cellular level. Gao et al. [97] had also developed semiconductor QDs coated with an amphiphilic triblock polymer and conjugated with PSMA monoclonal antibody for prostate cancer targeting and spectral imaging. *In vivo* uptake study showed accumulation of the ligand-conjugated QDs in the PSMA-overexpressing C4-2 prostate cancer xenografts in nude mice, which could be visualized by fluorescence imaging. Recently, Cheng et al. [98] synthesized tri-functionalized mesoporous silica NPs

incorporating cRGDyK peptides for  $\alpha_v\beta_3$  integrin-specific targeting, an NIR fluorescent contrast agent ATTO647N for imaging, a palladium-porphyrin based photosensitizer for PDT. The NPs were found to be cytocompatible and showed a significantly higher uptake by U87-MG human glioblastoma cells overexpressing  $\alpha_v\beta_3$  integrins, compared to the integrin receptor-deficient MCF-7 breast cancer cells. Similarly, Gianella et al. [6] developed multifunctional nanoemulsions encapsulating fluorescent dyes Cy7 and iron oxide nanocrystals for simultaneous NIR fluorescence and MRI for imaging-guided cancer therapy. The nanoemulsions further contained hydrophobic glucocorticoid prednisolone acetate valerate (PAV) as the therapeutic agent and RGD peptide for targeting tumor angiogenesis. The formulation was biodegradable, successfully imaged tumors *in vivo* and significantly reduced the tumor size.

Despite the increasing focus on a suitable nano-sized system specifically for diagnosis and treatment of several types of cancer, there has also been significant research in the development of theranostic NPs for treatment of other diseases. For example, McCarthy et al. [99] recently developed a multimodal crosslinked dextran-coated iron oxide (CLIO) nanoparticle for treatment of inflammatory atherosclerosis. The NIR fluorophore AlexaFluor 750 encapsulated within the CLIO can be used for NIR fluorescence imaging. Further, a chlorin-based photosensitizer used could be activated by light to display phototoxicity towards macrophages responsible for inflammation in the atherosclerotic lesions. Lim et al. [100] had also synthesized dextran-coated hollow-type gold NPs for imaging and treatment of macrophages. These NPs were highly uptaken by RAW274.7 macrophage cells and were capable of scattering NIR light for imaging followed by PTT. Further, the FDA has approved Visudyne as a light-activated liposome consisting of a photosensitizer (hydro-monobenzoporphyrin) and a phospholipid mixture (egg phosphatidyl glycerol (EPG) and dimyristoyl phosphatidyl choline (DMPC)) which can be used in the treatment of macular degeneration [3]. Another application of laser-activated NPs is tissue bonding as the heat generates by irradiation of the NPs via the appropriate laser resulting in reorganization of the components of the tissue, leading to wound closure and repair. For example, SPIO NPs (15 nm) have been used to produce heat and solder albumin onto rabbit aortic arteries *ex vivo* by applying radio-waves [101]. Also, small gold NPs are used as photo-thermal transducers to generate heat for non-invasive tissue bonding applications, ranging from skin lesions to explanted lens capsular tissues [102].

The applications of photo-based NPs extend to treatment of bacterial infections as well. For instance, gold NPs conjugated with anti-protein A antibodies were used to selectively target and kill Gram-positive *Staphylococcus Aureus*. Following uptake by the bacterium, the NPs were irradiated with focused laser pulses (420-520 nm) which were converted to heat resulting in irreparable damage of the bacterium [103]. Similarly, Huang et al. [104] developed multifunctional Fe<sub>3</sub>O<sub>4</sub>-gold nanoeggs with vancomycin immobilized on the surface, which are capable of absorbing NIR for PTT to treat bacterial infections. They further demonstrated that the use of an external magnet is useful in bacterial aggregation and more effective PTT.

## Conclusions and Future outlook

This paper has attempted to summarize the existing research in the field of photo-based nanomedicine. The continuous demand for effective clinical solutions to cure diseases with minimal patient discomfort has led many researchers to turn to the field of nanotechnology in order to develop systems that can be tracked/imaged following administration and used for targeted and localized drug delivery. Although extensive research is being conducted in this area, we still have a long way to go in terms of using these nano-systems in humans. Firstly, efforts must be taken to achieve mass-production of the NPs so as to translate the system from bench to bedside. Also, a comprehensive study of the biocompatibility of the nanoparticulate system and their interaction with cells and tissues needs to be done to ensure their safety before use in humans. Each component of these NPs would have to be tested extensively for immune reactions and toxicity *in vivo*. Theranostic NPs are slowly transitioning into clinical trials; however, these NPs can potentially overcome limitations of existing treatment techniques and provide a safe and reliable means of diagnosing and treating diseases in a minimally invasive manner with less patient discomfort and high accuracy. Photo-based theranostic NPs have thus emerged as a possible effective system that unites different aspects of chemistry, biology, bioinformatics, medical physics and various other fields to form a joint solution that can overcome various issues in human health today [105]. However, due to the nascent stage of this field of study, there are still several issues to be addressed before these photo-based theranostic particles can be translated to clinical use. Firstly, different modalities and nanoparticle components need to be tested to optimize the depth up to which NPs containing photosensitizers and metal-based imaging agents can be imaged. This would

be particularly necessary for deep tissue imaging of NPs following administration. Further, sensitive imaging techniques should be employed for accurate detection of signals from the NPs. The different photo-based therapies such as PDT and PTT using photo-based theranostic NPs should cause minimal damage to surrounding healthy tissue. Due to the toxic nature of some of the photosensitive payloads such as QDs, it is also imperative that the NPs be cleared from the system before complete degradation of the polymeric shell and subsequent release of encapsulated agents. Regardless, these nano-carriers have the potential to make significant strides towards diagnosis and treatment of different diseases due to their unique qualities such as good spatial resolution, controllable drug release and active targeting for efficient and target-specific delivery of the encapsulated agent.

## Competing Interests

The authors have declared that no competing interest exists.

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