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REVIEW ARTICLE

# Current Trends and Advances in Nanoplatforms-Based Imaging for Cancer Diagnosis

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## Abstract

The intersection of nanotechnology and biomedical imaging has ushered in a new era in the early detection and diagnosis of cancer which has revolutionized biomedical imaging by enhancing sensitivity, resolution, and targeting capability. This review presents a comprehensive overview of the latest developments and innovations in nanoplatforms-based imaging for cancer diagnosis, a burgeoning field that holds significant potential in improving cancer detection and treatment. Recently multimodal imaging techniques utilizing the unique properties of different types of nanoparticles are providing comprehensive diagnostic information. This multi-pronged approach allows for more precise tumor localization, size estimation, and growth rate calculation, offering a holistic view of the tumor and its environment. The primary focus of this review is on the recent progress in various types of nanoparticle-based imaging modalities, including optical, magnetic resonance, ultrasound, and nuclear imaging. Specific advancements in nanomaterial design for targeted imaging are highlighted, showing the improvement of precision targeting as an impact on the detection of cancer cells, even in early-stage tumors. A keen examination on the integration of diagnostic and therapeutic capabilities into single nano-based platforms for theranostics, underscoring their potential in personalized medicine is provided. The current challenges in the field, such as issues related to toxicity, biodistribution, and clearance of nanoparticles, and it explores ongoing research aimed at overcoming these hurdles. The growing body of research in this field highlights the promising future of nanoplatforms in improving the early detection and treatment of cancer.

**Keywords** Nanomaterials · Cancer diagnosis · Imaging modalities · Nanotechnology · Theranostics

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## Introduction

In recent years, the unique physical, biological, and chemical attributes of nanomaterials (NMs) have garnered significant attention from researchers, distinguishing them from their bulk counterparts [1]. These NMs are categorized based on attributes such as size, chemical composition, shape, and origin. Emerging from diverse sources, various types of NMs have been synthesized and classified as per these parameters [2]. Owing to industrial demands, their production has surged considerably. Broadly, NMs stem from two primary origins as engineered (synthetic) processes and natural occurrences. A nanomaterial is typically characterized by its external dimensions or its internal or surface structures being on the nanoscale [3]. By this criterion, many everyday materials could be considered nanomaterials due to their nanoscale internal modulations. A nanoparticle, on the other hand, is defined by having at least one external dimension on the nanoscale.

The term "nanoscale" encompasses sizes ranging from approximately 1 nm to 1000 nm [4]. This definition somewhat overlaps with microparticles, which start at around 1 micron. Although a lower limit of 1 nm is widely accepted, there's debate over the upper limit. Some authors cap it at 100 nm, but this restriction lacks empirical backing and could be overly restrictive for nanomaterial categorization [5].

In 2011, the European Commission offered a nuanced definition for "nanoparticle." They advocated considering the average particle size and its standard deviation. Nanoparticles typically consist of particle clusters, with sizes both above and below the 100 nm threshold. As per the European Commission's guidelines, for a material to qualify as a nanomaterial, it should contain 50% or more particles within the 1–100 nm range [3, 4]. The systematic categorization of nanomaterials (NMs) based on their chemical and physical properties are pivotal for grasping the vast diversity within this domain [2, 5]. Various methods such as ball milling, cutting, chipping, and extruding are employed to produce NMs. These techniques result in an array of structures, often with unique surface coatings, paving the way for their respective classifications. The distinguishing factors for categorization include dimension, shape, size, composition, and consistency [6]. Broadly, nanomaterials can be grouped based on their compositional characteristics and for a visual representation, refer to Fig. 1, which depicts the various classes of nanomaterials.

## Nanomaterials categorization

Nanomaterials (NMs) can be broadly segmented into four types, independent of their positional or chemical attributes.

(a) Categorization by Dimensional Properties

In 2007, a unique method for NM classification was introduced by Pokropivny and Skorokhod, focusing on their dimensional characteristics [8].

1. *Zero-Dimensional (0D) NMs*: With all dimensions confined to the nanoscale (typically up to 100 nm), this group encompasses entities like quantum dots (from carbon, graphene, and other inorganic materials) and spherical NMs (from noble metals, polymers, and more). Their unique properties, like optical stability and wavelength-specific photoluminescence, make them attractive for sectors like biomedicine and optoelectronics [9].
2. *One-Dimensional (1D) NMs*: Here, only one dimension exceeds the 100 nm threshold. Examples are nanotubes, nanorods, and nanowires made from diverse materials like polymers, metals, and carbon. Some other 1D NMs are veils and nonwovens, produced from polymer nanofibers. Their high surface area and minuscule pore size make them ideal for uses ranging from filtration to tissue engineering [8].
3. *Two-Dimensional (2D) NMs*: These have two dimensions beyond the 100 nm mark. They encompass

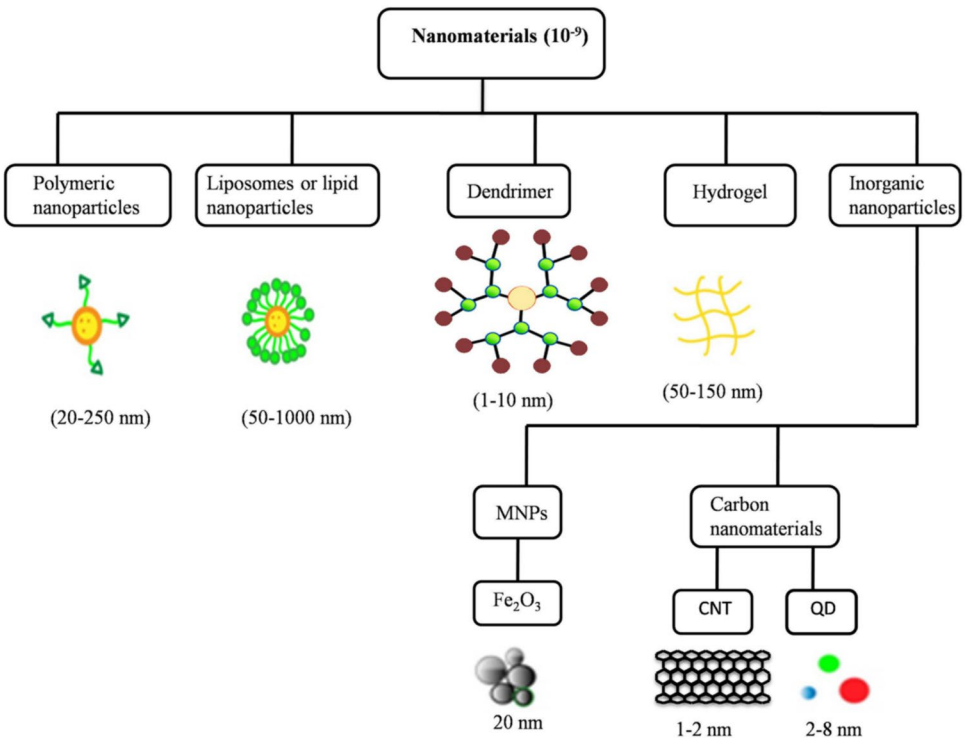


**Fig. 1** Various types of nanomaterials [7]

graphene variants, transition metal compounds, and other planar NMs. Their unique properties arise from their consistent shape and high surface area, among other factors.

4. *Three-Dimensional (3D) NMs*: These don't have any dimensions restricted to the nanoscale. This group includes structures like nanoporous powders and nanostructured electrodes. There's significant research into 3D NMs, especially for applications in storage devices and wastewater treatment. They also find roles in areas such as solar cells and biomedical equipment. 3D printing techniques promise to further expand their potential [8].

(b) Classification Based on Pore Size

As per the guidelines set by the International Union of Pure and Applied Chemistry (IUPAC), nanomaterials (NMs) can be segmented into three categories based on their pore dimensions: mesoporous, microporous, and macroporous NMs [10].

1. *Mesoporous NMs:* Characterized by pores that range from 2 to 50 nm, these NMs can be either structured or unstructured. Some highly porous carbon substances fall into the microporous category, enhancing the overall surface area. One common application of these highly porous materials is in the creation of adsorbents, usually featuring a carbon framework, with the specific structure and pore volume depending on their fabrication process [10].
2. *Microporous NMs:* These NMs have pores smaller than 2 nm, often referred to as nanopores. Examples include zeolites and metal–organic frameworks. Such materials are frequently employed in applications like air purification and gas separation, enabling the filtration of pollutants like mold spores and bacteria while allowing gas molecules to pass unhindered, ensuring a clean environment within confined spaces [10].
3. *Macroporous NMs:* With pore dimensions exceeding 50 nm, these NMs, such as macroporous arrays, stand out for their superior transport capabilities. These structured arrays are designed to maximize flow, ensuring that diffusion isn't a constraint. This is particularly vital for processes where easy access is essential, such as in drug delivery, sensing, catalysis, and absorption [10].

(c) Categorization Based on Nanomaterial Origins

Nanomaterials (NMs) can be classified by their source into four primary groups: natural, incidental, engineered, and bioinspiredas shown in Fig. 2 [11].

1. *Natural NMs:* These arise from inherent environmental processes, without human intervention. Examples include materials stemming from volcanic eruptions, forest fires, or organic structures like spider silk, butterfly wings, and human bone matrices. Some natural NMs, such as clays, owe their nanostructure to their unique crystal formation. Additionally, certain photonic crystals, owing to their nanoscale structure, fall into this category.
2. *Incidental NMs:* These are unintentionally produced as a byproduct of human activities. Emissions from vehicle engines, residues from welding or smoke from wood combustion are classic examples. Their form can be quite irregular, and their impact on the environment can be considerable, especially when juxtaposed with engineered NMs.
3. *Engineered NMs:* Designed for specific purposes, engineered NMs have consistent shapes and sizes. They differ from natural or incidental nanoparticles which typically exhibit inconsistent forms. The history of engineered NMs goes back several decades; for instance, fumed silica was commercialized in the 1940s, and silica nanospheres emerged in the 1960s [10, 11].
4. *Bioinspired NMs:* Crafted to imitate the structures or functionalities of natural substances or organisms, these NMs utilize sophisticated nanofabrication techniques to attain their desired attributes. A classic instance is how chameleons' dynamic color changes, resulting from the modification of guanine nanocrystals, inspire the creation of sensors that change color upon stretching or compression. Such innovations hold potential for diverse applications like wall designs, signage, and optical recording [11].

(d) Categorizing Nanomaterials Based on Chemical Composition.

Nanomaterials (NMs) can be constructed using various elements from the periodic table, particularly those used in creating natural and engineered NMs. Based on their composition, NMs can be segmented into carbon, organic, inorganic, and hybrid categories [12].

(1) Carbon-derived Nanomaterials: These NMs are sourced primarily from $sp^2$ carbon structures, examples include graphene, carbon nanotubes, and fullerenes. Unique methods such as laser ablation and arc discharge are utilized to produce them. One fascinating aspect of carbon-based NMs is their vast array of allotropes, making them a distinct type of organic NMs due to the dominant C–C bonds. Other members of this category include nanodiamonds and activated carbon. Carbon NMs have broad applications, including use in composite materials and energy sectors. Additionally, they are instrumental in the creation of electrodes for batteries and electrochemical sensors [6, 11, 12].

(2) Organic Nanomaterials: Predominantly composed of carbon and hydrogen, these NMs integrate other elements to

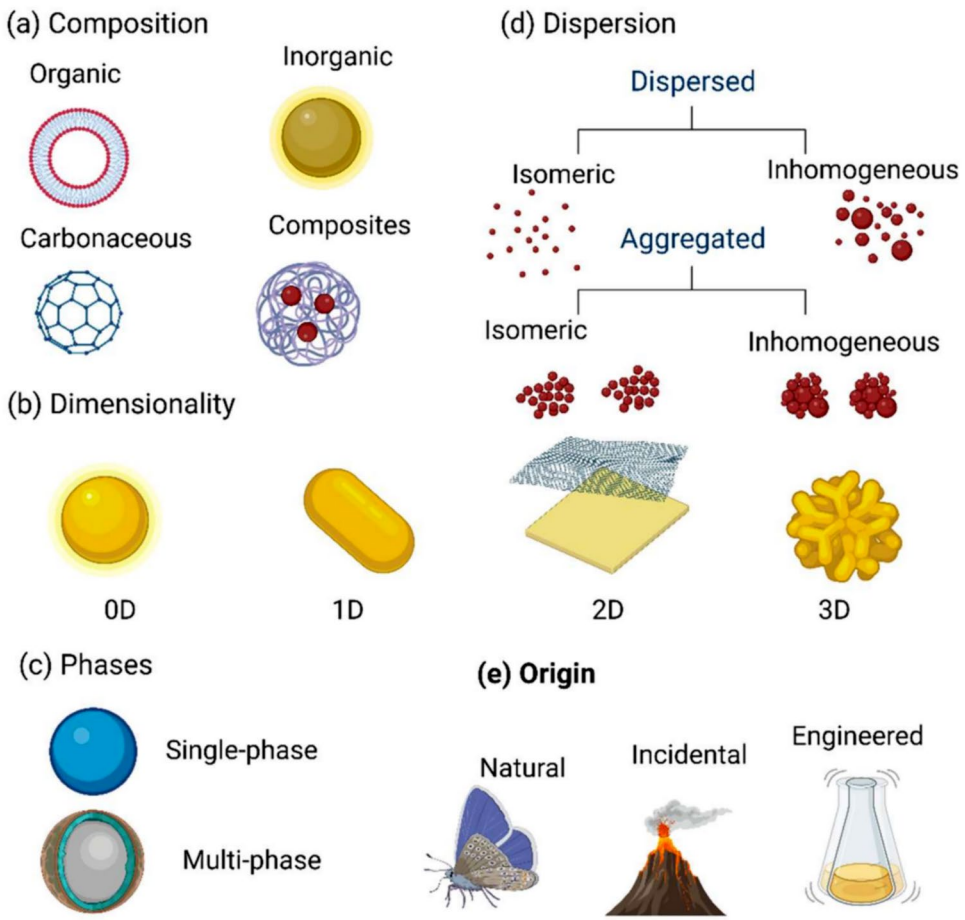


**Fig. 2** Classification of Nanomaterials based on composition, dimensionality, phases, dispersion, and origin [12]

gain specialized functionalities. This category encompasses dendrimers, micelles, and liposomes. Many of these, such as lipid and polymer nanoparticles, encapsulate substances within a nano-sized structure. Some structures, inspired by cellular lipid bilayers, are formulated using amphiphilic organic compounds that create micelles and liposomes with a characteristic hollow center [12].

(3) Inorganic Nanomaterials: These are characterized by the absence of carbon, featuring elements like metals and their derivatives. They exhibit a myriad of shapes due to atomic arrangements while retaining the crystallinity typical of metal compounds. Some inorganic NMs, like metal-based quantum dots, stand out for their unique properties at transitional stages between individual atoms and bulk material. Another notable subgroup includes magnetic nanoparticles known for their magnetic properties. Nanoclays and zeolites, both integral in environmental applications, also fall under this category [1, 12].

(4) Hybrid Nanomaterials: These represent an amalgamation of diverse phases, with at least one phase being nanodimensional. Polymeric nanohybrids incorporate organic or inorganic nanoparticles within polymer matrices. Furthermore, inorganic nanocomposites may merge multiple metals, resulting in alloys or core–shell structures. A notable member of this class is the composite combining carbon nanotubes with metal matrices, prized for its strength and conductivity. Interestingly, such hybrid structures are also observed in natural forms, evident in entities like abalone shells [6, 11].

## Role of Nanomaterials in *Cancer* Detection

(a) 2D Nanomaterials for Detection of Cancer

Cutting-edge nanotechnologies offer transformative benefits in advancing cancer theranostics. Two-dimensional nanomaterials (2DNMs) stand out as innovative materials, boasting multifaceted physicochemical attributes, paving the way for breakthroughs in cancer diagnosis and treatment. Research indicates that 2DNMs hold potential for various applications:

1. Recognizing cancer through their affinity for tumor indicators;
2. Enabling molecular imaging for targeted tumor treatments; and

3. Facilitating drug and gene transport, along with photothermal and photodynamic treatments for cancer [13].

Yet, employing these in the biomedical field prompts concerns due to the current lack of comprehensive knowledge about their in vivo behaviors, transformations, and potential side effects.

Cancer remains a significant global health challenge due to its high mortality rates and the profound impact on patients' lives. Even with considerable advancements in treatment, the five-year survival rate for many cancer patients is still not optimal, underscoring the need for more effective treatment approaches. As we gain a deeper understanding of the complex and varied nature of cancer, there's an increasing urgency to craft more targeted "precision medicine" that seamlessly blends diagnostic and therapeutic solutions. This has given rise to "nanotheranostics," a concept that amalgamates diagnostic imaging with therapy. In this landscape, innovative nanomaterials emerge as a beacon of hope, offering tailored treatment solutions. Notably, 2DNMs are gaining traction as frontrunners in the realm of cancer diagnosis and treatment [14].

Since 2004, when Geim and his team first unveiled graphene, a unique single layer of graphite, the intrigue surrounding 2D nanomaterials (2DNMs) has grown exponentially. These materials are characterized by their ultrathin nature, where one of their dimensions is less than 100 nm. Their distinctive structure affords them outstanding thermal, mechanical, chemical, and optical traits. As a result, researchers have extensively explored 2DNMs for their potential in sectors like energy storage, energy transfer, electronics, and mechanical device development. Furthermore, the biomedical arena recognizes the versatility of 2DNMs, given their exceptional drug and gene loading capabilities and their impressive photothermal and photodynamic attributes [6]. This makes them a promising avenue in the development of tailored cancer nanotheranostics solutions, spanning from sensing and imaging to gene/drug delivery and therapeutic applications. For instance, the inherent properties of rigid C-N planes in ultrathin g-$C_3N_4$ nanosheets make them apt for two-photon fluorescence imaging within cellular nuclei. Additionally, certain 2DNMs, like $WS_2$ and $MoS_2$, are known for their robust photothermal conversion and ability to penetrate deep tissue layers. Their expansive surface area relative to volume further positions them as ideal candidates in advanced drug delivery platforms [6, 15].

While 2D nanomaterials (2DNMs) exhibit pioneering attributes, their clinical application remains a considerable distance away. Several apprehensions persist around their in vivo behavior and safety: [16]

1. Some materials, such as graphene, tend to clump together in biological environments, leading to potential toxicity concerns.
2. The production process for these nanomaterials might introduce impurities like heavy metals, which could exceed human tolerance levels.
3. Slow metabolic rates could cause these materials to accumulate in vital human organs, presenting primary toxicity risks.

Numerous reviews have delved into the unique properties and possible uses of 2DNMs, with many spotlighting the vast biomedical potential, especially around graphene and its variants. Some studies also delve into the biological implications of graphene derivatives, encompassing their in vivo dynamics and safety profile [17]. Yet, there seems to be a lack of a holistic review concentrating specifically on the role of 2DNMs in cancer nanotheranostics. Moreover, the methods employed to adjust their physicochemical characteristics and the biological impacts of other 2D materials are yet to be exhaustively explored. As a result, a significant information void exists regarding the application-centric attributes of 2DNMs in cancer nanotheranostics and an updated overview of their role in oncology research.

## Nanotechnology in *Cancer* Imaging

Nanotechnology, the science of manipulating matter at an incredibly minute scale, holds the promise of revolutionizing cancer research and treatment. By crafting distinct structures and devices at the nanoscale, it can offer the tools necessary for enhancing cancer diagnostics, therapies, and preventive strategies. While the exploration of nanotechnologies in medicine began in earnest only in the 1980s, the field has witnessed substantial advancements. Today, several nano-enhanced therapies and diagnostic methods are in clinical use, with many more in the pipeline. This pioneering field brings together experts from various domains such as physics, chemistry, engineering, information technology, biology, and more. With its vast potential, nanotechnology is influencing diverse sectors, from electronics to biomedicine, driving a host of innovative solutions, particularly in the medical domain [17].

### Advantages of Nanotechnology in *Cancer* Treatment

Nanodevices are minuscule, ranging from one hundred to ten thousand times smaller than cells in the human body. Their size is comparable to large biomolecules, like enzymes. For context, hemoglobin, the oxygen-carrying molecule in our blood, is about 5 nm wide. Due to their petite size, nanodevices that are less than 50 nm can easily penetrate most cells. Those under 20 nm can exit blood vessels during circulation.

Their ability to interact with biomolecules both externally and internally in cells makes them uniquely positioned for innovative disease detection and treatments [18].

At its core, our biological processes operate at the nanoscale. Essentially, we're made up of countless biological nano-machines. Nanotechnology grants researchers the ability to observe and adjust macromolecules in real-time, especially during early stages of cancer. This technology can quickly detect cancer-associated molecules, allowing scientists to identify molecular variations even in a tiny cell fraction. Furthermore, nanotechnology can introduce innovative and effective treatment agents. Nanotechnology's significance in cancer treatment hinges on its customizable nature, its capacity to function as a diagnostic or therapeutic tool (or both), and its potential to accumulate around tumor areas, specifically target cancer cells, and cross challenging biological barriers in the body.

(a) Passive Tumor Collection

An ideal cancer drug delivery system would majorly concentrate on the tumor, leaving the surrounding healthy tissues unaffected. The natural tendency of many drugs to concentrate in tumors due to their movement through permeable blood vessels known as the Enhanced Permeability and Retention (EPR) effect is beneficial. Rapid tumor growth necessitates an equally rapid expansion of its blood supply. This rapid growth leads to vessels with larger pores, permitting the entrance of nanoparticles into tumor regions. The EPR effect ensures nanoparticles can target tumors. Present-day nano-based cancer treatments mostly rely on the EPR effect for effective drug delivery. But the EPR effect has its limitations. While effective for most tumors, central regions of larger tumors may not benefit from the EPR effect due to low oxygen levels. Thus, clinical methods have been developed to amplify the EPR effect [19].

(b) Active Tumor Targeting

While the EPR effect ensures nanoparticles concentrate in tumor regions, it doesn't guarantee their uptake by cells. However, certain treatments need intracellular activation. Additionally, the EPR effect's efficiency varies across tumors. Therefore, active targeting is viewed as crucial for advanced nanoparticle-based treatments. This can be achieved by modifying the nanoparticle surface with various molecules [20].

(c) Crossing Tissue Barriers

For nano-drugs to reach tumors, they must first overcome tissue barriers. These barriers can be biological, like tumor stroma, or functional, like tumor endothelium barriers. The tumor environment is complex, with abnormal blood vessels and various cells in an extracellular matrix. This environment presents challenges to drug delivery. One notable barrier is the blood–brain barrier (BBB), which safeguards our brain from harmful substances. Its protective nature also hinders therapeutic delivery to brain diseases. Nanoparticles' adaptability makes them ideal candidates for designing BBB-crossing delivery methods [21].

### Enhanced Detection and Diagnosis Techniques

A critical factor in combating cancer is its prompt detection. Thanks to nanotechnology, we now have advanced molecular contrast agents and tools that pave the way for swifter and more precise initial diagnoses. This also aids in the continuous monitoring of cancer treatments. Though nanoparticles are still not in clinical use specifically for detecting or diagnosing cancer, they're already employed in various medical screenings and diagnostic tests. A common application is the use of gold nanoparticles in over-the-counter pregnancy kits [22]. Furthermore, products like the Verigene® system by Nanosphere and T2MR system from T2 Biosystems incorporate nanoparticles and are in current hospital use for multiple purposes. In the realm of cancer, there's active research on nanodevices aimed at identifying biomarkers in the blood, such as proteins linked to cancer, floating tumor cells, and tumor-released DNA and exosomes. These nano-powered sensors boast impressive sensitivity, accuracy, and the capability to measure multiple factors simultaneously. The newer generation of these devices integrates genetic analysis, enhancing our understanding of an individual's cancer and illuminating potential treatments and the disease's trajectory. While nanoparticles are already recognized and used as contrast agents to highlight anatomical structures, the focus is now on harnessing them as molecular imaging tools. These can provide insights into cancer-specific genetic changes or the behavior of tumor cells. This crucial data assists doctors in determining or adjusting a treatment path. Another promising development is bioresponsive nanoparticles. Their ability to alter their properties based on internal body changes allows them to offer real-time, comprehensive insights into disease development and the efficacy of treatments.

(a) In Vivo Imaging Advancements

Traditional imaging techniques often detect cancers only after they've significantly affected tissue structures. By this stage, the cancer might have advanced and even spread to other parts of the body. Determining the type of tumor—whether it's benign or malignant—and its responsiveness to treatments typically requires invasive biopsies. Modern imaging struggles to identify tiny metastatic sites or cells that pre-seed metastatic locations. Additionally, surgical removal of tumors, a common treatment, poses the challenge of balancing between eliminating cancerous tissue and preserving vital healthy tissue. Achieving single-cell precision in cancer removal remains an aspiration [23]. It promises more refined contrast agents that can enhance tumor detection in real-time using established imaging techniques like MRI, PET, and CT. Furthermore, it introduces unconventional imaging methods, such as photoacoustic tomography

(PAT), Raman spectroscopic imaging, and multimodal imaging—where agents can function across multiple imaging techniques. The versatility of nanotechnology arises from its ability to hold various components at once, from targeted agents to contrast materials, and its inherent properties that amplify imaging signals.

Notable advancements have stemmed from research funded by the NCI. For instance, collaborations between Stanford University and Memorial Sloan Kettering Cancer Center led to the creation of nanoparticles that can outline brain tumor boundaries [24]. These particles can monitor tumor growth using MRI and assist surgeons during tumor removal, enabling precision down to individual cancer cells. For metastatic melanoma, teams from MSKCC and Cornell University have pioneered 'C-dots'—nanoparticles that offer both PET and optical imaging. Specifically designed to target melanoma, these nanoparticles have shown promise in early clinical tests [25]. Addressing prostate cancer, Stanford researchers are focusing on nanoparticles that reveal both the size and location of cancerous cells, and provide crucial insights to prevent overdiagnoses. Their innovations align with the recent introduction of a handheld imaging device, potentially revolutionizing how prostate cancer is diagnosed and treated. Gold nanoparticles are being explored to boost light scattering in endoscopic procedures like colonoscopies. The allure of nanotechnology in cancer care lies in its potential for 'theranostic' platforms systems that can both diagnose and treat. Emory University researchers are breaking barriers with a platform that penetrates pancreatic cancer's protective fibrous tissue, using magnetic cores for MRI imaging and direct drug delivery [26].

Furthermore, nanotech promises a more granular view of molecular markers, revealing the stages of cancer and even therapy-induced cell death. An innovative system from Stanford, called the Target-Enabled in Situ Ligand Assembly (TESLA), is based on nanoparticles that assemble within the body [27]. These particles emerge in response to enzymes produced during cancer cell death, enabling medical professionals to monitor treatment outcomes more effectively.

(b) In Vitro Sensing Through Nanotechnology

In vitro diagnostics enhanced by nanotechnology provide heightened precision and adaptability, enabling simultaneous testing for numerous targets. Utilizing familiar manufacturing techniques, like lithography, these diagnostic tools can be crafted into compact, on-site devices. At the core of such devices is a biosensor, equipped with a biological element that recognizes and reacts with a specific biological molecule. This interaction can stem from processes like antigen–antibody binding, DNA strand hybridization, or ligand-epitope attachments. The device's transducer then translates this biochemical activity into a measurable signal, employing mechanisms like optical, magnetic, or electronic responses. Many of these nanotechnology-powered devices can discern diverse biological markers from fluids or tissues. For example, the bio-barcode assay, conceived in Chad Mirkin's lab at Northwestern University [28], combines magnetic nanoparticles with gold nanoparticle probes to achieve femto-picomolar sensitivity levels. Another notable advancement comes from James Heath's lab at Caltech. Here, a unique method, DNA-encoded antibody libraries (DEAL), utilizes DNA to anchor antibodies in microchannels, enabling comprehensive protein assessments directly from blood [29].

Massachusetts General Hospital's Ralph Weissleder developed the Diagnostic Magnetic Resonance (DMR) sensor, a tool that capitalizes on magnetic field-induced changes in water molecules to detect labeled analytes [30]. Meanwhile, at Stanford University, Shan Wang's team crafted Giant Magnetoresistive (GMR) biosensors [31]. These tiny sensors adjust their electrical resistance based on local magnetic fields and have been optimized to detect proteins in diverse samples and gauge protein interaction dynamics. The aforementioned tools not only offer unparalleled multiplexing capabilities but also, through data analytics, allow the correlation of different biological markers. This can significantly improve patient treatment customization based on individual responses. Furthermore, with the growth of microfluidic technologies, it's now feasible to unite sample handling and processing with biosensing. This promises fully comprehensive diagnostic devices that can deliver conclusive medical insights from a singular sample.

(c) Assessing Therapeutic Reactions through Liquid Biopsies

Monitoring a patient's reaction to treatment during their illness journey is pivotal for tailored and predictive healthcare. Efficient and disease-specific tracking can guide more effective treatment strategies, such as adjusting drug combinations, dosing modifications, and early clinical choices (like discerning between those who benefit from a treatment and those who don't). It also aids in categorizing patients for clinical research. While in vivo imaging, tissue biopsy, and in vitro diagnostics have been the conventional methods, the emergence of the "liquid biopsy" promises non-invasive, repetitive testing using blood samples. Traditional biopsies, which obtain a small portion of tumor tissue, are invasive and don't provide the frequent sampling required to monitor changes in disease in relation to treatment [32]. In contrast, liquid biopsies harness the principle that tumors release certain materials (like cells, DNA, or specific biomolecules) into the bloodstream. The challenge is that the concentration of this tumor-released material is extremely low compared to other blood components [33]. This is where nanotechnology steps in, offering precise tools to identify, collect, and clean up the circulating tumor materials.

Recent innovations have combined intricate microfluidics with nanomaterials, paving the way for capturing circulating

tumor cells (CTCs), tumor DNA that's not within cells, microclots, vesicles, proteins, and newly recognized antigens with high accuracy [34]. For instance, some devices have successfully captured and then released CTCs, allowing these cells to be studied in detail afterward, like genomic sequencing or expansion in the lab. In some of these tools, magnetic nanoparticles enrich the blood before a magnetic separation process in a microfluidic system. Others use heat-sensitive nanopolymers that capture CTCs and release them upon temperature change after processing the blood. These methods provide excellent detection accuracy and a purity that outperforms non-nanotech devices. Additionally, as technology progresses, processing times have been speeding up, with current rates processing about 10 mL of blood in half an hour [35].

## Multimodal Imaging Techniques

Personalized medicine represents the future of patient care, with molecular imaging as a central component. Nanoplatform-based molecular imaging, an emerging interdisciplinary domain, combines elements from chemistry, engineering, biology, and medicine. These nanoplatforms enhance early disease detection, precise diagnosis, and tailored treatments. They're versatile, being utilized across various biomedical imaging techniques like optical imaging, MRI, and PET scans [36]. Their standout feature is multifunctionality; they can simultaneously carry targeting agents, imaging markers, therapeutic drugs, and more, enabling precise molecular imaging and therapy while overcoming biological challenges. Nanoparticles can be tailored as platforms to efficiently deliver drugs and imaging agents, navigating various biological and medical challenges. While advanced nanodevices like nanochips and nanosensors clearly outperform conventional assays for in vitro and ex vivo uses, in vivo applications face hurdles [37]. These include biocompatibility, in vivo behavior, targeting success, potential toxicity, and cost-effectiveness.

### Molecular Imaging Overview

Molecular imaging delves into understanding biological processes at both cellular and molecular levels. This technique offers comprehensive insights into an intact system, streamlining biomedical research, and drug development efforts by reducing time and costs. It supports early detection in patients, facilitates tailored treatments, and optimizes dosing. Modalities within molecular imaging range from optical bioluminescence and targeted ultrasound to advanced techniques like MRI, MRS, SPECT, and PET. There are commercially available systems that integrate multiple modalities, with several more in the developmental stages. While CT scans aren't typically categorized under molecular imaging, when using nanoparticles as contrast agents, they become relevant [38]. Figure 3 displays, a range of molecular imaging techniques is presented, each offering unique insights into the biological processes at the cellular and molecular levels.

Employing targeted nanoplatforms offers several advantages over traditional methods. A single nanoparticle can carry a multitude of imaging labels or even combinations tailored for different modalities, resulting in amplified signals. These nanoparticles can also be equipped with diverse targeting ligands to enhance binding precision. Additionally, these platforms can be designed to navigate biological obstacles, ensuring improved targeting efficiency. The ultimate goal is to couple various targeting ligands, imaging labels, therapeutic agents, and more to achieve controlled, real-time therapeutic delivery to patients. Multidisciplinary efforts in this realm promise advancements in molecular diagnostics and treatments.

**Optical Imaging Insights** Optical imaging stands as a cost-effective approach mostly tailored for small-animal research. When it comes to fluorescence imaging, a subject is illuminated with excitation light, and then the emitted light is captured at a different wavelength [40]. A significant drawback of this method is its inability to provide quantitative data, and the results usually emphasize the surface due to tissue absorption. Moreover, tissue autofluorescence often results in a high background signal. Yet, recent advancements like spectral imaging, which distinguishes fluorescence signals based on unique emission spectra, and fluorescence-mediated tomography,have enhanced data interpretation [40]. Figure 4 exhibits, the specialized spectroscopic optical probe is employed to measure and analyze the levels of specific blood factors present within breast tissue, facilitating a non-invasive diagnostic approach.

**Delving into Quantum Dots** Quantum dots (QDs) are at the forefront of optical imaging due to their unique properties. These inorganic, luminescent semiconductor nanoparticles outshine their organic counterparts in many ways. They have impressive fluorescence longevity, are resistant to photobleaching and chemical breakdown, and display a broad absorption range from UV to near-infrared (NIR; 700–900 nm). Their application spans across various in vitro and cellular studies. The noteworthy attribute of QDs is their massive two-photon absorption capability, making them apt for multiphoton microscopy in live organisms [42]. Research indicates there are two primary spectral windows apt for QD-based live-subject imaging: one ranges between 700–900 nm and the other spans from 1200–1600 nm. In practice, QDs without specific targeting have been useful for monitoring cells, imaging blood vessels, mapping sentinel lymph nodes, and visualizing neural pathways.For

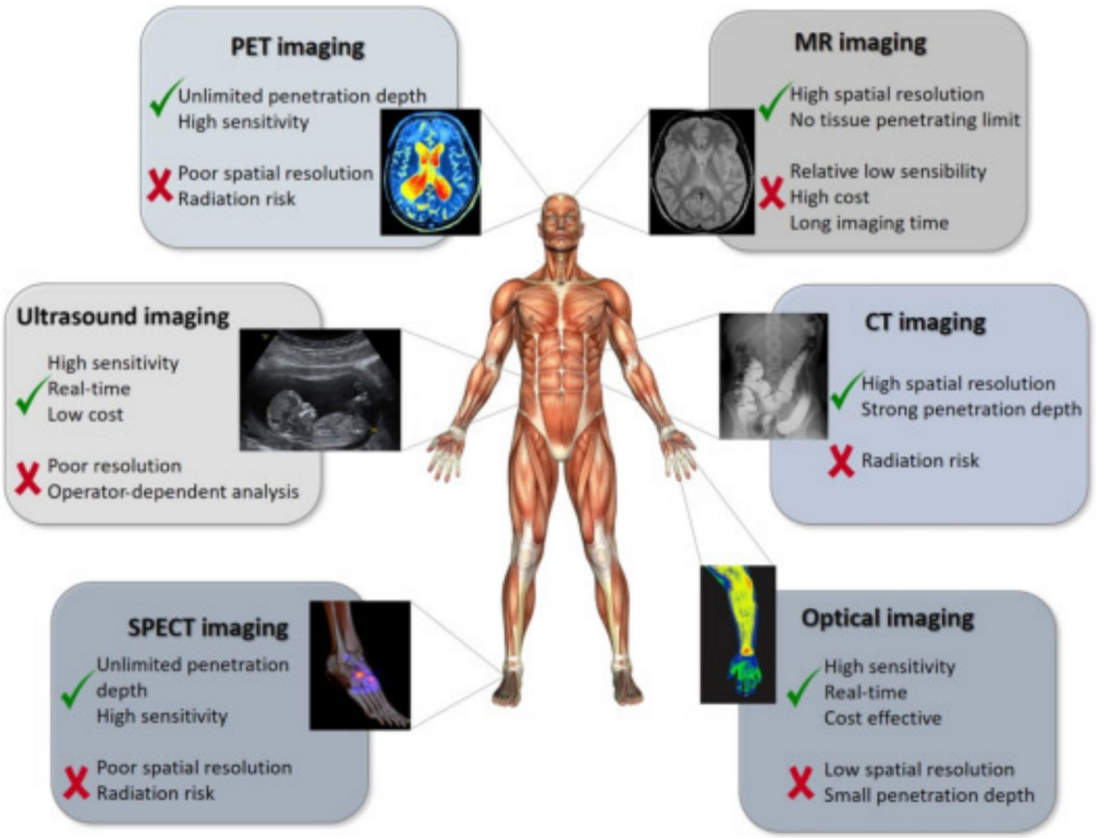


**Fig. 3** Illustrating various types of molecular imaging techniques [39]

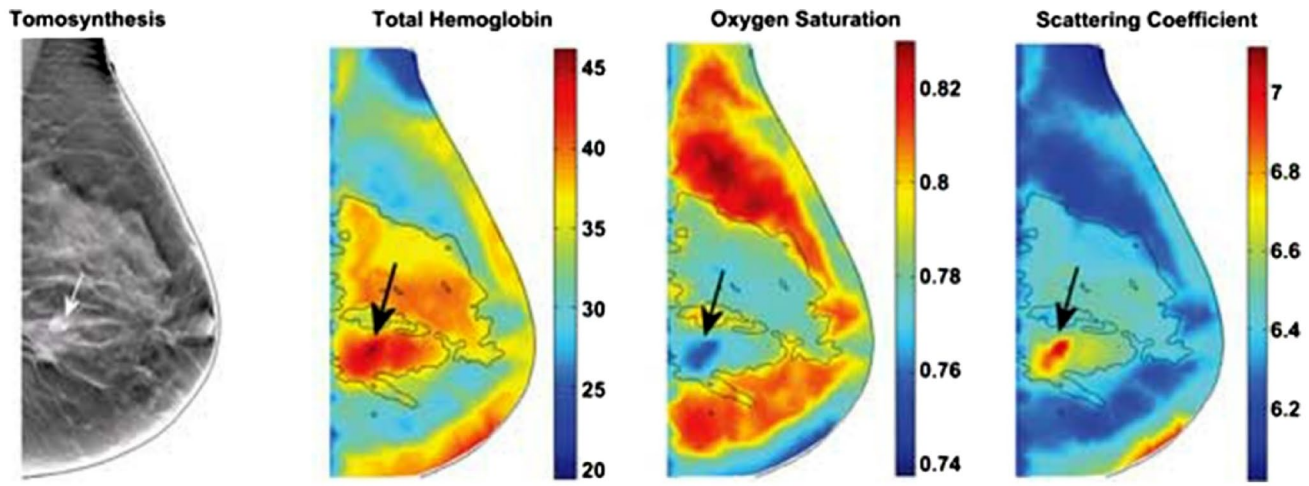


**Fig. 4** Spectroscopic Optical Probe for Assessing Blood Factor Levels in Breast Tissue [41]

quantum dots to be more impactful in the biomedical sector, they need to be precisely directed to particular organs or disease locations without interference. Although achieving this specificity involves integrating targeting molecules onto QDs, in vivo targeting remains a challenge due to QDs' size and short-lived circulation in the bloodstream. Despite these challenges, there are some documented successes in the field [43]. Figure 5 demonstrates, the dual-phase process utilizes the unique luminescent and magnetic characteristics of quantum dot-antibody conjugates for precise tumor imaging and the subsequent sensitive detection of circulating tumor cells in the bloodstream of an animal model.

**Quantum Dots with Peptide Linkers** Quantum Dot (QD) conjugates have been explored for targeted applications in living organisms, with peptides being among the earliest utilized targeting agents. An initial study indicated that when guided by specific peptides, these QDs could be directed towards tumor blood vessels and other desired targets. Although this study didn't encompass in vivo imaging, it validated the concept of harnessing QDs for targeted applications in living subjects, setting the stage for further research in this domain [45]. In a subsequent study into the potential of peptide-bound QDs for imaging specific targets within living organisms. A particular cell adhesion molecule, known as integrin avb3, is prominently present on certain active endothelial and tumor cells but remains elusive on inactive endothelial cells and in most regular tissues. Previous research had pinpointed integrin avb3 as a promising target for imaging studies. Given its presence on both tumor blood vessels and the tumor cells themselves, integrin avb3 became a prime candidate for QD-based imaging without the need for particle penetration into the tumor [46]. In their study, the researchers attached a peptide sequence—arginine-glycine-aspartic acid (RGD)—known for its integrin avb3-binding properties, to a specific Quantum Dot variant, QD705. Lab tests showed that this QD705-RGD complex was adept at recognizing and binding to integrin avb3. When tested in living organisms, specifically nude mice implanted with certain human tumor types, clear imaging results were achieved. The tumor's imaging signal peaked roughly 6 h post QD705-RGD injection. Due to the size constraints of the QD705-RGD complex, which measures around 20 nm, it primarily attached to the tumor's blood vessels, rather than the tumor cells. This was later validated through lab-based staining procedures. The predominant presence of integrin avb3 in the budding blood vessels of various tumor types underscores the potential of QD705-RGD as a versatile imaging agent for tumor-associated blood vessels in living beings [47]. Figure 6 illustrates, the innovative use of fluorescent quantum dots provides a multi-faceted approach to visualizing cellular interactions and targeted tumor identification within living organisms.

**Quantum Dots Linked with Antibodies** Quantum Dots (QDs) have been married with antibodies specific to the prostate specific membrane antigen (PSMA) to explore potential applications in imaging prostate cancer in mice. The study took a two-fold approach by employing both active and passive targeting techniques with QDs containing diverse surface ligands. However, the study fell short in its analysis, failing to conclusively determine whether the QDs focused on the tumor's blood vessels or the tumor cells themselves. In another innovative attempt, QDs were paired with an antibody against alpha-fetoprotein (AFP, a known marker for certain liver cancer cells). Through advanced fluorescence imaging systems, the study visualized liver cancer and mapped out the inconsistent spread of the QD probe within the tumor [49]. Nonetheless, the study's credibility was tainted by the absence of concrete proof of the antibody's attachment to the QD.An interesting development was the tracking of a solitary QD-antibody combination within a tumor using a sophisticated microscope setup. This technique showcased the intricate journey of the QD, from entering the bloodstream to binding to a specific receptor on the tumor cell. However, the study missed an important data point it failed to quantify the number of QDs that actually penetrated the tumor, leaving an information gap about the typical behavior of such conjugates inside a living organism.Scaling QD-based imaging from small animals to humans presents challenges, mainly because of the optical limitations in penetrating human tissues [50]. In clinical scenarios, QDs might find applications in imaging superficial tissues, areas reachable by endoscopes, and during surgeries. Despite their promise, there are hurdles to surmount

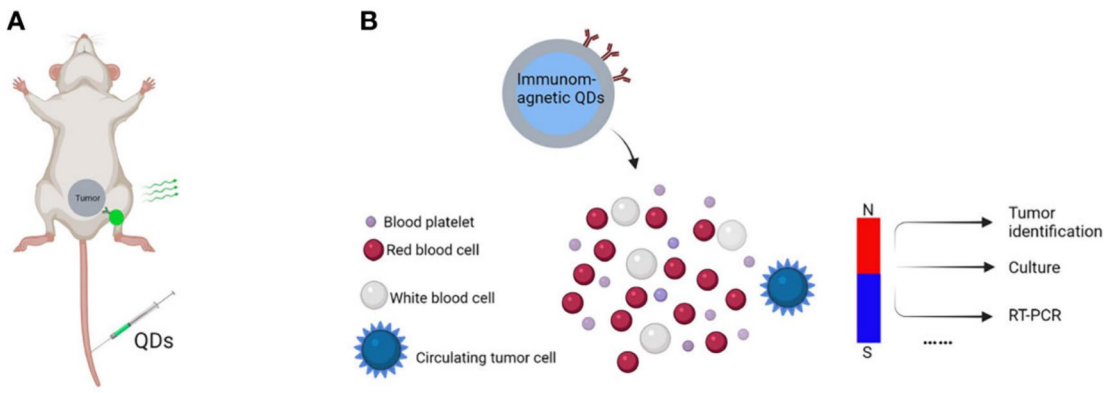


**Fig. 5** The schematic depicts two phases of in vivo tumor imaging and circulating tumor cell detection: first, the injection of quantum dots-antibody conjugates through the tail vein for efficient tumor targeting, and second, the use of these conjugates' fluorescent and magnetic properties to non-disruptively detect CTCs in animal bloodstreams [44]

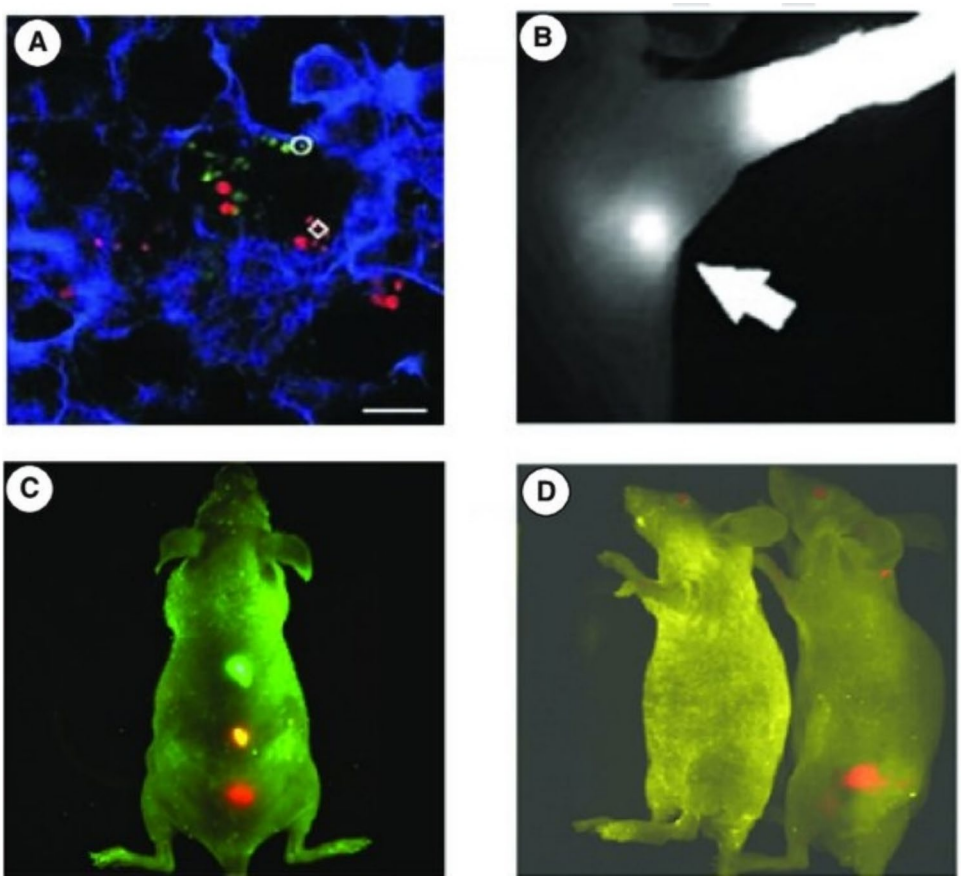


**Fig. 6** **A** A micrograph showcasing fluorescently labeled tumor cells within the lung tissue of a mouse. **B** An image demonstrating the absorption of fluorescent quantum dots by lymph nodes. **C** A photograph of a living mouse showing the injection site of fluorescently labeled microbeads. **D** A live mouse imaged to reveal the specific location of a prostate tumor marked by fluorescent quantum dots attached to antibodies. [48]

before QDs see clinical use, including concerns over delivery efficiency, potential side effects, and accurate quantification. However, optimism remains. As technology evolves, yielding smaller, safer, and more multifaceted QDs, along with refined conjugation techniques, the clinical potential of QDs in effective tumor targeting with a safe profile seems attainable in the foreseeable future [51].

**Other Nanoparticles** Optical coherence tomography (OCT) is a sophisticated imaging method capable of achieving resolutions between 10–15 mm, facilitating real-time and sectional views of biological tissues. This method functions by measuring the depth of reflections from a directed low-coherence light within a tissue. In the realm of OCT imaging, studies have indicated the potential of nanoshells and gold nanocages. The unique resonance characteristics of these nanoparticles not only offer enhanced contrast for in vivo optical imaging but also present possibilities for treating diseases using light-based therapies [52]. However, challenges remain, the critical step of targeted nanoparticle delivery in a living system remains elusive. Before these nanoparticles can be widely adopted for medical use, rigorous evaluations of their biological interactions and impacts in animals are necessary. Another intriguing development is the emergence of ligand-attached, near-infrared (NIR) marked low-density lipoprotein (LDL) nanoparticles. Recent findings suggest these can redirect LDL away from its usual receptors, potentially paving the way for new cancer diagnosis and treatment methods using LDL particles [53].

**Computed Tomography (CT) Overview** CT imaging creates a 3D visual of an object's interior by processing numerous 2D X-ray images taken from various angles around the object. Although it isn't fully adapted for molecular imaging due to the absence of target-specific contrast agents, most of the CT contrast agents today are based on substances like iodine and gadolinium, which distribute non-specifically and quickly in the body. Recent advances have shown iodinated nanoparticles acting as CT contrast agents. A notable development involved the detection of macrophages in certain rabbit conditions after using an iodinated nanoparticle-based contrast agent, suggesting potential clinical applications for coronary artery evaluations. Still, most contrast enhancements in CT rely on non-specific targeting [54]. A new injectable CT contrast agent based on polymer-coated $Bi_2S_3$ nanoparticles has shown promising attributes like enhanced X-ray absorption, prolonged in-body circulation, and a competitive efficacy and safety profile. Given the widespread use

of CT, it's anticipated that molecular CT will soon become a clinical standard [55].

**Focused Ultrasound** Ultrasound is popular in clinical settings due to its safety, affordability, accessibility, and simplicity. It works by emitting high-frequency sound waves from a probe onto the body and capturing the reflected sound waves to produce images. The imaging quality is influenced by various factors, including sound speed and the imaging technique employed. Clinically, ultrasound contrast agents, often in the form of micro-sized particles, are employed for various applications, enhancing imaging quality. These agents, called microbubbles, vibrate in response to the ultrasound beam, producing improved image contrast [56]. For specificity, these agents can be chemically modified or attached with disease-specific ligands. However, their size restricts them from penetrating tissues, necessitating vascular changes for imaging. Examples include targeted ultrasounds that focus on specific molecules using ligand-adorned microbubbles. An innovative study used ligand-covered nanoparticles to detect certain expressions in pig arteries, showing promising ultrasound applications. While ultrasound provides detailed images, its depth penetration and sensitivity can be challenging. Also, the majority of the current molecular targets pertain only to vasculature due to the size of the agents used [57]. As Fig. 7 highlights, the differential response of carotid arterial walls to tissue factor-targeted nanoparticles post-angioplasty is captured through high-frequency ultrasonic imaging, with marked acoustic contrasts distinguishing the treated areas from those receiving a control substance.

**Molecular MRI Insights** MRI is a non-intrusive technique that visualizes tissue by evaluating the interaction of specific atomic nuclei within the tissue. Different tissues produce distinct contrasts based on their relaxation times. To amplify this natural contrast, external agents can be used. Adjusting the imaging parameters can focus on either T1 or T2 contrasts. Historically, compounds like gadolinium chelates were employed to enhance T1 contrasts. However, newer agents, like gadolinium-inclusive liposomes and superparamagnetic iron oxide nanoparticles, are emerging with superior capabilities. MRI stands out for its high-resolution imaging without using radiation [59]. However, its sensitivity is comparatively low, which can be improved using higher magnetic fields, lengthier data acquisition, and external contrast agents.

(a) General MR Contrast Agents

Iron oxide nanoparticles stand out as the primary choice for nanoparticle-based MR contrast agents. Typically, these nanoparticles are indiscriminately absorbed by the reticuloendothelial system (RES), and their size often dictates their destination, such as the liver, spleen, or lymph nodes. Consequently, non-specific iron oxide nanoparticles have found applications in imaging of the liver, spleen, and lymph nodes. These particles might also congregate at tumor sites due to abnormalities in the tumor blood vessels or from macrophage activities. Given the significant involvement of macrophages in certain neurological conditions, iron oxide-based agents have been utilized to capture images related to strokes, multiple sclerosis, brain tumors, and specific arterial plaques [60]. Their sensitivity is so remarkable that even single cells or individual particles can be detected. In recent studies, these nanoparticles have been adopted to mark cells to monitor their movement and distribution in the body using MRI. There's also a mention of FeCo/graphitic-shell nanoparticles that display

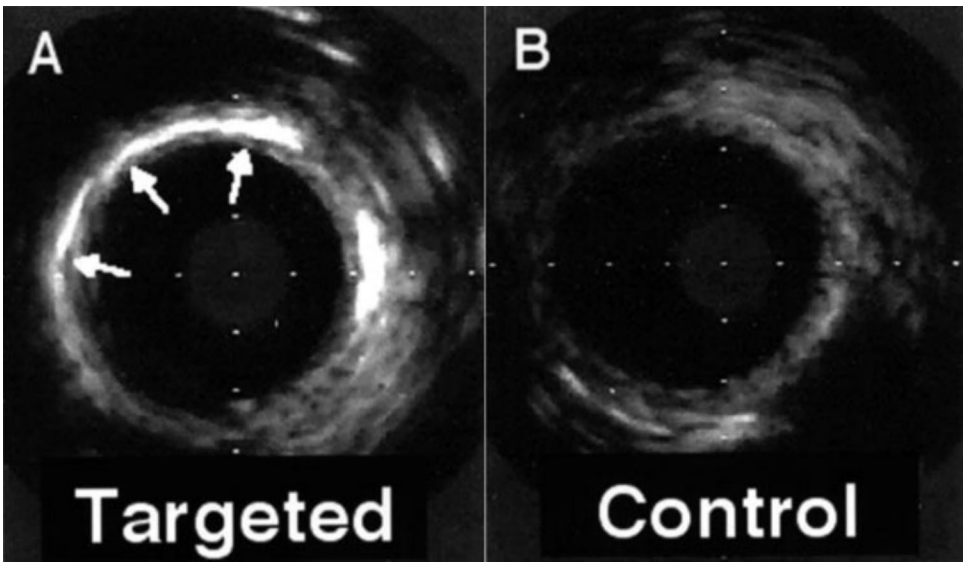


**Fig. 7** Ultrasonic intravascular images captured at a high frequency showing the carotid arteries following angioplasty and exposure to nanoparticles aimed at tissue factors versus a control group. **A** shows enhanced acoustic signals (indicated by arrows) in specific areas of the arterial wall, which align with uneven dilation damage caused by the balloon. In **B**, these acoustic signals are absent in the arterial walls following the administration of a control contrast agent. This pattern was consistent across three separate animal subjects [58]

impressive magnetic characteristics, promising potential for combined diagnostic and therapeutic applications [61].

(b) Molecular MRI Targeting Integrin avb3

Integrin avb3 stands as a frequently researched target for molecular MRI. The pioneering approach employed antibody-coated liposomes infused with Gd3 + ions to image this specific integrin expression. These paramagnetic liposomes, when combined with a particular antibody, showed promising results in imaging specific cancer cells in a rabbit. Additionally, nanoparticles of varying sizes were utilized to enhance the contrast in angiogenic vessels. By attaching a specific antagonist to magnetic nanoparticles, images at standard clinical field strength became more defined, notably in specific tumor types [62]. Notably, despite their considerable size, these nanoparticles were able to permeate tumor blood vessels but were restricted from moving significantly into surrounding tissue. Subsequent experiments showcased these integrin-targeted nanoparticles in imaging certain tumors in animal models. A more advanced study combined imaging with drug delivery, using integrin-targeted nanoparticles. These particles carried an antiangiogenic medication, proving their effectiveness in visualizing treatment areas, monitoring drug delivery, and assessing local treatment reactions [63]. This method underscored the potential of melding molecular imaging with targeted drug delivery, offering a comprehensive, non-invasive approach to disease treatment and monitoring.

(c) Molecular MRI for Diverse Targets

Figure 8 elucidates, the comparative MRI scans taken before and during immunotherapy capture the hallmarks of pseudo-progression, with changes in various imaging sequences that collectively suggest an increase in tumor size which does not correspond to actual tumor growth.Studies on $Gd^{3+}$loaded nanoparticles tailored for fibrin detection have been executed in canine models. The results indicated a heightened ability of these fibrin-specific paramagnetic particles to spot and pinpoint fibrin deposits. This ability offers potential for early detection of problematic plaques, aiding in timely medical intervention.Explorations have also been made into vascular smooth muscle cell (VSMC)-oriented nanoparticles for delivering drugs, especially post-angioplasty to curb restenosis [64]. Nanoparticles aimed at tissue factors, containing either doxorubicin or paclitaxel (both renowned cancer-fighting agents), exhibited heightened efficacy in hindering cell proliferation. Advanced MRI techniques captured these nanoparticles adhering to VSMCs, and further examinations could quantify and non-invasively assess the administered drug dosages.Further developments

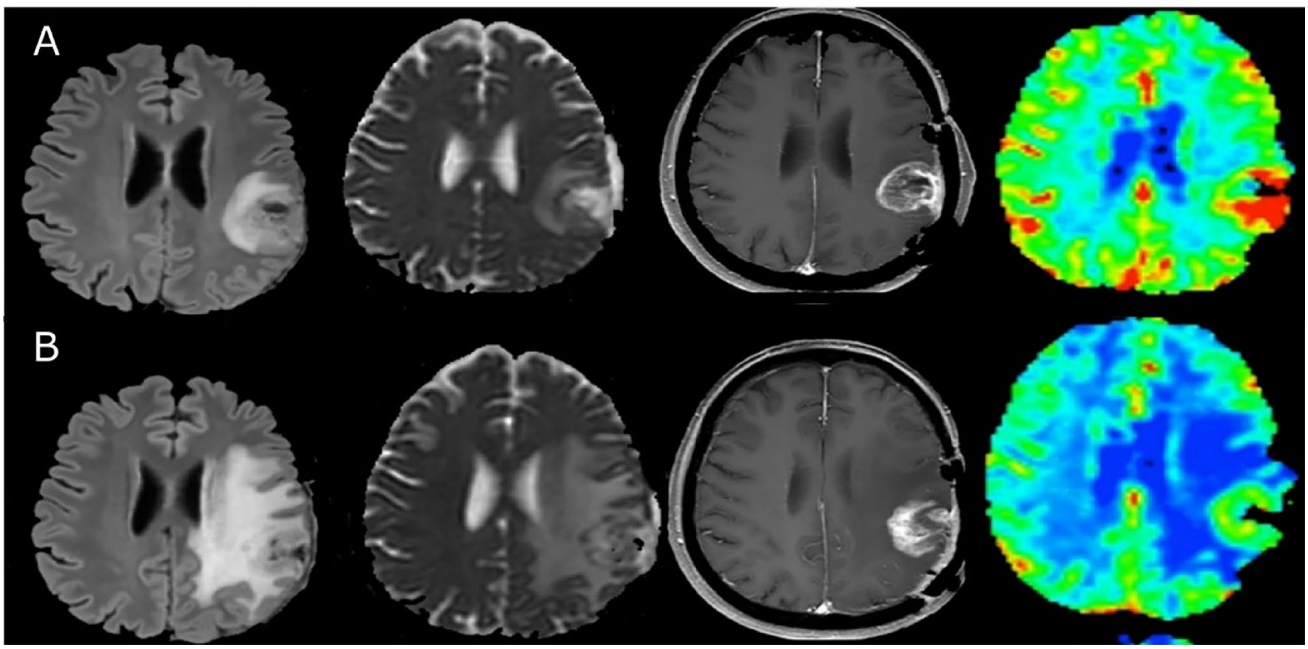


**Fig. 8** An illustration of a single case demonstrating the radiographic characteristics of pseudo-progression during immunotherapy, as observed through standard and advanced MRI techniques. From left to right, the images display FLAIR, ADC, post-gadolinium T1, and CBV sequences. The initial MRI scan (**A**), conducted post-surgery and radio-chemotherapy but prior to starting immunotherapy, shows notable T1 enhancement and increased CBV. An edema is observable in the FLAIR image, along with a reduced ADC, indicating heightened cellularity. In contrast, the MRI set taken four months into immunotherapy (**B**) reveals an apparent enlargement in tumor size on the T1 image. However, a reduction in CBV and elevated ADC values, compared to (A), denote diminished vascularity and lessened restriction. These alterations imply that the perceived tumor growth is actually deceptive, attributable to pseudo-progression rather than actual tumor progression [66]

include latex nanoparticles, enhanced with tomato lectin (known for its robust binding to rodent blood vessels) and Gd3 + elements, for refining MRI vascular contrasts [65]. Additionally, a nanoparticle derivative grounded on high-density lipoproteins (HDL) was employed to detect atherosclerotic plaques. A cutting-edge nanosystem, housing MRI and photodynamic therapy (PDT) components, showcased amplified MRI contrast and PDT outcomes.

In a groundbreaking approach, Mn-doped magnetism-adapted iron oxide (MnMEIO) paired with trastuzumab (a specific anti-HER2 antibody) illuminated small HER2-positive tumors in mice models.Interestingly, a similar combination using cross-linked iron oxide (CLIO) wasn't as successful. This discrepancy was attributed to CLIO's larger size and inferior magnetic attributes, making its detection at minimal concentrations by MRI challenging.Despite its promise, molecular MRI remains in a nascent phase. There is often a lack of in-depth ex vivo analyses to understand the precise locations of these targeted MRI contrast agents within the body [67]. Most of the current particles might predominantly interact with the vasculature due to their large sizes. Future innovations may see the design of smaller nanoparticles (< 20 nm ideally) with extended circulation durations, allowing them to permeate the tumor's abnormal vasculature more effectively. Particles coated with smaller molecules or peptides might outperform those with antibodies due to their size and higher ligand count. Given MRI's natural sensitivity limits, the next wave of contrast agents might target cells in conjunction with vasculature, boosting the MRI signal, thus expanding the potential of molecular MRI in biomedicine [68].

**Radionuclide Imaging Techniques** Radionuclide imaging encompasses techniques like SPECT and PET, which employ internally administered radiolabeled pharmaceuticals to produce images. Radionuclide-based molecular imaging stands out for its sensitivity, quantifiability, and unrestricted tissue penetration. However, its resolution (usually > 1 mm) is inferior compared to other imaging methods. Often, nanoparticles are labeled with radionuclides to non-invasively study their pharmacokinetics via SPECT or PET [69]. Figure 9 depicts, the process of receptor-targeted molecular imaging employs intricately designed probes that deliver radioisotopes to breast cancer cells, enabling precise imaging through the specific attachment of ligands to overexpressed cellular receptors.

(a) SPECT Imaging with Nanotechnologies

Gamma-ray emissions serve as the primary source for SPECT images. These gamma rays, emanating from the decay of radioisotopes, are captured by a gamma camera to yield 3D images. A collimator first encounters these gamma photons, directing them along specific paths towards the detector. This ensures accuracy in pinpointing the gamma

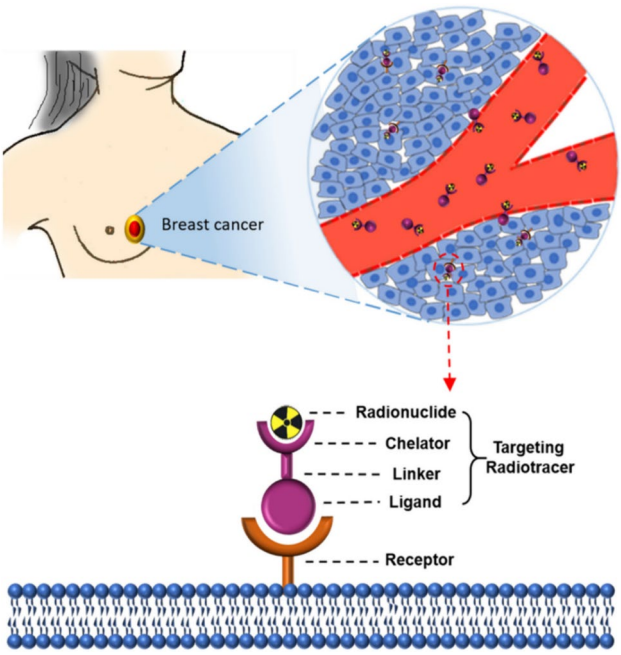


**Fig. 9** Diagrammatic representation of targeted molecular imaging in breast cancer via receptor-specific probes. These probes are composed of a targeting ligand, a connecting linker, a binding chelator, and a radioactive tracer. The targeting ligand, specific to receptors that are abundantly present on breast cancer cells, is attached to the chelator via the linker. The chelator's role is to facilitate the incorporation of radioisotopes like 68 Ga and 99mTc, which are connected through the linker [70]

rays origin. Due to the collimator's role in determining incidence angles, SPECT's detection efficiency is quite low often less than 0.01% of the emitted gamma rays [71]. As Fig. 10 illustrates, the diagram provides a clear overview of the various radiolabeling strategies for nanoparticles and their corresponding applications in advanced medical imaging techniques such as SPECT and PET, highlighting the mechanisms by which radionuclides are integrated with nanoparticles for diagnostic purposes.In one study, researchers explored the properties and therapeutic potential of 111In-labeled ChL6 monoclonal antibody-combined iron oxide nanoparticles in mice with human breast cancer HBT 3477 tumors. These nanoparticles were coated with dextran and conjugated to carboxylated PEG. It was inferred that the nanoparticles circulated long enough to exit blood vessels and target cancer cells. When subjected to an externally induced alternating magnetic field (AMF), tumor necrosis was observed 24 h post-AMF treatment. Subsequent studies revealed that after nanoparticle introduction, tumor growth delay was significant post-AMF treatment compared to controls, as determined through SPECT imaging [72].

Another investigation employed integrin avb3-targeted 111In-labeled PFC nanoparticles to detect tumor angiogenesis in rabbits implanted with Vx-2 tumors. Those

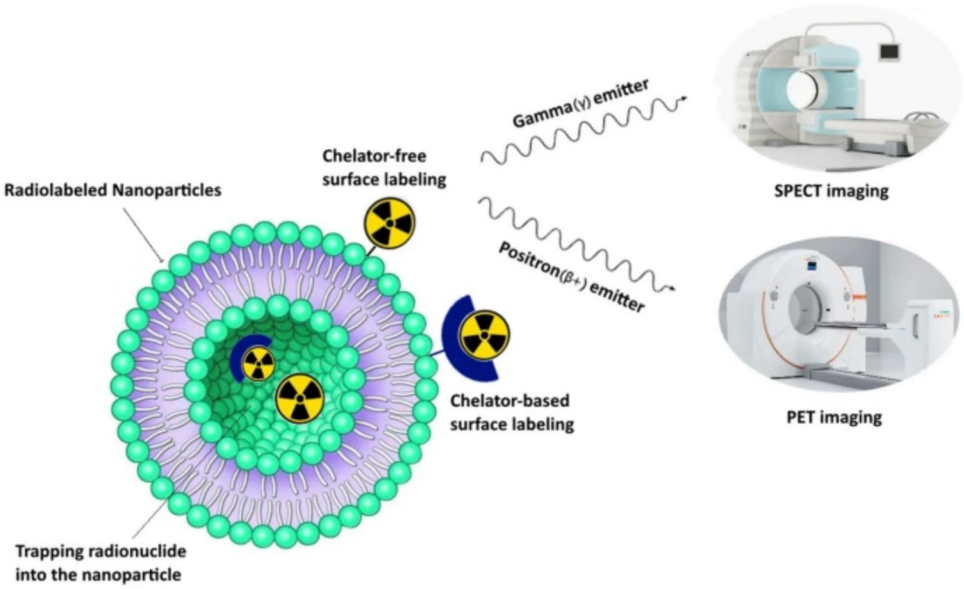


**Fig. 10** Nanoparticle Radiolabeling and Imaging Techniques **A** Schematic of a nanoparticle showing radiolabeling methods, with chelator-free surface attachment and chelator-based surface attachment of radioisotopes. **B** Depiction of a gamma (γ) emitter and its use in Single Photon Emission Computed Tomography (SPECT) imaging. **C** Illustration of a positron (β+) emitter used in Positron Emission Tomography (PET) imaging. **D** Visualization of the encapsulation process for trapping radionuclide within the nanoparticle core [73]

nanoparticles with about ten 111In atoms per particle were more effective in distinguishing tumors than those with just one 111In atom [74]. After injection, targeted nanoparticles showed roughly four times higher tumor activity than non-targeted controls. The spleen emerged as the primary clearance organ.One edge that SPECT holds over PET is its potential to simultaneously image multiple radionuclides, given the unique energy signatures of gamma rays from distinct radioisotopes. Yet, no research has showcased dual radioisotope imaging using radiolabeled nanoparticles, leaving questions about its advantages over single-isotope SPECT [75]. Over recent years, the adoption of PET imaging has surged in both research and clinical contexts. Figure 11 displays, the imaging contrasts between nontargeted and targeted nanoparticles, with the targeted nanoparticles demonstrating a clear accumulation in the tumor site, providing evidence of their enhanced specificity and potential for improved diagnostic imaging.

(b) PET Imaging via Nanotechnology

PET harnesses molecules labeled with positron emitters in trace amounts to capture and assess the workings of biological processes with minimal disruption. PET stands out with its heightened sensitivity in comparison to SPECT, primarily due to the absence of a collimator. The introduction of microPET scanners tailored for small animals enhances the in vivo imaging capabilities across species, like mice, rats, and monkeys. Such advancements ease the clinical application of novel PET agents, bridging the gap between animal models and human trials. However, studies focusing on PET imaging of targeted nanoparticles remain limited [75].

One substance of interest for biological applications, due to its distinct size, shape, and physical attributes, is the

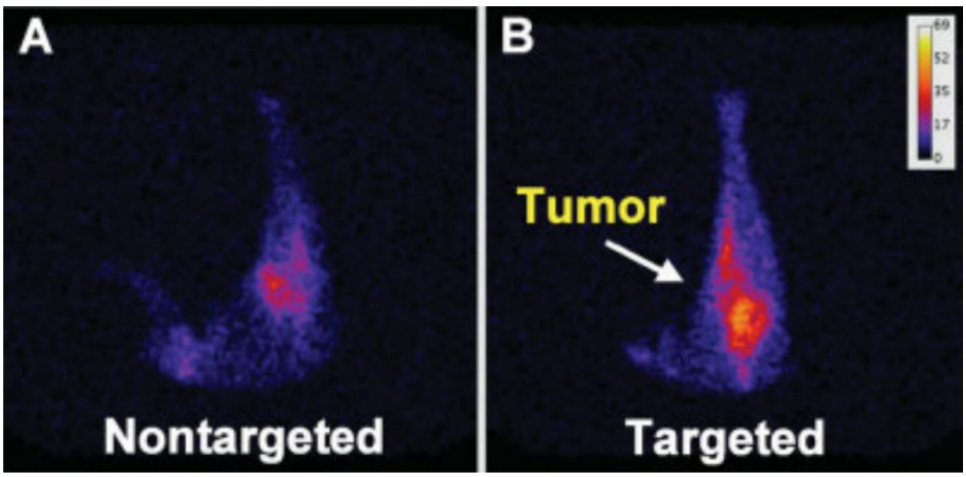


**Fig. 11** A comparison of 18-h post-injection planar scintigraphy images (15-min duration, 128 × 128 resolution) of rabbits, which had Vx-2 tumors implanted approximately 12 days earlier, following intravenous administration of 22 MBq/kg of either non-specific (**A**) or AMB3 peptide-specific (**B**) indium-111 (111In) nanoparticles, each containing roughly 10 111In atoms per nanoparticle. Image intensities have been normalized to the same scale, as indicated in image [74]

single-walled carbon nanotubes (SWNTs). A study by Liu and colleagues delved into understanding the distribution of SWNTs labeled with 64Cu in mice using PET, alongside biodistribution studies and Raman spectroscopy as shown in Fig. 12. Results indicated the in vivo stability of SWNTs, with findings highlighting that the length of surface PEG chains could influence circulation and distribution patterns. SWNTs, when modified optimally with PEG, showcased an extended circulation period (approximately 2 h) and minimal uptake by the reticuloendothelial system (RES) [76]. A targeted approach towards tumors in mice was achieved when these SWNTs were layered with PEG chains attached to cyclic RGD peptides. Further validation of these findings was done using the Raman signatures of SWNTs to confirm the radionuclide-based imaging results [77]. Given their potential, SWNTs are being evaluated for unified applications in molecular therapy and multimodal imaging.

Comparatively, radionuclide imaging offers superior tissue penetration capabilities than both ultrasound and optical imaging, along with a heightened sensitivity relative to MRI. A unique advantage it presents over other imaging techniques is its capacity to chronologically measure the concentration of radionuclides across different organs. Such data is precious for discerning the pharmacokinetics and overall distribution of the radiolabeled nanoparticles. However, it's essential to understand that radionuclide imaging specifically targets the radiolabel rather than the nanoparticle itself, rendering the nanoparticle's distribution an indirect measure. Hence, potential discrepancies might arise between the distribution of the nanoparticle and the radionuclide if there's any separation between the two [78]. This emphasizes the importance of thoroughly evaluating the stability of the radiolabel on the nanoparticle. Additionally, verifying the nanoparticle distribution becomes imperative

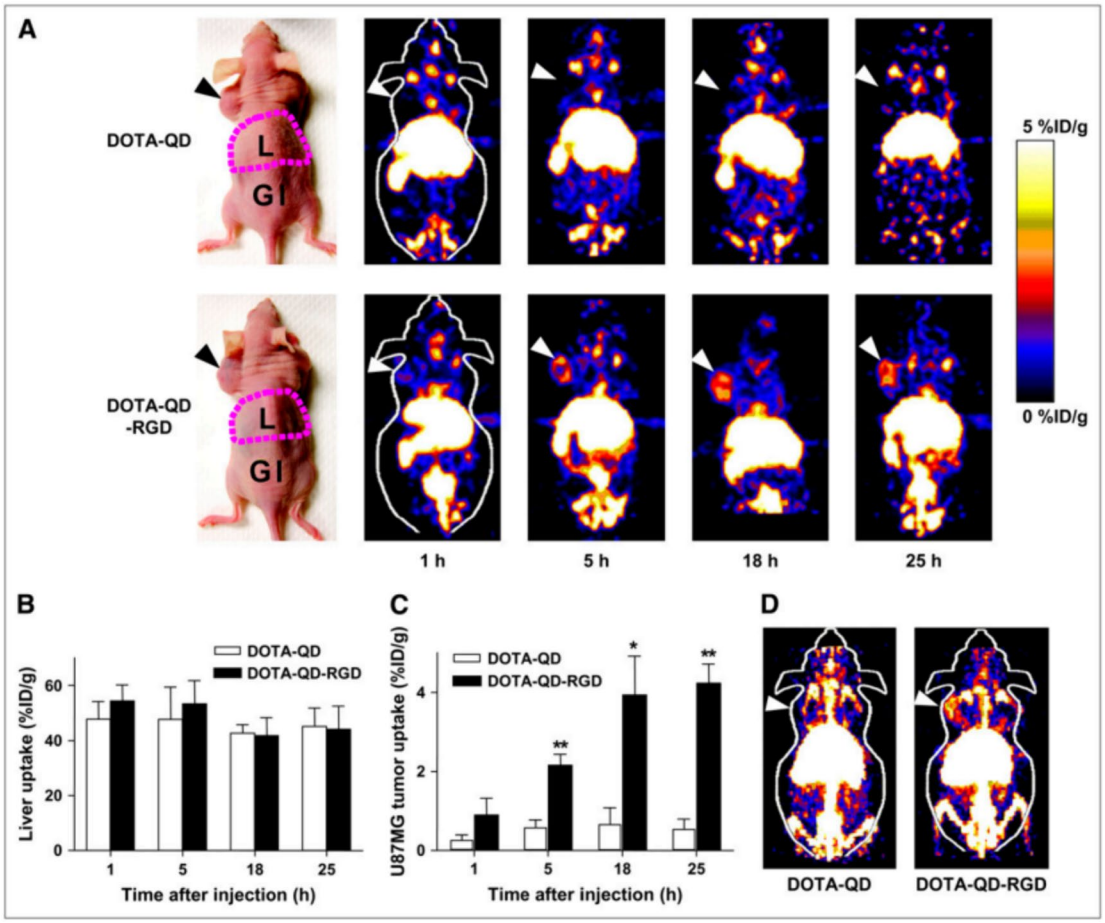


**Fig. 12** Positron Emission Tomography Imaging in Mice with U87MG Tumors Using a PET/NIRF Dual-Functional Probe. **A** Sequential coronal PET images of mice post-injection at 1, 5, 18, and 25 h with 64Cu-tagged DOTA-QD and DOTA-QD-RGD, with the tumor sites marked by arrowheads. The images represent 1 mm slice thickness. The gastrointestinal tract is labeled GI; the liver is denoted as L. **B** Graph showing the liver's uptake of both probes over time, determined by region of interest (ROI) analysis from the PET scans, with three subjects in each group. **C** Graph of U87MG tumor absorption of the two probes as time progresses, also measured by ROI analysis from the PET scans with three subjects per group. (D) Bi-dimensional whole-body projections of the same two mice featured in A, taken at 5 h post-injection, with tumors indicated by arrowheads. Statistical significance is noted where $^{*}P < 0.05$ and $^{**}P < 0.01$

to validate radionuclide-based results, as indicated in the aforementioned study. Advancements in nanotechnology paired with evolved bioconjugation chemistry suggest that radiolabeled nanoplatforms can be the cornerstone for seamlessly integrating targeted molecular therapy with non-invasive quantitative imaging.

#### Integrated Molecular Imaging Techniques

No single molecular imaging technique is without its limitations, and none provides a comprehensive view on its own. For instance, accurately quantifying fluorescence signals in living organisms, especially deep tissues, poses challenges. MRI boasts superior resolution but is limited by its sensitivity. On the other hand, radionuclide-based methods offer exceptional sensitivity but come up short in resolution. By merging various molecular imaging modalities, one can harness the strengths and counter the weaknesses of individual techniques. For instance, juxtaposing optical imaging with three-dimensional tomographic methods like PET, SPECT, or MRI, results in more precise and sensitive noninvasive imaging in living subjects.Small molecules prove tricky for multimodality imaging due to their limited attachment points and potential binding interference [79]. However, nanoparticles, with their expansive surface areas, are equipped to handle multiple functionalities, making them suitable for integrated molecular imaging. There have been instances where nanoplatform-based probes, designed for both MR and optical imaging, were used in experimental setups. For example, RGD peptide-adorned, MR-detectable and fluorescent liposomes were explored for their potential in live tumor imaging. Analyses revealed specific associations of these probes within the tumor environment, distinguishing between activated tumor endothelium and other regions.

Despite these advancements, some dual-modality probes only achieved single-modality noninvasive imaging. Yet, having a secondary imaging modality available serves as a reliable method for ex vivo confirmation, even if it isn't utilized for live imaging.In a remarkable study, *Cai* et al. designed a probe using quantum dots (QDs) for both NIRF and PET imaging [80]. By modifying QD surfaces with particular peptides and chelators, the probe could target specific tumor components and be detectable through PET after 64Cu-labeling. This innovation permitted tumor contrast imaging at much lower concentrations, thus minimizing the potential adverse effects of certain QDs, and opening up possibilities for biomedical applications [79].While most prior studies showcased the potential of dual-modality imaging, it was more typical for one mode to act as a validation for the other. However, recent innovations have started to fully exploit the combined strengths of both modalities.

Gene regulation via short interfering RNA (siRNA) has emerged as a potent tool for studying gene function in mammalian cells. A groundbreaking probe was introduced, which not only facilitated siRNA delivery but also permitted concurrent imaging of its accumulation in tumors through both MR and NIRF methods [81]. This groundbreaking research paves the way for combining therapeutic interventions with dual-modality imaging, leveraging the benefits of nanotechnology.

#### Targeted Drug Delivery Systems

Cancer remains a leading global cause of death, and its prevalence is on the rise despite efforts to reduce risk factors. Current treatments, like chemotherapy and radiotherapy, often harm rapidly dividing healthy cells, leading to significant side effects. Additionally, the poor solubility, stability, and metabolism of many anticancer drugs contribute to toxicity, inefficacy, and limited reach within the body. Thus, there's an urgent need for targeted treatments that effectively combat cancer while preserving healthy tissues. Nanotechnology offers promising solutions in the fight against cancer. Nano-based methods enhance transport across biological barriers, specifically targeting malignant cells and allowing sustained drug release. A variety of nanomaterials, ranging from 1 to 100 nm in size and made from organic, inorganic, lipid, and protein compounds, have been developed to address issues related to cancer drug delivery [82]. These nanocarriers offer several advantages:

1. Overcoming solubility and stability issues of anticancer drugs.
2. Protecting drugs from enzymatic degradation and extending their half-life.
3. Improving drug distribution and targeting.
4. Ensuring sustained drug release at cancer sites.
5. Delivering multiple drugs, reducing drug resistance.

Therefore, nanotechnology is reshaping the approach to drug delivery and the overall treatment landscape for cancer. This review will delve into the development of these "smart" nanomaterials, focusing on drug targeting and sustained release [75]. It will also cover the design of various nanomaterials like liposomes, dendrimers, inorganic nanomaterials, and polymeric nanomaterials, and will discuss the challenges and future prospects of nanomaterials in cancer management.

## Drug Delivery Approaches, Targeting, and Release in *Cancer* Therapy

The efficacy of anticancer drugs can diminish before they reach the target, leading to ineffective treatment and unwanted side effects. For optimal results, the drug dosage must be accurate and perform maximally within cancer cells.

To achieve this, nanomaterials designed to target tumor cells should enhance the drug's concentration at these sites, thereby minimizing toxicity to healthy cells. Two primary methods to achieve this efficient delivery are passive and active targeting.

### Passive Targeting [68]

Most nanomaterial-based anticancer drugs are introduced via intravenous injection, bypassing the absorption step essential after oral intake. The disrupted vascular barrier in tumors allows nanocarriers to accumulate at these sites. The size of these gaps in tumor vasculature varies from 200 to 2000 nm. Due to inadequate lymphatic functionality, nanoparticles aren't swiftly cleared, leading to accumulation in tumor tissues. This phenomenon, known as the enhanced permeability and retention (EPR) effect, is the cornerstone of passive targeting as shown in Fig. 13. The accumulation of these nanocarriers largely depends on characteristics like size, shape, surface charge, and chemistry [83]. The size influences the nanomaterial's accumulation and delivery rates at the tumor, with factors like blood flow, interactions with biomolecules, and immune system clearance playing roles. Successful passive targeting applications have led to breakthroughs in clinical treatments. The first FDA-approved nano-drug is a PEGylated liposome containing doxorubicin, used against HIV-related Kaposi sarcoma and ovarian cancer. This encapsulation decreases the drug's toxicity, leading to better drug behavior, distribution, and release. Another recent FDA-approved formulation, CPX-351 Vyxeos™, encapsulating a cytarabine–daunorubicin combination, extended overall patient survival from 6.0 to 9.6 months in high-risk acute myeloid leukemia cases [83].

Numerous formulations targeting cancer therapeutics have received approval, paving the way for innovative treatments. Enhancements in nano-formulations can be made by adding functionalities that optimize their distribution and reduce immune system interference. Such refinements are further discussed under active targeting in this review. The efficacy of passive targeting is influenced by the variability of the Enhanced Permeability and Retention (EPR) effect across diverse tumors. The inconsistency in endothelial gaps, caused by aggressive tumor growth, leads to irregular entry of nanoparticles into the targeted region. While it's believed that nanoparticles should have higher permeability in the oxygen-deprived tumor core than its edges, some studies challenge this notion [85]. This variability complicates passive targeting strategies. Furthermore, factors like blood flow rate in the tumor's vicinity, which varies both spatially and temporally, pose challenges. Once nanomaterials pass through the blood vessels, their deeper infiltration into the tumor is obstructed by the tumor's structural matrix. Here,

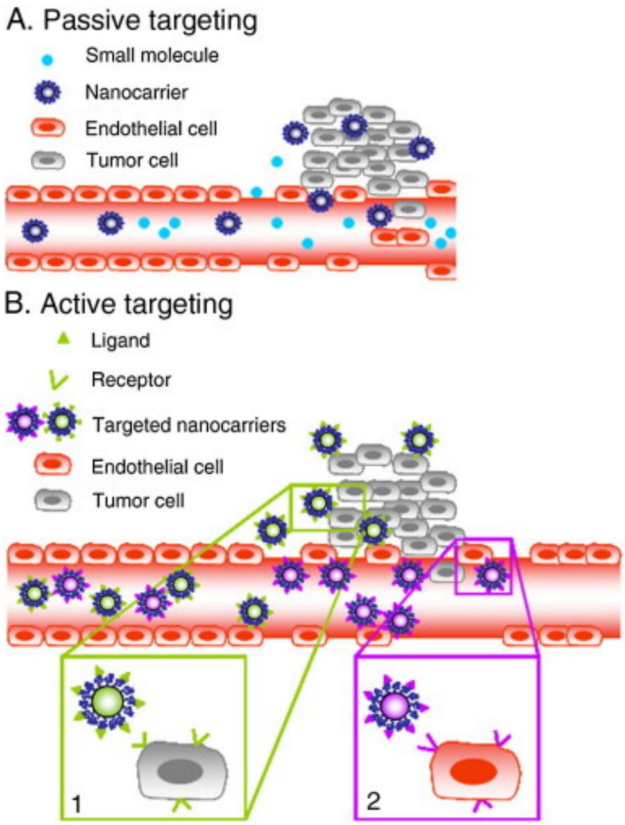


**Fig. 13** Strategies for nanocarrier drug delivery: passive versus active targeting mechanisms [84]

the nanoparticle's size and its associated attributes play a crucial role in enhancing its reach.

Innovative solutions, like the one proposed by Wong and colleagues, involve developing nanosystems that adjust size based on tumor location [86]. They designed gelatin particles that shrink from 100 to 10 nm upon entering the tumor, aided by enzymes found in the tumor environment. However, our comprehension of the EPR effect is limited due to the absence of accurate solid tumor models for humans. Current models, which are based on certain aggressively-growing subcutaneous tumors, may give an exaggerated perspective of passive targeting's effectiveness. Regrettably, there's a significant shortage of patient-specific data on EPR. To push the boundaries of cancer therapeutics, a deeper understanding of tumor biology and the nuances of the EPR effect across diverse tumors is essential [87]. Such insights will guide the development of tailor-made nanomaterials for more effective, personalized treatments.

### Active Targeting

Active targeting, often termed the ligand-directed method, hinges on the affinity-based identification, anchoring, and expedited absorption by designated cells as shown in

Fig. 13. The chemical affinity in active targeting revolves around distinct molecular associations, including receptor-ligand interactions, charged interactions, and specific motif-based connections with base molecules [88]. A variety of entities can serve as ligands, ranging from antibodies and proteins to nucleic acids, peptides, sugars, and even certain organic compounds like vitamins. Potential targets might include surface molecules on afflicted cells, proteins, saccharides, lipids within organs, or molecules in the vicinity of diseased cells. Cleverly engineered nanosystems harness the multiplicity of ligand interactions with target antigens, intensifying the nanoparticles' affinity for their specific targets. The process is amplified as the binding of one ligand molecule often promotes the attachment of subsequent molecules, cumulatively boosting the binding efficiency and subsequent activities [89].

The essence of the active targeting strategy is to amplify nanoparticle assimilation by target cells, thereby elevating drug delivery potency. One investigation highlighted that liposomes outfitted with anti-HER2 targeting ligand elements amplified the cellular absorption of these nanoparticles in HER2-positive cancer cells. On the flip side, liposome nanoparticles lacking these specific targeting elements mainly congregated in the tumor's surrounding areas, where they were promptly purged by macrophages, leading to insufficient tumor cell absorption. In a separate research, Zhou and colleagues engineered a magnetic iron oxide nanoparticle (IONP) system equipped with the therapeutic agent doxorubicin and conjugated with insulin-like growth factor 1 (IGF1) [90]. When introduced into a model of pancreatic cancer, this setup demonstrated outstanding tumor targeting and penetration. This IGF1-IONP-doxorubicin combination effectively stalled the progression of pancreatic tumors, demonstrating a promising avenue for enhanced treatments. Additionally, the team utilized magnetic resonance imaging (MRI) to monitor the position and assimilation of these nanoparticles, leveraging the contrasting attributes of IONPs. Moreover, these magnetically-targeted systems offer potential in photothermal therapy. Their precise positioning within tumors can generate localized heat to annihilate surrounding tumor cells when exposed to an alternating magnetic field [91].

#### Precision Targeting in Nanotherapy

Targeting strategies often harness the distinct molecular expression variances between cancerous and healthy cells. For instance, the epidermal growth factor receptor (EGFR), vital for epithelial cell growth and stability, displays increased expression in cancer cells due to their rapid proliferation. This differential in cellular expression serves as a foundation for studies pinpointing cancer cells with elevated EGFR levels. Given the intricate nature of administering nanoparticles and potential unwanted molecular interactions, merely differentiating nanoparticles' affinity for cancer versus regular cells isn't always adequate for precise and effective drug delivery [92]. Contemporary strategies have delved into targeting multiple cellular receptors using single nanoparticle systems affixed with various ligands. Bhattacharyya and colleagues, for example, developed dual receptor nanosystems employing gold nanoparticles [93]. They targeted both EGFR and folate receptor (common in ovarian cancer) using specialized antibodies. Results indicated this dual system outperformed its single-ligand counterparts in nanoparticle delivery.

Nevertheless, ensuring effective implementation of active-targeted cancer therapies requires optimizing several variables. The number of ligands on a nanoparticle can influence its binding strength, emphasizing the importance of how these ligands are attached. While covalent bonding methods are popular, systems relying on affinity connections via physical absorption have also shown potential. A central challenge is ensuring the longevity of these attachments in the body's challenging environment. Interestingly, a direct correlation between ligand density and overall affinity isn't always observed. Factors such as molecular saturation, ligand misalignment, bond limitations, and spatial hindrances might play roles in this unexpected result. Additionally, excessive nanoparticle hydrophobicity can increase its vulnerability to macrophage absorption, negating any benefits in targeting speed [94].

Two key elements underpin the efficacy of an active-targeted nanoparticle system: targeting precision and drug delivery capability. "Specificity" encapsulates the effectiveness of the nanoparticle's interaction with its target versus unintended interactions. During distribution in the body, a myriad of minor molecular interactions, like van der Waals forces, might impede the nanoparticle's path. External proteins and biomolecules can also latch onto the nanoparticle's surface, altering its identity and function—a phenomenon often referred to as Vroman's effect. These changes might make the nanoparticle less effective in reaching its intended location. Given that these systems depend on proximity to their target sites, it's crucial to understand their distribution profile. Elements such as immune responses and kidney filtration play a role in this process. Due to the typically low blood flow in tumors, mere ligand affinity isn't sufficient [95]. As such, nanoparticles designed for active targeting should exhibit prolonged circulation times and biocompatibility, combined with a neutral exterior to minimize unintended molecule binding.

The primary interaction sites for nanoparticle ligands are usually located outside blood vessel linings in tumors. Hence, active targeting heavily depends on endoplasmic retention effects. The degree of this retention varies among cancers, dictating the size and type of nanoparticles used.

But endoplasmic retention is just one component of a multifaceted tumor environment. Challenges for nanotherapies also arise from factors like slow tumor blood flow, tumor-associated macrophages, and the surrounding cellular framework. Grasping these intricacies can guide the development of more effective nanoparticle-based therapies, whether they utilize active or passive targeting.

### Nanotherapy Drug Release Approaches

The effectiveness of drug delivery is contingent upon the efficiency with which drugs are stored and the methodologies for releasing them within nanosystems. Few nanomedicine techniques to combat anticancer drug resistance are as given in Fig. 14. The efficacy of "storing" drugs within these systems relies on encapsulation or efficient drug linkage. Various nanoparticles have distinctive methods for housing drug compounds, which will be detailed later. A crucial strategy revolves around regulating the drug's release speed, either spontaneously or in reaction to specific triggers. This ensures both sustained release and the consistent presence of the therapeutic dose over a given duration. Nanosystems can be broadly categorized into two types based on their drug release triggers: open-loop control systems and closed-loop control systems [96]. Open-loop systems depend on external stimuli like magnetic or thermal changes, sound waves, or electrical fields to initiate drug discharge. On the other hand, closed-loop systems adjust the drug's release based on inherent stimuli detected near target locations. Several contemporary methodologies focus on the intrinsic properties of nanosystems. These systems can respond to changes in pH or temperature, degrade due to specific chemical surroundings, initiate drug release through oxidation–reduction reactions, or harness enzyme-driven release mechanisms [97].

(a) Redox-Triggered Drug Discharge

Redox-triggered drug release mechanisms involve nanocarriers that are sensitive to redox changes. These carriers have functional groups that react when exposed to oxidizing or reducing agents found around cancerous cells, such as peroxides, GSH, and free radicals. This reaction results in the breaking of chemical bonds. The ensuing chemical alterations may also influence the water-repellent properties of the polymer, affecting the stability of nanoparticles and causing the drug to be released. For instance, nanoparticles composed of the poly(propylene sulfide) polymer possess disulfide bonds [99]. When these bonds interact with

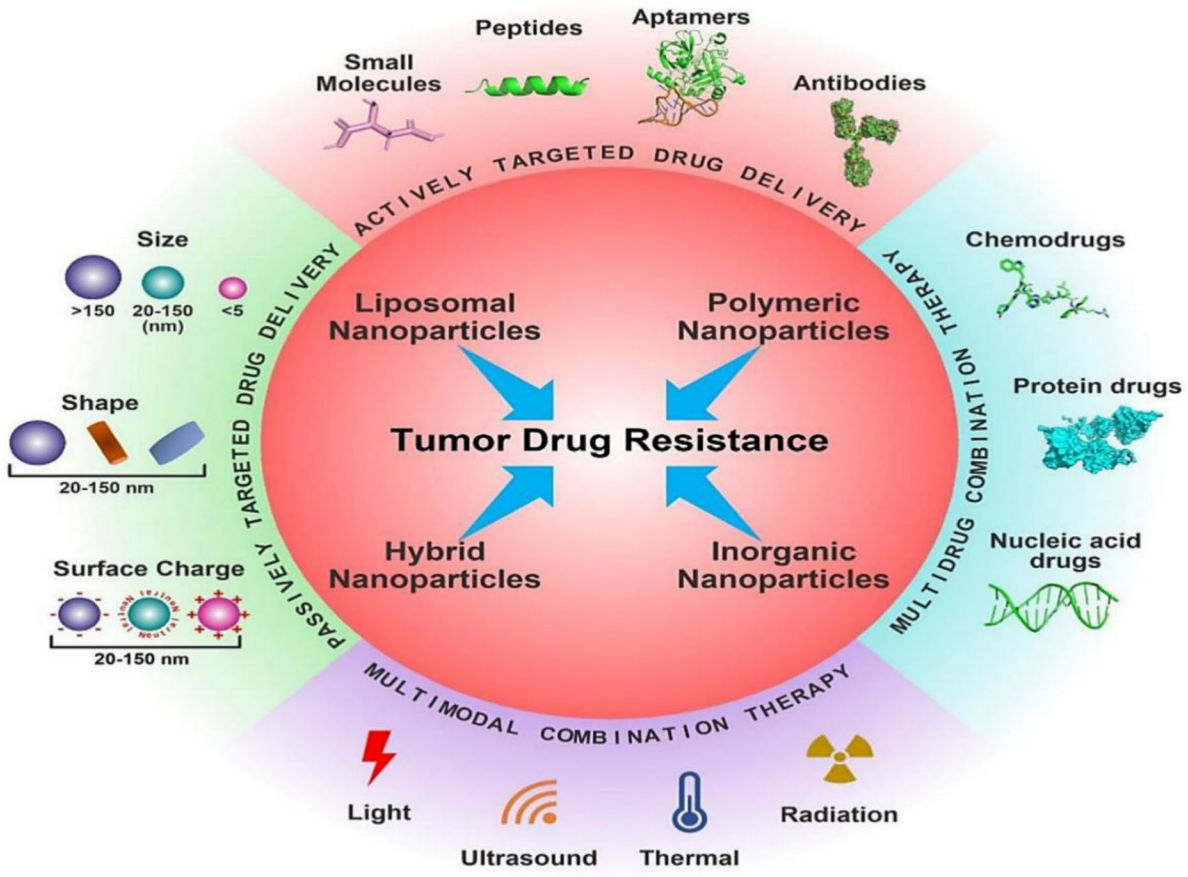


**Fig. 14** Varied Nanomedicine Techniques to Combat Anticancer Drug Resistance [98]

$H_2O_2$, the polymer's water-repellent nature alters, causing nanoparticle degradation and subsequent drug release. Furthermore, redox-sensitive components can react in intricate ways, ensuring they respond rapidly to high stimuli concentrations but remain stable at lower levels, thus maintaining targeted specificity.

(b) Drug Release Triggered by pH

Tumor tissue environments often possess a unique acidic profile, a result of the lactic acid released from glycolysis in conditions of oxygen deficiency. Research indicates that pH levels can decrease to approximately 6.5 from the usual physiological level of 7.4 during tumor growth or metastasis. This pH difference between healthy and diseased cells provides an opportunity for precise drug delivery. Various chemical bonds are researched that can facilitate this targeted release process. Chemical bonds that are sensitive to pH changes, such as the benzoic-imine bond, the 1,3,5-triazaadamantane (TAA) group, and the hydrazone bond, have been explored as part of these pH-responsive systems. For example, Gao and his team utilized benzoic-imine bonds to connect α-cyclodextrin to mesoporous silica nanoparticles (MSNs) [100]. These bonds underwent partial hydrolysis in the tumor's extracellular space and complete hydrolysis within endosomes having a lower pH, approximately 5. The team then evaluated the progressive release of the drug doxorubicin at varying pH levels to verify the system's efficacy [101].

(c) Additional Stimuli-Responsive Mechanisms

Researchers have delved into various other stimuli for regulating drug release. These include thermally-activated release using magnetic fields, systems activated by light exposure, ultrasound-triggered mechanisms, and those activated electrochemically. Thanks to the rapid progression in molecular biology and advances in enzyme technology, the potential for modifying or functionalizing nanoparticle surfaces is vast. The use of biomolecules to modify and conjugate can also greatly contribute to the creation of precise drug delivery systems, potentially overcoming the limitations of other techniques. Table 1 showcases an array of nanocarriers filled with drugs that are dispatched to tumor sites in response to specific activating stimuli [102].

## Impact of Nanocarrier Physicochemical Attributes on Drug Delivery Efficiency

Creating effective nanocarriers for drug delivery involves a detailed process, considering their physicochemical characteristics. Numerous materials serve as the foundation for these nanocarriers. Key attributes, such as size, shape, composition, surface charge, and biocompatibility, play pivotal roles in designing them for controlled drug release. These physicochemical traits determine how nanocarriers interact with cells, including how they adhere, behave, and accumulate. Such interactions can result in either therapeutic benefits or adverse effects. Therefore, optimizing these attributes is crucial to enhance their efficiency in medical applications [131]. The following section delves into these physicochemical aspects, highlighting their implications in both therapeutic and diagnostic contexts.

(a) Dimensions and Form of Nanoparticles

The dimensions (both size and shape) of nanomaterials play a pivotal role in how they amass in tumors and distribute throughout the body. The size particularly affects how cells take up the drug and how these nanoparticles interact with specific tissues for therapeutic objectives. Furthermore, these dimensions dictate the drug's encapsulation, release, and the overall stability of the nanomaterials. Recent research underscores how the dimensions of gold (Au) nanoparticles, specifically their size and shape, can sway the efficiency in delivering small interfering RNA (siRNA). In a study involving U87 glioblastoma cells, different sizes and forms of siRNA-linked gold structures were crafted, namely 13 nm spheres, 50 nm spheres, and 40 nm stars. Interestingly, larger structures were taken up by cells more readily than the smaller 13 nm spheres, underscoring the role of size and shape in cellular uptake dynamics and intracellular distribution [132].

Another study looked at Au nanoparticles of varying sizes, between 2 to 15 nm, covered with tiopronin. The findings suggested that smaller particles were more prone to accumulate in tumor tissues of mice. Similarly, when mesoporous silica nanoparticles of varying sizes were tested on HeLa cells, the 50 nm-sized particles showed optimal cellular uptake, marking their potential as drug delivery agents. Shape, alongside size, is vital. Research by Chithrani and colleagues emphasized that the cellular uptake of Au nanoparticles varies depending on their shape—spherical versus rod-like. Spherical nanoparticles showcased better uptake than their rod-shaped peers [133]. Additionally, when silicon-based nanoparticles of different shapes were examined, discoidal particles showcased broader organ distribution compared to other shapes.

(b) Surface Charge Dynamics of Nanoparticles

The surface charge of nanomaterials critically influences their medical effectiveness and suitability. One of the key elements guiding nano-bio interactions is this surface charge, dictating how nanoparticles engage with biological systems. Notably, a nanoparticle's cellular uptake is directly influenced by its surface charge, with studies indicating that nanoparticles possessing a negative charge often have better accumulation within tumor sites. Research exploring micellar nanoparticles revealed that tumor cells predominantly uptake negatively charged nanoparticles. These particles enter cells through various endocytosis pathways, such as clathrin-mediated, caveolae-mediated, and macropinocytosis. When examining in vivo distributions, it was observed

**Table 1** Stimulus-activated drug dispensation using diverse nanocarriers

| Stimuli | Nanocarriers | Drug | Target | References |
|---|---|---|---|---|
| pH | Hybrid micelles | Doxorubicin | Breast cancer | [103] |
| | Mesoporous silica nanoparticles | Doxorubicin | Cervical cancer | [104] |
| | Dendrimers | Doxorubicin | Breast cancer | [105] |
| | Coordination polymer mesoporous silica nanoparticles | Topotecan | Cervical carcinoma | [106] |
| | Gold nanocages | Doxorubicin | Breast cancer | [107] |
| | Chitosan nanoparticles | Tamoxifen | Breast cancer | [108] |
| | Polymeric nanoparticles | Cisplatin | Ovarian cancer | [109] |
| | Titanium dioxide nanoparticles | Daunorubicin | Leukemia | [110] |
| Redox | Mesoporous silica nanoparticles | Doxorubicin | Glioblastoma | [111] |
| | Polymeric conjugates | Doxorubicin | Hepatocellular carcinoma | [112] |
| | Polymeric nanoparticles | Camptothecin, doxorubicin | Breast cancer | [113] |
| | Magnetic micelles | Doxorubicin | Hepatocarcinoma | [114] |
| | Chitosan nanoparticles | Methotrexate | Cervical cancer | [115] |
| | Gold nanoparticles | Doxorubicin, methotrexate, 6-mercaptopurine | Cervical, lung carcinoma | [116] |
| Magnetic Field | Block copolymer nanoparticles | Doxorubicin | Lung cancer | [117] |
| | Magnetite nanoparticles | Doxorubicin | Multiple myelomas | [118] |
| | Magnetic nanoparticles | Doxorubicin | Liver cancer | [119] |
| | Iron oxide nanoparticles | Homocamptothecin | Squamous cell carcinoma | [120] |
| | PMAM-magnetite nanocrystallites | Cisplatin | Colon adenocarcinoma | [121] |
| | Superparamagnetic iron oxide nanoparticles | Valrubicin | Prostate cancer | [122] |
| | Magnetic nanoparticles | Doxorubicin | Cervical cancer | [123] |
| Light | Mesoporous bamboo charcoal nanoparticles | Doxorubicin | Breast cancer | [124] |
| | Telluride PEG co-block polymeric nanoparticles | Cisplatin | Breast cancer | [125] |
| | $TiO_2$–iron oxide nanoparticles | Artemisinin | Breast cancer | [126] |
| | Chitosan-based nanocarrier | Camptothecin | Breast cancer | [127] |
| Temperature | Liposomes | Tamoxifen, imatinib | Breast cancer | [128] |
| | β-Cyclodextrin star polymer | Paclitaxel, doxorubicin | Liver cancer | [129] |
| | Polysaccharide based nanogels | Doxorubicin | Cervical cancer | [130] |

that liver cells primarily absorbed highly charged nanoparticles. However, the uptake was diminished for negatively charged particles, suggesting a negative charge might minimize unintentional clearance by the liver [37].

Surface charge also plays a definitive role in the mechanism of cellular internalization and endocytosis. Typically, positively charged nanomaterials can more readily penetrate cell membranes due to the inherent negative charge present on cell surfaces. This characteristic can be harnessed for therapeutic avenues, especially when using nanoparticles for drug or gene transport. In recent research endeavors, cationic liposomes were formulated, and these were observed to favorably accumulate within tumor tissues. Furthermore, investigations by Villanueva and colleagues showed that the retention of magnetic nanoparticles within HeLa cells hinged on the surface charge and exposure duration. Remarkably, cationic magnetic nanoparticles remained within cells for extended periods without causing harm [134]. Another innovation in the realm of nanoparticle research is the development of charge-switchable nanoparticles. These are designed to alter their surface charge in response to specific external triggers, which has proven beneficial in boosting cellular uptake.

(c) Nanoparticle Surface Chemistry Insights

Designing the surface chemistry of nanoparticles is paramount when crafting them for specialized medical uses. By refining the surface attributes, one can not only enhance the stability of these nanoparticles but also mitigate their potential toxic effects. The cell interactions of polymer-rich nanomaterials, for instance, are notably shaped by their surface constitution.Recent innovations in the sphere of PLGA (poly(lactic-co-glycolic acid)) nanoparticles underline how apt surface modifications can boost their circulation time in the bloodstream, thereby amplifying their efficacy against tumors. In certain studies, nanoparticles with specific drug coatings successfully halted lung tumor progression in mice, showing greater effectiveness and fewer side effects compared to standalone drugs. Moreover, surface variations in PLGA nanoparticles, using coatings like polyvinyl alcohol

(PVA) or vitamin E TPGS, revealed discernible differences in cellular absorption rates [135].

Gold (Au) nanoparticles have emerged as a focus of cancer research. Investigations into the impact of surface coatings on Au nanorods have highlighted how certain coatings, like poly(diallyldimethylammonium chloride) [PDDAC], can modulate cellular uptake rates. PEG-topped Au nanoparticles, enhanced with specific drug compositions, have demonstrated increased effectiveness against particular cancer cell lines. Furthermore, mesoporous silica nanoparticles, when adorned with various functional groups, display diverse endocytic pathways, emphasizing the role of surface chemistry. Carbon nanotubes, when equipped with specific chemical features, have proven to be potent carriers for several therapeutic agents, including small interfering RNA (siRNA) and certain anticancer drugs [136].

It's evident that the surface chemistry of nanoparticles can fine-tune their therapeutic impact, dialing down unwanted reactions and amplifying the desired outcomes. But, it's crucial to meticulously analyze these surface modifications before transitioning to clinical applications. Presently, there's a plethora of tools and techniques to optimize nanoparticles for targeting specific cells, including the use of antibodies, peptides, aptamers, and other ligands. The choice of ligand is pivotal for enhancing tumor cell targeting. Additionally, after modifying the nanoparticle surface, it's essential to analyze the subsequent effects on toxicity, specificity, and sensitivity.In conclusion, elements like size, shape, and surface charge, coupled with surface chemistry, govern a nanoparticle's cellular interactions, therapeutic behavior, and distribution. Other factors, like nanoparticle purity, stability, chemical makeup, and aggregation state, among others, also play significant roles in shaping their biological interactions and outcomes [137].

#### Nano Drug Delivery Challenges and Prospects [138]

While the integration of varied nanomaterials in drug delivery has showcased potential in revolutionizing cancer treatment, it's not without its challenges. The primary obstacles include:

1. Safety Concerns: The interaction of nanomaterials with biological systems can present toxicity concerns, impacting both their biocompatibility and efficacy. There are concerns that some nanocarriers might interact negatively with biomolecules, which could compromise the effectiveness of nanomedicines.
2. Manufacturing and Consistency: Scaling up the production of nanomedicines is technically challenging. With their intricate production processes, there are concerns about consistency between batches and the overall cost of production.
3. Regulatory Hurdles: The current regulatory landscape lacks specific guidelines tailored for nanomedicines. This absence can prolong the evaluation and approval process, hindering commercialization.
4. Clinical Translation: The journey from lab to clinic is fraught with challenges, primarily due to gaps in understanding the nano-bio interactions and the pharmacokinetics of drug-carriers.

While nanomedicine promises a new horizon in cancer treatment, its practical implementation is riddled with complexities. A deeper understanding of the interactions between nanoparticles and biological systems is pivotal. Future strategies must focus on design innovations, targeted deliveries, and more comprehensive regulatory guidelines to fully harness the potential of nanomedicines in cancer care.

### Bioimaging Techniques

Cancer, a major global health concern, often remains undetected in its early stages due to the absence of clear genomic markers and its complex nature. However, the advancement in biophotonics offers promise in early cancer detection. This approach leverages the fluorescence, scattering, and absorption properties of cells and tissues. The diagnostic methods discussed herein are particularly sensitive to the cellular and tissue changes during disease advancement. Techniques like confocal and two-photon fluorescence (TPF) recognize shifts in fluorescence signals in cells or tissues. Moreover, second harmonic generation (SHG) microscopy identifies morphological alterations in specific tissue structures, such as collagen. Raman spectroscopy, a non-invasive approach, acts as a fingerprinting tool, distinguishing between benign and malignant tissues. Finally, photoacoustic techniques offer molecule-specific detection, combining high resolution with deep tissue penetration [139].

#### Fluorescence Spectroscopy in Cancer Diagnosis [140]

Fluorescence spectroscopy is a vital diagnostic technique that leverages the emission spectra from tissues to pinpoint cancerous changes. These spectra represent intensity changes against varying wavelengths. The method offers two main forms: steady-state and time-resolved. Among them, steady-state fluorescence is popular due to its ability to analyze emission spectra without needing external fluorescent labels, facilitating in vivo studies without any tissue preparation. Figure 15 shows the quantification of tumor fluorescence during intraoperative optical cancer imaging.

The application and parameters of fluorescence vary based on its purpose—whether to diagnose an unidentified lesion, track its growth, assess the effectiveness of a treatment, or guide surgical interventions [142]. Effective

cancer detection using this method depends on the tumor's structure, chemical composition, and metabolic activity. For in vivo studies, factors like tissue pH, temperature, and cellular ionic balance play a role in the fluorescence outcome. Remarkably, certain molecules, such as serotonin, shift their emission peaks based on pH levels, potentially acting as markers for the acidic environment found in tumors [143].Oxygen deprivation or hypoxia, a common trait in solid tumors, influences cellular metabolic processes. These results in changes in the ratio of primary fluorescent coenzymes like nicotine adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), which can be detected through their fluorescence patterns. Moreover, cancer cells often exhibit increased metabolism, demanding a higher absorption of tryptophan from surrounding areas [144]. This amino acid's fluorescence changes with pH fluctuations, making it a potential cancer indicator. Notably, tumors degrade the tissue's extracellular matrix, leading to decreased fluorescence from structural proteins like collagen and elastin.

Interestingly, one early discovery in light-induced fluorescence (LIF) was the alteration in endogenous porphyrin fluorescence, which signaled pathological changes ranging from infections to cancer. By comparing fluorescence spectra between healthy and malignant tissues, researchers found differences in the fluorescence emission patterns. This highlighted the accumulation of porphyrins in cancerous tissues. In addition, laser-induced autofluorescence (LIAF) lifetime spectroscopy has shown its potential in differentiating between healthy and cancerous tissues in various cancers. For instance, the average fluorescence lifetime was identified to be distinct between healthy and malignant tissues [145].As cancer progresses, tissues undergo significant morphological and chemical alterations. These changes lead to noticeable variations in the fluorescence spectra, serving as potential markers for early detection or even predicting tumor progression. The application of fluorescence spectroscopy spans various organs, from the lungs and brain to the skin, gastrointestinal tract, and cervix [146].

### Fluorescence Microscopy Techniques [147]

Confocal fluorescence microscopy (CFM) and Two-Photon Fluorescence (TPF) microscopy are advanced imaging techniques that offer non-invasive, high-resolution views of untreated tissues in real-time. CFM achieves this by using a pinhole to filter out-of-focus light, producing sharper three-dimensional images. Depending on the pinhole's size, it enables precise focus on specific tissue planes. CFM has proven to be especially effective both in lab settings on tissue samples and in real-time clinical procedures, particularly in gastrointestinal investigations [148].

For CFM, the most commonly employed excitation wavelength is 488 nm. This wavelength primarily highlights structural proteins like collagen and elastin in tissue samples. In a living organism, coenzymes like NAD and FAD also become visible. Despite its effectiveness, CFM has limitations like significant photobleaching [149]. In contrast, TPF, which requires two photons to simultaneously interact with a molecule, offers deeper tissue penetration with less damage, achieved using ultra-short pulse lasers. The TPF technique is particularly advantageous because of its ability to function within the therapeutic window of 600–1000 nm, allowing for more profound tissue exploration.In practical applications, a confocal endoscopy system was employed to inspect colon tissues, revealing potential for high accuracy in detecting neoplastic alterations during colonoscopies. Another study by Clark and team demonstrated that confocal techniques could discern features of oral cancers, potentially aiding in real-time cancer detection and treatment tracking [150].

TPF, on the other hand, has been employed in investigations involving tumors tagged with green fluorescence

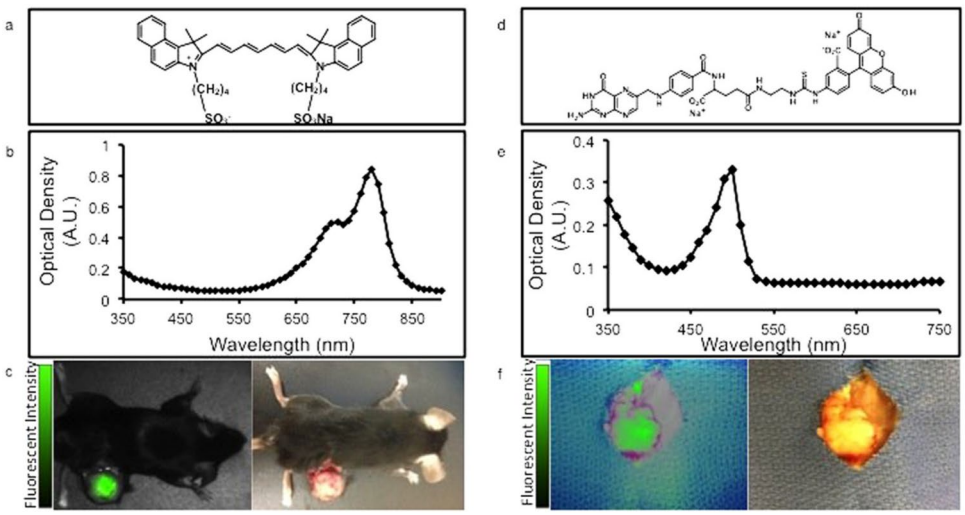


**Fig. 15** Quantification of tumor fluorescence during intraoperative optical cancer imaging [141]

protein (GFP). Results showed that as GFP-expressing cells increased, there was a correlating surge in fluorescence signals [151]. This technique also proved effective in identifying specific tumor targets in certain mouse models. Additionally, studies conducted by Allocca's team revealed that combining confocal and TPF techniques can yield significant insights into cellular interactions in bone cancers. Despite its potential, TPF's effective imaging depth is currently restricted due to various factors, necessitating further technological advancements [152].

#### Nonlinear Imaging with Second-Harmonic Generation Microscopy [153]

While traditional microscopy relies on a direct proportional relationship between a sample's optical response and incident light intensity, second-harmonic generation (SHG) microscopy employs a nonlinear approach [154]. In SHG microscopy, signals arise from the interaction of multiple photons with non-centrosymmetric molecules within biological samples. One of the notable advantages of this method is its minimal photon toxicity, allowing for detailed 3D visualizations of tissues and organs [155].A primary source of SHG signals in the extracellular matrix (ECM) is collagen. Collagen's structural alterations during cancer's advancement play a critical role in diagnosing and predicting the disease's course. By evaluating the shifts in collagen's SHG signals, researchers can gain insights into cancer progression [156]. Figure 16 illustrates, the application of P-SHG microscopy reveals distinct differences in tissue characteristics at the tumor boundary, providing detailed visualization of the transition from cancerous to normal tissue in human lung samples.

Burke and colleagues demonstrated the ability of SHG signals to predict metastasis in specific cancer patients. They focused on determining the risk in lymph node-negative (LNN) and estrogen receptor-positive (ER+) patients, hoping to better tailor treatments. Their approach, which centered on the SHG signal ratio from fibrillar collagen, showed promise as a supplementary tool to traditional cellular diagnostic methods.Furthermore, the use of polarization-resolved SHG microscopy has gained traction in oncology [158]. This advanced method can shed light on the minuscule structural changes in tissues. Researchers have used this technique, specifically Double Stokes-Mueller polarimetric SHG imaging, to differentiate between normal and cancerous breast tissues. The findings suggested that shifts in collagen orientation during cancer evolution could serve as crucial indicators of the disease.

*Zhuo* et al. utilized multiphoton microscopy, a combination of TPF and SHG techniques, to delve into the early detection of cervical cancer [147]. This study highlighted the structural intricacies of cervical tissues and explored metabolic markers to discern between healthy and malignant states.Another innovation in this field is the development of an endoscope designed for SHG imaging. This tool offers high-resolution insights into cervical collagen and its alterations, which are linked to conditions like preterm birth. Initial studies showed that this non-invasive approach provided more accurate data than traditional benchtop SHG devices, especially regarding collagen shifts during pregnancy. Further research showcased SHG's potential in studying collagen changes in post-menopausal women, shedding light on the physiological changes during menopause [156, 158].

#### Raman Spectroscopy [159]

Raman spectroscopy, which examines molecules based on their scattering characteristics, has garnered significant interest due to its non-invasive nature and precision at the molecular level. A standout feature is its ability to function without prior sample preparation, facilitating in vivo studies. The resulting Raman spectra offer insights into a molecule's vibrational mode density, which is then translated into its chemical composition [148]. Such molecular "fingerprints" pave the way for distinguishing benign from malignant tissues across various cancers.A noteworthy application has been in skin cancer detection. By leveraging confocal Raman spectroscopy, researchers have pinpointed differences between Basal Cell Carcinoma (BCC) and adjacent healthy tissues, especially focusing on unique Raman spectral variations. These differences have highlighted the technique's potential for diagnostic applications.Further, the technique has been extended to detect lung cancer. Using confocal Raman microscopy, researchers have employed Principal Component Analysis (PCA) to discern between healthy and malignant lung tissues [160]. This combination yielded promising results, allowing for differentiation based on the subtleties in spectral intensities.

Figure 17 demonstrates, the combined use of white light micrography, Raman spectroscopy, and cryo-transmission electron microscopy offers a multifaceted approach to the preparation and analysis of tumor tissues and biological fluids for the early detection and study of cancer. Advanced Raman processes, such as Coherent Anti-Stokes Raman Scattering (CARS) and Stimulated Raman Scattering (SRS), have emerged with heightened sensitivity, contrast, and functionality. Specifically, SRS employs a laser-driven approach to induce specific molecular vibrations, resulting in either gain or loss of photons [161].There's an increased emphasis on augmenting the selectivity of imaging. By fusing single-frequency SRS imaging with traditional Raman spectroscopy, researchers can achieve this. For instance, specific spectral ranges can be attributed to DNA, proteins, and lipids. Thus, by identifying these distinct Raman shifts,

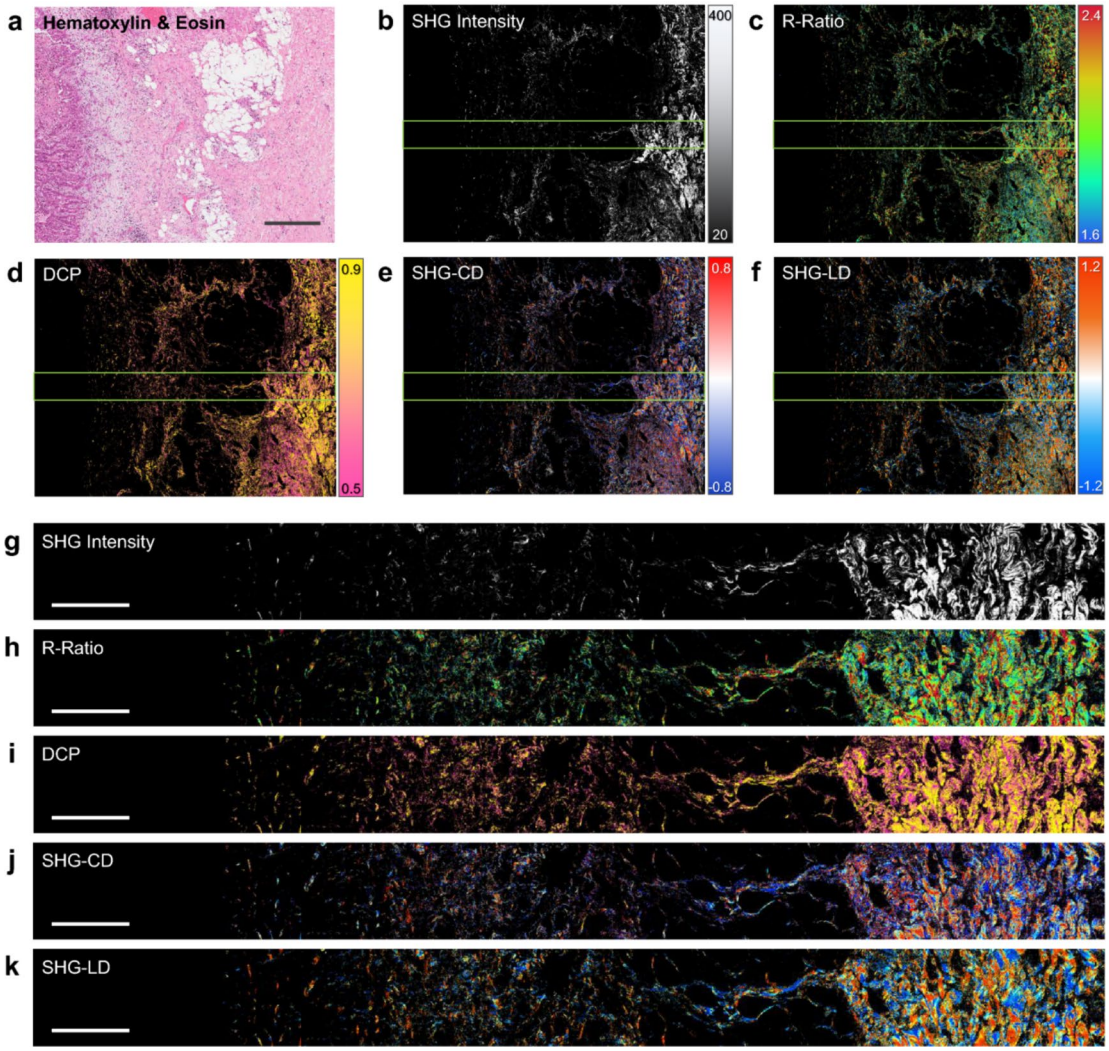


**Fig. 16** Multimodal P-SHG Microscopic Analysis of a Tumor Margin in Lung Tissue. Presented are images of an extensive section of human lung tissue: **a** a white-field image with Hematoxylin and Eosin staining, **b** visualization based on Second-Harmonic Generation intensity, **c** R-ratio mapping, **d** Degree of Circular Polarization, and images demonstrating **e** SHG circular dichroism and **f** linear dichroism. The scale bar represents 500 µm. Enlarged views within the green-outlined area highlight the polarimetric parameter variations between the tumor tissue (left) and the adjacent normal-appearing tissue (right), in figures (**g**)–(**k**). The scale bar for these close-up images is 200 µm [157]

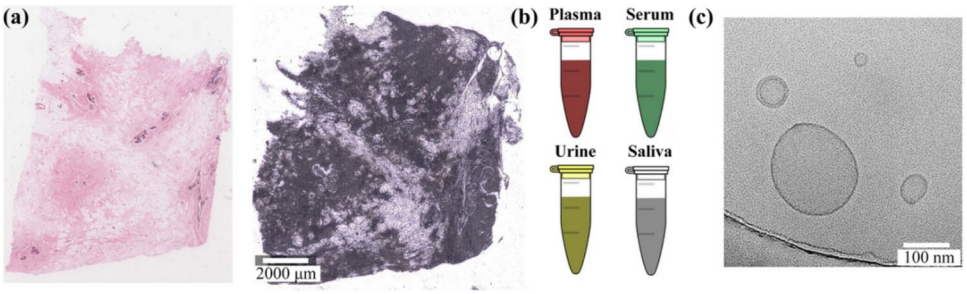


**Fig. 17** Preparatory Methods for Raman Spectroscopic Analysis of Tumor Tissues: **a** White light micrographs of both stained and unstained frozen sections of healthy breast tissue; **b** Raman spectral analysis of various human body fluids (plasma, serum, urine, saliva) for cancer detection; **c** Cryo-transmission electron microscopy visualization of extracellular vesicles (EVs) [162]

scientists can differentiate healthy cells from cancerous ones.A fascinating observation using SRS imaging was the unusual accumulation of certain fats in cancerous tissues. Specifically, there were two predominant fat types in the cancerous liver tissue. One type exhibited characteristics of saturated lipids, while the other showcased traits of unsaturated lipids. This stark contrast, combined with the analytical prowess of SRS, offers a promising avenue for in-depth cancer diagnostics [148].

In a vein similar to SRS, CARS microscopy provides a detailed analysis combined with high-definition 3D imaging. It is especially adept at identifying lipid-rich structures, making it a valuable tool for studying cellular content. Studies have noted an increase in lipid content in specific cancers, further accentuated by hormone treatments [147, 148]. CARS microscopy has played a pivotal role in identifying and characterizing these cellular lipid accumulations.In essence, Raman spectroscopy, with its diverse applications and techniques, stands as a beacon in molecular analysis, especially in the realm of cancer diagnostics.

#### An Overview of Photoacoustic Spectroscopy (PAS) [163]

Photoacoustic spectroscopy (PAS) operates on the principle of molecule absorption of modulated optical radiation, which subsequently generates an acoustic signal. This acoustic signal can be captured using a microphone, especially if the modulation frequency matches the molecule's non-radiative relaxation period [164]. The PAS technique produces an absorption spectrum when a laser beam spans a specific wavelength range over a spectral period.The intensity modulation essential for generating the acoustic signal can be achieved through techniques such as mechanically interrupting a continuous laser beam or employing lasers with nanosecond pulse durations. The design of the photoacoustic detector (PAD) plays a pivotal role in determining the sensitivity of the PAS method.Laser sources, particularly Optical Parametric Oscillators (OPOs), have been widely adopted in PAS due to their tunable nature across extensive spectral ranges. For instance, an OPO-based PAS instrument was developed to detect gases like propane, ethane, and methane, boasting an impressive detection limit of 100 ppm. The obtained results showed good agreement with established databases, such as HITRAN, with minor deviations in relative error [165].

PAS has been instrumental in detecting Volatile Organic Compounds (VOCs) related to lung cancer. VOCs like isoprene, styrene, and hexanal were evaluated as potential lung cancer biomarkers using an OPO laser source. When compared to the National Institute of Standards and Technology (NIST) reference data, the observed spectra for certain compounds displayed minor shifts.Researchers have also explored the potential of PAS in diagnosing respiratory diseases by analyzing exhaled air. A study involving patients with lung cancer, pneumonia, chronic obstructive pulmonary disease, and healthy volunteers used the PAS technique combined with advanced analysis tools such as the support vector machine (SVM) and Principal Component Analysis (PCA). The results exhibited commendable binary classification accuracy.In another study focusing on breast cancer monitoring, PAS was used to analyze the progression of breast tumors in mice. Researchers pinpointed specific frequencies that allowed them to differentiate tumor stages with high accuracy. Similarly, PAS has been employed to study ovarian tissues, differentiating healthy samples from those with anomalies. Multiple algorithms, including ANN, k-NN, and SVM, were utilized for data analysis, demonstrating high levels of specificity and sensitivity [164]. Figure 18 reveals, through the use of photoacoustic imaging, one can distinctly observe the physiological differences within tumor environments, such as variations in oxygen saturation, offering a non-invasive method to study tumor hypoxia and vascular structure.

PAS has also proven effective in distinguishing between blood samples from breast cancer patients and healthy individuals. This differentiation was achieved using a combination of wavelet analysis, PCA, and logistic regression applied to selected frequency regions.However, there are certain limitations to PAS. The detection limit is tethered to the laser radiation source's power, making high-power lasers more desirable. Effective PAS requires standard pressure conditions, as its sensitivity wanes significantly under reduced pressures. Additionally, ensuring accurate measurements mandates regular calibration of PAS instruments.

#### Photoacoustic Imaging Techniques and Applications [165]

The last decade has seen a surge in the adoption of photoacoustic microscopy and tomography for various imaging purposes, including single cells, tissues, and live specimens [99]. This imaging approach combines optical excitation and acoustic detection. It branches into three specific techniques: photoacoustic computed tomography (PACT), acoustic-resolution photoacoustic microscopy (AR-PAM), and optical-resolution photoacoustic microscopy (OR-PAM) [167].The depth and resolution of PAM are primarily determined by the optical beam's focus. A tighter focus results in enhanced spatial resolution but reduced imaging depth. Generally, there's an inverse correlation between imaging depth and PAM's spatial resolution, with an approximate ratio of 200:1. The choice of transducer also influences the axial resolution. PACT stands out for its ability to achieve micrometer-level spatial resolution over extensive depths, reaching several centimeters. Meanwhile, OR-PAM and AR-PAM differ based on their reliance on optical or acoustic focusing. Specifically, OR-PAM boasts a finer lateral resolution, while

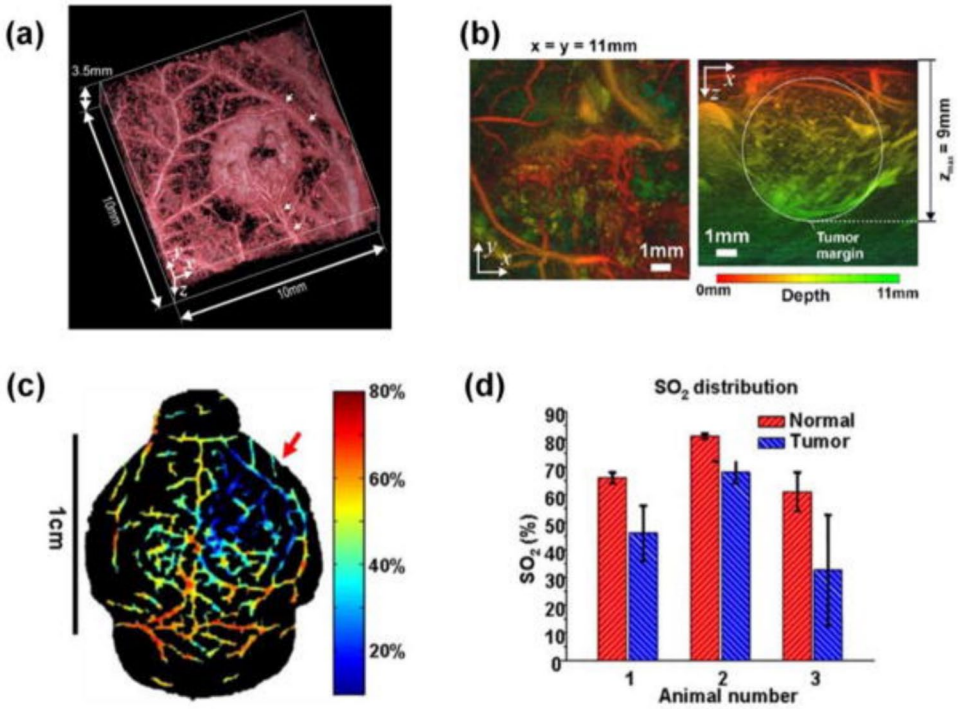


**Fig. 18** Photoacoustic (PA) Imaging of Tumor Structures with Intrinsic Contrast: **a** Three-dimensional PA tomography of an LS174T tumor, 8 days post-inoculation, captured at 600 nm with a step size of 70 µm. **b** x–y and x–z plane maximum intensity projection images of a larger SW1222 tumor, 12 days post-inoculation, obtained at 758 nm, with imaging depths reaching at least 9 mm. **c** Oxygen saturation ($SO_2$) mapping of a glioblastoma xenograft in a nude mouse brain via PA imaging, indicating hypoxic areas within the brain tumor; the tumor location is marked with a red arrow and is characterized by lower $SO_2$ levels compared to the adjacent vascular structures. **d** A graph showing the comparative $SO_2$ levels between normal and tumor vasculature in three mice, distinctly revealing the reduced oxygenation in tumor regions [166]

AR-PAM shines in axial resolution. Depending on the experimental setup, PAM can operate in either transmission mode (where the light source and detector are on opposing sides of the sample) or reflection mode (where they are on the same side) the latter being more apt for in vivo studies [168].

Several factors influence PAM's detection threshold, including laser intensity, tissue properties, and the ultrasound transducer's efficiency. Notably, PAM can analyze tissues without any labels, leveraging inherent chromophores like hemoglobin, melanin, and cellular DNA/RNA. By adjusting the excitation wavelength, researchers can target specific chromophores. For instance, the differential absorption between oxyhemoglobin and deoxyhemoglobin can be utilized to determine blood oxygen levels with impressive precision.Furthermore, PAM has shown potential in detecting glucose levels in tissue, paving the way for molecular-specific imaging [169]. However, water absorption in tissues can overshadow other signals in PAM, necessitating techniques like stimulated Raman processes to overcome this.

Another innovative application is the photoacoustic flow cytometry (PAFC), which excels in analyzing cells or nanoparticles in blood flow. Its sensitivity has been highlighted in detecting circulating tumor cells in vivo, showcasing its superiority over traditional in vitro methods.The versatility of photoacoustic imaging extends to vascular imaging, allowing for detailed analysis of angiogenesis a process linked with tumors. The heightened vascular density in malignant tissues offers increased PAM contrast, making tumor detection more straightforward [168]. Innovations like photoacoustic angiography further enhance the precision of vascular imaging, with resolutions capturing vessels less than 100 µm in diameter.In conclusion, photoacoustic imaging techniques, due to their exceptional resolution and penetration capabilities, hold significant promise for various medical applications. These range from monitoring brain activity and diagnosing skin conditions to assessing tumor progression and aiding surgical procedures.

### Terahertz Spectroscopy in Medical Diagnostics [170]

Terahertz (THz) waves have a unique interaction with water, largely because of the absorption by hydrogen bonds in this frequency range. This characteristic has been exploited in the detection of cancerous regions, which often have elevated water content due to heightened metabolic activity. Figure 19 shows, the THz spectrum system and THz

imaging system are pivotal in biomedical analysis, offering a detailed spectral assessment of biological molecules and precise imaging diagnostics for different cancer types.However, the strong attenuation posed by water molecules presents a challenge for THz waves. To counteract this, various methods like tissue freezing, embedding, vaporization, and the use of penetration boosters have been explored. Despite these hurdles, THz spectroscopy has been effectively used to analyze biological fluids like blood, saliva, and urine.For instance, the presence of sialic acid in saliva, an early breast cancer marker, was emulated using sialic acid powder dissolved in deionized water, combined with silver (Ag) and gold (Au) nanoparticles. THz investigations in the range of 0.2 to 1.8 THz showed that the nanoparticles slightly amplified absorption at 1.32 THz [171]. Concurrently, time-domain THz spectroscopy was used to identify oral lichen planus (OLP), a potential precursor to oral cancer. A detailed examination of 30 patients yielded exceptionally accurate results in distinguishing between different forms of OLP.

Another intriguing application of THz spectroscopy lies in the detection of microRNAs in biological fluids. These small, single-stranded RNA molecules can act as markers for various cancers, especially the miR-200 family. Additionally, exosomes, tiny vesicles produced within cells, were also analyzed using THz laser spectroscopy, showcasing the potential of this technique in diagnosing colorectal cancer. Blood studies have further highlighted the versatility of THz spectroscopy [172]. For instance, when examining the blood serum of rats with certain cancers, changes in protein levels and biochemical composition became evident. Another study focused on homocysteine, a compound linked to cancer risk, found in blood and urine. This compound was analyzed using a Fourier transform infrared (FTIR) spectrometer, offering a detection limit of about 10 µmol/L. When juxtaposed with Raman spectroscopy, THz spectroscopy exhibited superior correlation metrics [173].

## Nanotoxicity and Safety Considerations

The surge in nanomaterial production poses potential risks to both human health and the environment. The potential harmful effects of nanomaterials, especially in the realm of biomedical applications, raise significant concerns. Many initiatives have been launched to delve into nanotoxicity and its interactions with biological entities. Yet, evaluating nanotoxicity within living systems remains a complex endeavor. Our focus is to explore the complexities surrounding the toxicity assessments of nanomaterials. This review delves into the challenges faced in examining the effects of nanomaterials on various biological platforms, including in-vitro, in-vivo, and in-silico studies. Each method of assessing toxicity has its unique challenges, especially when dealing with nanomaterials in different states—powder, dissolved, or when interacting with biological entities. Proper tools and methodologies are crucial in navigating these challenges, and it's essential to consider factors like the shape, size, and morphology of nanoparticles during cytotoxic evaluations [174].

The unique properties of nanomaterials (NMs) arise from their diminutive size and increased surface area. However, when these materials come into contact with biological systems, they can elicit unexpected biological reactions. Notably, their minute size allows them to traverse further within the body compared to larger materials. As the production and application of NMs expand, so does the risk of occupational exposure. There are also growing concerns about potential disruptions to the environment and natural ecosystems [1–4]. Given these issues, researchers have been motivated to delve into the potential negative impacts of NMs

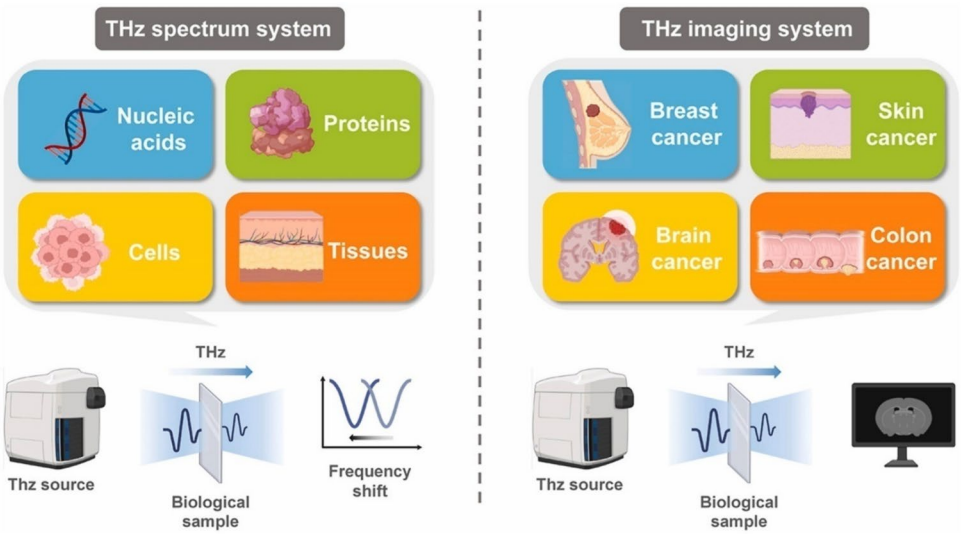


**Fig. 19** Terahertz (THz) Technology in Biomedical Analysis. The left panel (THz spectrum system) illustrates the spectral analysis of biomolecules such as nucleic acids, proteins, cells, and tissues using THz radiation. The right panel (THz imaging system) displays the application of THz imaging in diagnosing various types of cancer, including breast, skin, brain, and colon cancer, by creating detailed visual representations of the biological samples

on health and the environment. While numerous studies on NM toxicity have emerged over the years, comprehensively understanding the interactions between nanoparticles and biological systems remains intricate. This complexity often stems from the challenges in experimental methodologies and accurate characterization during toxicological evaluations. Alarmingly, as nanotechnology products become more prevalent, there's an escalating risk to human health upon exposure or consumption through various means [6].

### Issues in Detailing and Analysis

Understanding the toxicity of chemical entities necessitates thorough detailing and analysis of the materials. Today's technologically advanced landscape offers an array of methods for detailing nanomaterials in various states, such as powder, film, or solution, and for examining their interaction with biomolecules [3]. However, the intricacies multiply when dealing with nanomaterials. Their varied forms, encompassing diverse shapes, sizes, surface areas, charges, chemistries, crystalline structures, porousness, agglomerative tendencies, and solubility, call for more meticulous attention [4]. Ensuring consistent and reproducible results in experimental nanomaterials is paramount. Comprehensive examination of nanomaterials demands state-of-the-art equipment and adept professionals. The nuanced characteristics of nanoparticles and their associated toxicological implications remain largely uncharted. Hence, for nanomaterials, a holistic examination, covering facets such as size distribution, form, surface attributes, crystalline nature, porosity, agglomeration tendencies, charge, solubility, and more, is advocated to discern the interplay between their physical and chemical attributes and the resultant biological responses. However, researchers often operate within the constraints of their available resources and tools, underscoring the pivotal role that detailing methodologies play in deciphering the essence of nanomaterials. In terms of toxicity evaluation, the size of a nanomaterial can profoundly influence its behavior and interactions with biological entities [5, 6]. Techniques like Brunauer–Emmett–Teller (BET), dynamic light scattering (DLS), and transmission electron microscopy (TEM) aid in determining nanoparticle size [4]. A persisting hurdle, though, is to accurately ascertain average sizes and their spread, as various methods often yield disparate results. This stems from the distinct underlying principles of these techniques. Moreover, discrepancies can arise due to variations in sample preparation or instrumental procedures. Such disparities can lead to confusion in pinning down the precise size metrics, underscoring the importance of a solid grasp over the fundamentals and technical intricacies of the tools employed.

### Evaluating Nanomaterial Toxicity: In-Vivo, In-Vitro, and In-Silico Techniques

The journey and persistence of nanomaterials within organisms, including their assimilation methods, are better understood by examining the behavior and interactions of nanoparticles (NPs). Various methodologies exist to track the movement and impacts of NPs within organisms, predominantly categorized into in-vivo, in-vitro, and in-silico evaluations [6].

**In-Vitro Techniques [175]** In-vitro methodologies are often favored for toxicity testing due to their reliability, cost-effectiveness, broad applicability, extensive accessibility, and ethical advantage, as they often reduce the need for animal testing. These approaches attempt to replicate cellular interactions, providing insights into how organisms might respond. Such tests play a pivotal role in determining safe dosage levels and understanding the behavior of foreign substances within the body. By exposing various cell cultures to nanomaterials and subsequently evaluating cell growth and metabolic reactions, researchers can gain valuable insights. While drawing physiological inferences and predicting the behavior of foreign agents can be intricate, initial evaluations usually leverage in-vitro techniques due to their straightforwardness and ready accessibility [176]. The widespread adoption of in-vitro methods for rapid and thorough examination is testament to their efficacy. Various tests, differing mainly in how they discern cellular responses and their detection methods, are employed to investigate cytotoxicity.

In-Vitro Toxicity Testing: Popular Assays

(a) MTT Assay [6]

This colorimetric assay measures cell viability based on cellular metabolic activity. Active cells convert the MTT compound, a yellowish substance, into a purple-colored formazan product. The darker the color, the more viable cells are present. However, it's essential to note that some studies have revealed discrepancies, highlighting potential overestimations of viability in certain contexts [177].

(b) LDH Release Assay

When cellular damage occurs, the enzyme Lactate dehydrogenase (LDH) leaks from cells. By measuring LDH levels outside the cells, researchers can gauge cellular membrane damage and infer cytotoxicity. Nanoparticles might interfere with LDH activity, which requires careful consideration [178].

(c) Trypan Blue Staining

A classic method, the Trypan blue dye is absorbed only by dead cells, making it a tool to discern viable cells from non-viable ones based on color differences [179].

(d) Apoptosis Detection.

Apoptosis signifies programmed cell death. Several techniques, like TUNEL and Apostain, detect different apoptosis stages. TUNEL focuses on DNA fragmentation at apoptosis's end stages, while Apostain spots early-stage apoptosis by identifying specific enzymes [180].

(e) DCFH-DA Assay

This assay evaluates oxidative stress in cells, a factor often associated with toxicity. The non-fluorescent dye DCFH-DA enters damaged cells and, upon reacting with reactive oxygen species (ROS), emits fluorescence. The fluorescence intensity gives an idea of ROS levels and thus cell stress [181].

(f) Comet Assay

Also known as single-cell gel electrophoresis, this assay evaluates DNA damage within cells. Cells with DNA damage spread out in an electrophoresis gel, resembling a comet's shape, with the comet's tail indicating DNA fragments, offering insights into the extent of DNA damage [181].

**In-Vivo Methods** In-vivo evaluations remain the gold standard in toxicity assessments. These evaluations use live animals, which raises ethical concerns. However, they offer real-world insights, and the findings are more consistent with how the human body might respond, offering advantages in both time and cost.The outcomes from in-vivo assessments differ from in-vitro evaluations because of the complexities of living organisms. These include hormonal fluctuations, interactions between cells, and long-term chronic effects. These cannot be replicated in vitro. But in-vivo evaluations come with their challenges. The dosage given to animals must be precise, ensuring nanoparticles are dispersed uniformly. The potential of nanoparticles to cluster together can lead to inconsistent distribution and unpredictable outcomes. Once inside an organism, nanoparticles might interact with proteins, changing their characteristics, behavior, and distribution [182].

In a study by Chen and colleagues, the effects of 21 nm gold nanoparticles (AuNPs) were observed in male C57BL/6 mice. They used SEM for visual observations and real-time PCR to observe macrophage numbers and proinflammatory cytokine expressions. They found that AuNPs were largely compatible with tissues. They noted an accumulation in abdominal fat and traces in the liver, which led to fat reduction in treated mice.Another study by Rizzo et al. aimed to align in-vitro results with in-vivo observations using zebrafish embryos. They tested various nanoparticles, noting that those coated with biocompatible polymers were less toxic. While in-vitro studies on various cell lines showed minimal toxicity, zebrafish embryos exhibited lethal reactions to the same nanoparticle doses. They proposed that the lethal effects might be due to the aggregation of uncoated nanoparticles, which might block vital passages in the embryos [183].

Another research focusing on zebrafish embryos highlighted stage-dependent toxic effects of AgNPs ($97 \pm 13$ nm). Different developmental stages exhibited varied sensitivities to nanoparticle concentrations. The earliest stages showed prominent abnormalities, such as deformities in head and eyes, while later stages were less affected. This suggests that early developmental events like cell signaling and gene transcription are highly sensitive to nanoparticles. These nanoparticles were found in various parts of the embryo, with their specific locations verified using LSPR spectra [184].

**Digital Simulations in Toxicity Analysis** Given the urgency for time-efficient, ethical, and reliable results, researchers are veering towards alternative methods of toxicity evaluation. In-silico analysis emerges as a pioneering technique compared to traditional approaches, relying on theoretical predictions and computerized simulations of molecular properties. Although rich in advantages, in-silico methods are not without challenges, especially when they need to be validated experimentally. The gap in data and a limited scope in assessing the risk of nanomaterials on online platforms remains a concern. Present techniques focus on the production and manufacturing stages but often neglect the usage and disposal phases of nanomaterials. Modified computational methods like nano-QSAR or QNAR are being tailored to cater to nano-based models.In-silico techniques employ models that have a well-documented history or are at the cutting edge of toxicity assessment. Models based on structural alerts and rules help predict toxicity. While structural alerts identify specific chemical structures indicating toxicity, rule-based models are anchored in human expertise, literature, or data-driven simulations, relying heavily on statistical likelihood [185].

European projects, such as the GUIDEnano tools and the SUNDS, offer insights into the practical application of tools for assessing the full life cycle of nano-based products. These online resources are invaluable for the holistic assessment of nanomaterials, from production to disposal. It's crucial to adopt a life cycle analysis to gauge the environmental implications of nanomaterials. Risk prediction tools can be enhanced by assimilating data on nanomaterials' properties, behavior, and transformations.Nevertheless, some assays produce unreliable results due to issues like nanoparticle solubility, clumping, sedimentation, and protein corona formation. A standardized protocol, adaptive to the diverse nature of nanomaterials, can rectify this. Metallic nanoparticles and carbon-based nanotubes, for instance, display varying behaviors in physiological conditions, necessitating different approaches in assessment [186].

### Key Parameters for Evaluating Nanomaterial Toxicity

Nanomaterial characteristics are intrinsically tied to their cellular interactions. Recent breakthroughs in material science have enabled the precise creation of nanomaterials with specific attributes. Factors like size, surface texture, charge, and composition play pivotal roles in determining nanotoxicity [187].

(a) Importance of Size

The size of nanomaterials is integral in influencing their interactions within cells. Notably, smaller nanoparticles are frequently associated with heightened cytotoxic effects [83, 85]. Bharadwaj and team highlighted how varying nanoparticle sizes, between 20 to 500 nm, demonstrated differential accumulation in brain tissue sections. Moreover, the toxicity relationship between quantum dot size and their production methods has been observed [188].

(b) Surface Morphology

The shape of nanoparticles dictates their uptake and subsequent cellular impacts. Nanomaterials exist in diverse forms like spheres, rods, and needles. These different shapes can cause cellular membrane alterations, sometimes leading to ionic imbalances. Chithrani et al. documented that rod-shaped nanoparticles show a 500% increase in uptake when compared to spherical ones [189].

(c) Coating and Surface Charge Dynamics

Coatings on nanoparticles serve as the bridge between nanomaterials and cellular interfaces, influencing factors like cellular contact and internalization. Surface charges can modulate a material's cytotoxicity [190]. Predominantly, positively charged materials exhibit heightened toxicity levels compared to their neutral or negatively charged counterparts. Coatings can be classified into three categories based on interaction types. Furthermore, coatings can have pivotal roles in applications ranging from drug delivery to imaging. It's worth noting that coatings can mitigate or heighten the toxic effects of nanomaterials, as seen in the case of chitosan-coated CuNPs and $Fe_2O_3$ NPs [191].

### Looking Ahead and Concluding Thoughts

The rapid advancements in nanotechnology are ushering in a plethora of new materials. This underscores the importance of ensuring their safe application. Therefore, understanding the ecological implications and biological impacts of these materials is paramount, not to merely discard potentially harmful ones but to engineer safer alternatives. While various methods have been established to gauge the toxicity of nanomaterials, the varied nature of nanoparticles and the multiplicity of factors influencing their behavior complicate a straightforward understanding of their toxic effects. Generally, nanotoxicity investigations are bifurcated into two domains: environmental and biological.

Environmental factors, like temperature variations, ionic concentration, and internal transformations within biological systems, play a pivotal role in determining nanomaterial toxicity. It's crucial to study the behavior of nanoparticles in their aggregated form, the physicochemical alterations they undergo, and their interactions at the nano-bio interface. Many assessments focus on the toxicity of pristine nanoparticles. Yet, it's the transformed or decomposed versions that typically enter the environment, and these warrant attention, particularly concerning carbon-based materials on biological systems [192].

The second realm of study revolves around the biological destiny of nanoparticles. This demands high-end instrumentation, advanced culture mediums, and reliable in vitro and in vivo tests. While traditional viability tests remain foundational, we must also consider other metrics, such as the nanoparticles' stability within cells, their degradation potential, and excretion routes. Addressing nanotoxicity demands a harmonized, interdisciplinary approach to delve into the intricacies of nanoparticle-cell interactions. Especially challenging are the intracellular molecular processes and their connection to overarching biological functions. Contemporary methods lean towards exploring adverse outcome pathways, seeking more efficient, predictive mechanisms. These pathways shed light on early-stage molecular events, potentially traceable using sophisticated electroanalytical techniques. Parallelly, in-silico methods for assessing nanotoxicity have emerged as a promising, more ethically sound complement to traditional in vivo and in vitro tests. While computational simulations often rest upon experimental results, they offer a prospective avenue to predict nanotoxicity effects prior to production. With evolving technologies, it's envisioned that the future holds distinct predictive models for correlating nanomaterial properties with biological outcomes. Key challenges entail shifting from mere correlational studies to causative analyses, mitigating nanoparticle interference for clear in vitro assessments, deciphering cellular responses at a single-cell level, and elucidating the kinetic parameters governing nano-bio interactions [193].

## Clinical Translation and Commercialization

Nanotechnology stands as a beacon of promise in the fight against cancer. Its applications extend beyond the medical realm, potentially offering solutions to reduce pollution, decrease energy consumption, mitigate greenhouse gas emissions, and stave off diseases. NCI's Alliance for Nanotechnology in Cancer emphasizes the responsible development of these groundbreaking technologies. While nanotechnology might seem novel, the concept of nanosized particles isn't new. Nature has produced nanoparticles for eons, evident in phenomena like volcanic ash and sea spray. Additionally, human activities, such as the use of fire, have produced nanoparticles

in the form of smoke and soot. Given the pervasive presence of natural nanoparticles in our environment, scientists often face challenges in exposure studies, distinguishing between natural and engineered particles [194].

Safety remains paramount when exploring any novel technology and nanotechnology is no exception. The unique properties of nanomaterials that make them attractive for biomedical and industrial uses have also sparked discussions regarding their environmental, health, and safety (EHS) impacts. Notably, recent debates have arisen over the potential adverse effects of certain nanomaterials, such as carbon nanotubes (CNTs), after they were linked to tissue damage in animal studies. However, the prevailing sentiment suggests that nanoparticles, as a material category, don't inherently possess unique toxicities. Many engineered nanoparticles exhibit toxicity levels substantially below that of everyday products, like household cleaners, pet insecticides, or common anti-dandruff solutions [195]. Notably, nanoparticles utilized in drug delivery systems, particularly for chemotherapy, prioritize safety, ensuring drug transportation to tumors while sparing healthy tissues.

The NCI Alliance for Nanotechnology in Cancer extends the capabilities of its Nanotechnology Characterization Laboratory (NCL) to bolster nanotech safety research. This specialized unit within the Alliance performs rigorous safety and toxicity assessments of nanomaterials in controlled laboratory environments and through animal studies. The NCL's comprehensive evaluation approach delves deep into the interaction, biocompatibility, and efficacy of nanoparticles tailored for cancer treatments and diagnoses. The laboratory's extensive work has allowed them to assess over 125 distinct nanoparticles intended for medical purposes. Collaborating with esteemed institutions like the U.S. Food and Drug Administration (FDA) and National Institutes of Standards and Technology (NIST), the NCL aims to create relevant experiments and validate testing procedures for various nanomaterial types. This collaboration also aids in framing consensus-driven standards to gauge the environmental, health, and safety implications of nanotech applications consistently [196].

In summary, while nanotechnology offers immense potential benefits, it is crucial to navigate its development with an emphasis on safety. By proactively addressing both real and perceived risks, we can harness the transformative power of nanotechnology, especially in arenas like cancer research and treatment.

## Conclusions

The merger of nanotechnology with biomedical imaging has unveiled groundbreaking opportunities for early cancer detection, greatly refining the sensitivity, resolution, and targeted capabilities of imaging techniques. This review offers a comprehensive exploration of the state-of-the-art innovations in nanoplatforms-based imaging for cancer diagnosis. Through a detailed categorization of nanomaterials, from their dimensions to their origins, the review illustrates their critical roles in cancer detection, with 2D nanomaterials being prominently spotlighted. As we delve deeper, it becomes evident that nanotechnology not only enhances the quality of cancer imaging but also provides unique advantages in cancer treatment, enabling precise tumor targeting and facilitating the crossing of tissue barriers. The multimodal imaging segment brings forth an integration of various imaging modalities, emphasizing the potential of quantum dots and the revolutionary benefits of molecular MRI and radionuclide techniques. Furthermore, the review shifts its focus towards the strategic delivery of drugs, presenting a clear demarcation between passive, active, and precision-targeting in nanotherapy. While discussing drug delivery, the importance of nanoparticle physicochemical attributes and their implications on therapeutic efficiency are elucidated. The incorporation of bioimaging techniques, ranging from fluorescence spectroscopy to the innovative terahertz spectroscopy, reinforces the expanding horizons of diagnostic possibilities. However, while the advantages of these nanoplatforms are evident, the review doesn't shy away from addressing the pertinent concerns regarding nanotoxicity. Through rigorous in-vivo, in-vitro, and digital simulation methods, the review underscores the need for a meticulous evaluation of nanomaterial safety. Collectively, this exhaustive overview highlights the transformative potential of nanoplatforms in cancer diagnosis and treatment, but it also emphasizes the necessity for rigorous research, especially pertaining to safety considerations. The momentum in this field holds a promise, hinting at a future where early and accurate cancer detection becomes a standard, rather than an exception.

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**Author contribution** Kovuri Umadevi: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (equal); Methodology (equal); Writing-original draft (lead); Writing-review & editing (equal). Dola Sundeep: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (equal); Methodology (equal), and Writing-review & editing (equal). Alluru Raghavendra Vighnesh: Conceptualization (equal); Methodology (equal); Formal analysis (lead); Investigation (equal); Writing-review & editing (lead). Aroonima Misra: Formal analysis (lead); Investigation (equal); Methodology (equal); Writing-original draft (lead); Writing-review & editing (equal). Alluru Gopala Krishna: Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

**Data Availability** Data will be made available on request.

## Declarations

**Conflict of interest** All the authors declare that they don't have any financial or any other competing interests.

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