

# Chapter 3

## Evolution Towards Theranostics: Basic Principles



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### 3.1 Introduction

Nanotechnology is defined as the science of tiny particles lying within the scale of a few nanometers [1, 2]. The emerging fields in nanotechnology brought scientists together to develop a prognosis by merging diagnosis and therapeutics, leveraging their advantages. This provided insight into some major discoveries in effectively treating patients through early detection. Several deadly disorders including neurological and cardiovascular disorders could now be diagnosed and treated simultaneously [3, 4]. Moreover, personalized medicine for different diseases like cancer which shows heterogeneity has started to evolve with the developments in therapeutics modalities [5, 6]. To go deep into the approaches of many such case studies, we require an understanding of the phenomena behind the evolution of this therapy.

A single nanoparticle working as a diagnostic, therapeutic, and imaging agent can be substantiated in point-of-care testing. The term theranostic was proposed to define this aspect of treatment [7]. Surprisingly, although seems newly evolved, “Theranostic” was coined over 50 years ago when scientists struggled to determine a better way for simultaneous analysis and cure of thyroid cancer. The first treatment has been reported to incorporate nuclear medicine of Iodine-131 which is a radioisotope [8]. Undergoing various phases of development, it is now quite an effective

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and safe method of treatment. This paved the way for the discovery of many nanotheranostic agents further enhancing the potential of medical care [9, 10].

In practice, not only theranostics but also theragnostics materials are being widely used in hospitals and various markets worldwide [11]. Several companies have many theranostic agents in the pipeline, and the future will bring innovative advances giving a detailed perspective on this type of technology. While many nanoparticle-based theranostic agents have FDA approval, a few are undergoing clinical trial licensing under specific categories of imaging or therapeutic agents [12]. However, to develop an effective, reliable, and well-designed theranostic medicine, science has still to go a long way. It is important to establish universally acceptable, safety, and toxicity profiles to get regulatory approvals for launching multifunctional theranostic materials as a point-of-care diagnosis in the market.

Through this chapter, we will be enhancing our primary understanding of theranostics as a point-of-care diagnostic and therapeutic platform. Further heading towards the characteristics and mechanisms involved with special emphasis on intrinsic properties. We also wish to provide insight into behavioral studies in biological environments based on toxicological fate. The chapter will take the reader through the challenges to the solution and a future scope ending on a summary of a point-of-care theranostic device.

Overall, the chapter sheds light on a topic of new found interest, wherein we see the seamless integration of theranostics and point-of-care devices. The basic concepts of theranostics have been introduced at first, initially advocating the need for constant monitoring and patient centered therapeutics, which is then followed by unraveling the components involved in theranostics and its applications in point-of-care devices. The chapter then focuses on the use of carriers in theranostics, particularly nanoparticles and their route of administration, followed by the targeting approaches involved in ensuring that the carrier reaches the site of the infected or diseased cells. Recent advancements in the field have been reviewed with a focus on the affordability of such devices, advantages of these technologies over the already existent conventional ones, and the challenges involved in developing and commercializing such technologies. With personalized medicine becoming a topic of major interest, point-of-care devices having theranostic potential is picking up as an area of research in the foreseeable future and the chapter aims in exploring this domain and identifying the pros and cons of this medically advanced diagnostic and therapeutic platform amalgamated in a single device. The chapter provides comprehensive information about the types of theranostic materials, their applications, advantages, and challenges.

### **3.2 Basic Principle of Theranostics in POC**

Theranostics covers a wide range of topics that help optimize a vast range of therapies in the medical sector [13]. One such advancement of theranostics is its extension to real-time analysis which focuses on point-of-care testing. Point-of-care

testing is a type of healthcare made available on an immediate basis at any given point of time with the desired effect. Initially, this was utilized for an accurate on the spot diagnosis specific to a patient. However, with further development, therapy-based diagnosis has emerged for patient care in conjugation with professional consultation and advice. Since then many individually programmed kits have been utilized to rectify of a patient's disease and disorders [14]. The design, strategy, and novelty of such dynamic yet systemic approaches depend on deep apprehension and clinical proficiency. As we move towards the mechanism and different properties of nanoparticles used in POC, we have summarized the definition and the principle aspects of theranostics.

### ***3.2.1 Fundamental Prospects***

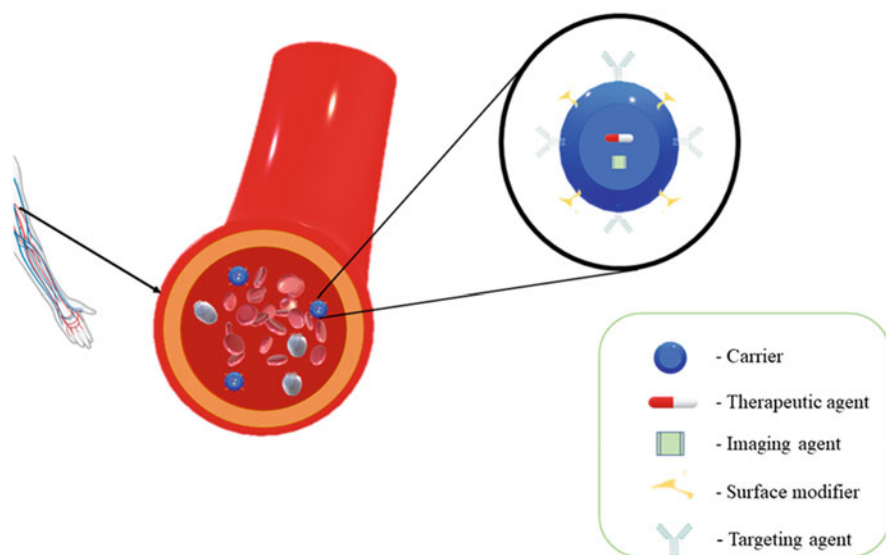
Biodistribution and therapeutic efficacy marked their occurrence soon after disease detection, where undesirable variation in therapy may have led to treatment failure. Medical imaging and therapeutics synergistically define theranostics as an integration of a biocompatible material package to diagnose and treat the patient. With theranostics, both the pharmacokinetic and pharmacodynamic fate of a drug inside the body can be traced and enhanced systematically [15]. The conceptual basis of theranostics in point-of-care testing is based on the principle interplay between pharmacogenetics, proteomics, and biomarker profiling of the patient. Pharmacogenetics assists in understanding the genomic sequence centralized on adequate biomarker response for an optimal outcome. A biomarker is a protein that acts as a biochemical indicator to monitor and diagnose every disease development stage during molecular treatment. This modulates designing a better drug moiety as a theranostic agent [16]. Moreover, the mechanisms of physiological pathways need to be determined for increasing the knowledge of an altered disease state. The changes in a gene sequence can adversely impact the drug binding and function [17]. Hence, another crucial part of patient care is predicting the protein infestations in revealing the encoded isoforms by the genome to get information about the physiological behavior of a cell. This provides the key to examine cellular function, localization, and expression of proteins. Different types of proteins behave differently in varying environments giving better prediction of variable biological information [18]. In contrast with community settings where generalized treatment is said to be dispersive, patient-centered treatment drifts evidence-based care improving accuracy and precision, possibly with the help of biomarker tracers. It includes molecular characterization, biomarker function, biomarker validation, and effective clinical utilization including bioinformatics tools [19].

The major concept behind extending these approaches has given a way to tackle physical clues associated with asymptomatic infections, supporting therapy. Organ-specific tests combined with electrical medical records and real-time image analysis have enhanced early detection of the target infestations [20]. Overall, this standardized shift from “one medicine for all” to a “fit for purpose” mindset has

revolutionized the healthcare sector. POC has principally been investigated to satisfy demand, seeking more improvised prognosis, diagnosis, and treatment [21].

### 3.2.2 Components

Due to their tiny characteristic dimensions, nanomaterials possess the ability to carry both the therapeutic and diagnostic agents along with the targeting moiety for drug delivery to a specific site. This has led to nanomaterials becoming an integral part of theranostics, which encouraged the use of the term “nanotheranostics”. Nanotheranostics in conjugation with point-of-care devices aim to utilize the principles of nanotechnology in advanced theranostics. Characteristics of the therapeutic agent, diagnostic agent, effect, size, shape, and type of material define a theranostic, as shown in Fig. 3.1. Implementing all the properties in a single module for achieving effective treatment has brought about various nanoparticles and their chemical functionalization. Surface modified liposomes, dendrimers, metal and inorganic nanoparticles, carbon nanotubes, solid lipid nanoparticles, micelles, and biodegradable polymeric nanoparticles have been specifically designed to circumscribe patient-specific medicines. Also, recent developments in synthetic biology have propagated the use of cellular systems such as bacteria, viruses, and mammalian cells as theranostic agents. However, they require strong validation due to the characteristic cell-based therapies employed for individuals [22]. The advent behind the theranostic nanoparticles’ design strategy is based on Ferrari’s classification which comprises three major components, namely a biomedical payload, a carrier,



**Fig. 3.1** Different constituents of a theranostic as a carrier system (created with Biorender.com)

and a surface modifier. The biomedical cargo consists of therapeutic agents, imaging agents, contrast agents, quantum dots, etc. After being processed, nanoparticles serve as carrier systems with inorganic or organic particles relinquishing sufficient protection to the therapeutic agent from biological invasion under physiological conditions for the therapeutic agent to be delivered efficiently. Surface modifiers increase the time of circulation by enhancing uptake and allowing easy penetration by crossing heterogeneous barriers for site-specific binding [13]. Upon further exploration, the types of technologies that come under the realm of point-of-care devices consist of label-free biosensors, microfluidic devices, lab on a chip technologies, lateral flow assays, smartphone-based applications, and wearable technologies. Gold nanoparticles used in lateral flow biosensors have contributed to improving the sensitivity of the methods as the formation of a sandwich occurs between the primary biomarker, the analyte, and the secondary biomarker tagged to a nanoparticle resulting in the appearance of color in the test zone. This could be attributed to the surface plasmon resonance effect. In the case of silver nanoparticles, the variation in nanoparticle morphology and associated optical properties results in different wavelengths, forming different tones of color in the testing zone which can be used in multiplex point-of-care tests. Electrochemical-based sensor nanoparticles have been explored to prepare inks for the fabrication of electrodes such as gold, silver, and carbon nanoparticle tubes. Also, magnetic nanoparticles employed in nuclear magnetic resonance imaging provide high precision diagnosis, owing to the low background signal. Nanowires of copper, nickel, platinum, gold, silver, and silicone are used in patient-centered medical support devices. The introduction of nanowires into paper substrates used for electrocardiogram monitoring depicts an application of using nanowires in POC devices [23].

Another aspect of theranostics has been the developing image-guided drug delivery by using imaging agents coupled with techniques like positron emission tomography (PET), fluorescence molecular tomography (FMT), and ultrasound. Apart from this, photothermal therapy (PTT) with magnetic resonance imaging (MRI) induces apoptotic cell death wherein near infra-red (NIR) visible light excites photoabsorber molecules and converts the energy of the incident light into heat by increasing temperature. Photodynamic therapy is another emerging modality where the reactive oxygen species produces a photo-necrotic effect at a suitable wavelength. The photosensitizer drug and the imaging moiety enable image-guided treatment of the diseased person, thus increasing the chance of better treatment [24].

### ***3.2.3 Point-of-Care Devices***

Point-of-care diagnosis technology has propelled the biomedical field towards engineering micro- and nanodevices amalgamating many clinical steps in a single build. Reliability, efficacy, portability, and affordability with ease of operation follow the basis of validation during clinical and personal use [25]. While many nanodiagnostic tools apply advanced optical, mechanical, electrical,

electrochemical, and mechanical concepts, there is no limit to improving the design of POC devices detection of diseases. Through nanoport technology, devising nanosensors with translating nucleic acids a lot of progress in the field of digitalization-based biosensors, for on the spot to recognizable electric signals and immediate mapping with already available datasets is possible [26]. Diseases like cancer whose tumor growth and development requires precise detection for effective treatment have also been explored as part of this domain. Antibodies carrying SERS (surface-enhanced Raman scattering/spectroscopy) nanotags with Raman reporters have been investigated to detect CD44, TGF $\beta$ RII, and EGFR biomarkers during in vivo tumor targeting, showing active signals for a longer time. Detection techniques involving SERS offer higher specificity and multiplexing simultaneous detections capabilities in a single test [27]. Alternatively, quantum dots (QDs) [28] can assist in detecting changes in the pH of the body's physiological and acidic tumor microenvironment [29]. Recently, many lab-on-a-chip miniaturized nanoparticle-based devices for real-time analysis have been developed to diagnose and treat infectious diseases. Quantum dot-based fluorescent nanoparticles, such as iron oxide due to its magnetic and metallic properties when labeled with various biological targets have shown wide scope in increasing sensitivity for immunoassay POC devices already existent in the market. Many such approaches have been extended to readily available and extensively used devices in cell phone-based polarized light microassay platforms. Diagnostic based magnetic resonance systems offer nuclear magnetic resonance (NMR) based rapid multiplex detection. However, its application is limited due to its affordability and portability. Other metallic POC nanodiagnostic tools including paper and magnetic barcode-based systems utilize lateral flow microfluidics, effectively displaying visible readouts by simultaneous detection of multiple infections [30]. However, many criteria remain unfulfilled and the following sections reveal the importance of integrating this promising technology with various upcoming developments.

### 3.3 Biological Factors Involved in Theranostic Applications

The developments in nanoparticles for point-of-care applications need to consider variety of biological factors to transform into a functionalized personal medicine. While dealing with theranostic applications, the design scheme involves two basic precision steps in nanoparticle formulation. Firstly, for diagnosis purposes, the nanoparticle design is crucially important in disease identification and treatment progression after administration. It is combined with various techniques like NIR fluorescence, PET/SPECT, and MRI. Second factor is related to the use as therapeutic by photodynamic, chemotherapy, gene therapy, hyperthermia, and radiation therapies [21, 31].

### 3.3.1 Administration of Nanoparticles

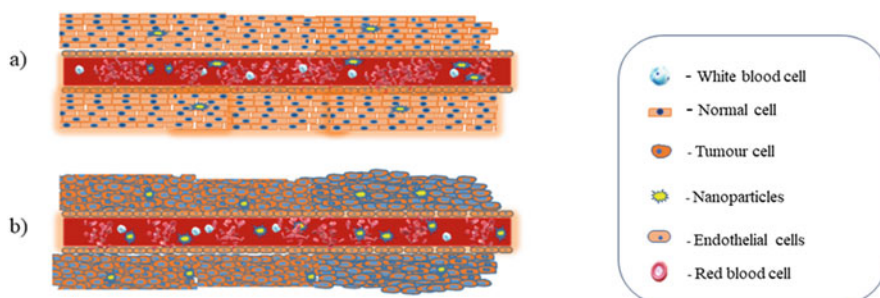
Path guided nanoparticles targeting a specific site is highly dependent on the route of administration. This purpose is served mostly by localized and systemic administration. In localized administration, nanoparticles are injected directly into the disease-affected sites. Similarly, the drug is particularly delivered to the tumor microenvironment (intratumoral region). However, in the case of systemic treatment, the nanoparticles circulate through body fluids hence have to encounter and overcome many barriers to reach the desired site [32, 33].

The accumulation of nanoparticles at the target site depends on engineered nanoparticles conjugating ligands (antibodies, enzymes, aptamers, or small molecules). The type of surface modifications depends on molecular markers expressed on the disease or disorder being treated. The property of enhanced permeability and retention (EPR) shown in Fig. 3.2 should be presented by drug for effective cancer treatment [34].

The state of action is the disease microenvironment being biologically different from the normal tissues acting as a mode for the targeted delivery of nanoparticles. The targeted delivery can further be classified as active, passive, and physical briefly described [35].

#### 3.3.1.1 Passive Targeting

In passive targeting, the nanoparticles are accumulated at the target site by an intrinsic property of the disease environment. The permeability factor is responsible for the crowding of nanoparticles. The neovasculature, developed due to angiogenesis at the diseased site, is different from that of the normal cells. They have high permeability. Such vascularization represents one characteristic property of blood



**Fig. 3.2** The accumulation of nanoparticles is less in normal cells. (a) as compared to the tumor cells. (b) The event termed as enhanced permeability and retention (EPR) due to wrapped vessels surrounding the tumor

vessels at the tumor. This distinctive feature is known as EPR supporting the accumulation of nanoparticles at the target sites [35].

### 3.3.1.2 Active Targeting

Active targeting utilizes affinity interaction as a key player in nanoparticle accumulation at target sites. Active targeting is more specific than passive targeting as it increases the reliability of drug-loaded nanoparticles. This requires actual chemical moiety for surface changes of the nanoparticle to ease proper interaction with ligand. Some examples include the conjugated ligands acting against folate, transferring, and human epidermal receptors. Biologically dynamic conditions like angiogenesis factor, uncontrolled cell growth, and tumor target have also been studied. One development is designing the nanoparticles by inhibiting the growth factors for preventing tumor progression [35].

### 3.3.1.3 Physical Targeting

Nanoparticle delivery involving internally and externally assisted approaches to enhance penetration is stated as physical targeting. Electroporation, ultrasound, and magnetofection are few examples of externally employed physical techniques used in nanoparticle delivery. Different physical properties of body homeostasis can also be utilized to configure nanoparticles. Physiological properties of the disease environment ought to act as a stimulant for nanoparticles, thus improving the drug molecules' delivery. pH, temperature, light, and hypoxia are a few factors that have been explored in passive targeting [35].

## 3.3.2 *The Journey of Nanoparticles to the Target Sites*

The systematically injected nanoparticles before reaching the target site have to pass through multifaceted biological elements on its way. Sequential events involve corona formation, blood, extravasation, tumor penetration, and intracellular trafficking [36]. Introduced nanoparticle first interacts with the biomolecules of blood components forming structure known as "corona." The corona formation depends on the physicochemical property and travel time of nanoparticles. Proteins present in corona mask the function of the engineered surface of nanoparticles. This even restricts the antibody exposure, hence resulting in reduced release of encapsulated drug molecules at the target site. Additionally, this might also activate the cascading effect of the immune response leading to hypersensitivity. However, not all protein interactions hamper the activity of nanoparticles. Proteins like dysopsonin and albumins reduce the phagocytic capability of the immune response against nanoparticle facilitating its release. Half-life and effectiveness of circulating nanoparticle



depend on the permeability of tumor vasculature. A shorter half-life during circulation is sufficient for nanoparticle accumulation in the target site because of increased vascular permeability at the tumor environment. In contrast, a longer circulation time is required for those having low permeability of the vasculature. Polyethylene glycol (PEG), present on the surface of nanoparticles increases blood circulation time and hence shows more effect. Moreover, nanoparticle interaction with protein reduces due to steric hindrance, rendering it less prone to immune attacks. During cancer progression, the presence of arteries and veins formed by the process of angiogenesis possesses higher permeability than those of normal cells. “Dynamic vents” could be helpful in the intake of nanoparticles to the tumor. Dynamic vents open momentarily, assisting penetration depending on the tumor permeability, nanoparticle size, and other physicochemical properties. Also, the conjugated ligand antibody against the biomarker is beneficial for tumor penetration. The higher the affinity between antibody and biomarker, the more is the penetration. Strong interactions lead to internalization of nanoparticles, whereas weak interactions of the antibody have extensive diffusion within the tissues exposing to intracellular trafficking. Likewise, some of the nanoparticles are engineered with RNA interference (RNAi). RNAi requires a cytosolic environment to be functionally active. Overall, all the processes cause nanoparticle for further modifications and might interfere with actually intended properties, thus are required to be studied thoroughly [36].

### 3.4 Recent Advancements in Theranostics

The prospects of theranostics for various diseases have been extensively investigated worldwide for the last two decades. Herein a few of the recent concepts leading the researchers towards the multipurpose probes and tools for affordable developments are listed. Organic, inorganic, composites, biomimicking, and biomaterial-based are all utilized to meet these demands and serve efficacious therapy. Few examples are represented in Table 3.1.

Smart and demand-responsive integration of biomaterial science with imaging modalities and disease biology has built the strong platform of theranostics. They can detect, sense, image, trigger, activate, block, and convey according to their design approach sufficing the demands. Theranostic platforms have revolutionized the clinical conclusions of various diseases and also the traditional methods to treat them.

Moderation of the traditional techniques gives the advantage of providing quick treatment rendering sufficient time for other latest technologies to emerge and gain validation for advanced treatment. In radiotherapy and brachytherapy, there is enormous scope for modifications and improvements in the probes. Designing new theranostic probes can lead to improved cancer treatment [37, 38]. Similarly, the theranostic eye lens is an excellent example of a wearable theranostics, with surface modification of lens surface for diagnosis and providing antiviral treatment

**Table 3.1** Overview of nanosystems for theranostic applications

S. no.	Targeted disease	Theranostic agents	Active agent	Platform implemented	References
1.	Cancer	64Cu-D3R-C18-DOTA And 64Cu-D3R-C16-DOTA	Cu and Lu	PE/TCT scan-based imaging and then brachytherapy dosimetry	Wang et al. [37]
2.	Medulloblastoma Cancer	90Y-DOTA-tyr3-octreotide (90Y-DOTATOC) And 68Ga-DOTA-tyr3-octreotide (68Ga-DOTATOC)	Yttrium and gallium	Targeting cell surface with a peptide attached radio isotope for imaging via PET followed by radiotherapy	Waldherr et al. [38]
3.	Breast cancer TNBC	Bioresponsive hydrogel nanoprobes	5-FU drug	Nanodevice sense, silence the specific gene sequence, and release the active drug	Conde et al. [39]
4.	Eyes	Theranostic eye lenses	Antiviral poly-sulfonate	Theranostic lenses to defend eyes with help of antiviral coating and detect IL-1 $\alpha$	Mak et al. [40]
5.	Gastrointestinal stromal tumors (GIST)	Cancer theranostic H-dots	Imatinib loaded H-dots	Image-guided surgery of GIST, with lowered immune uptake and increased tumor suppression with efficient drug delivery	Kang et al. [41].
6.	Bacterial infections	Siderophore based DOTAM theranostic scaffold	Siderophore conjugated catechol and DOTAM scaffold	Imaging the bacterial growth via a fluorogen activating protein and treat the infection by the antibiotic loaded in the cargo	Ferreira et al. [42]
7.	MDR bacterial infections	Sonotheranostic based nanoliposomes for MDR	Purpurin loaded in MLP <sub>18</sub> liposomes	Distinguish bacterial infection from inflammation or cancer site and treat resistant bacteria using a sonosensitizer	Pang et al. [43]
8.	MDR bacterial infections	Luminescent nanodrugs of ciprofloxacin	Ciprofloxacin based nanodrugs	Diagnosing the bacterial infection with help of nanosaggregates which are luminescent as well as antibacterial	Xie et al. [44]

9.	Bacterial infection	Fluorescent pH sensitive antimicrobial nanoparticles	Poly(poly-(ethylene glycol) methyl ether methacrylate)- <i>b</i> -poly(2-diisopropylamino) ethyl methacrylate- <i>co</i> -Hydroxyethyl methacrylate-chlorine e6 (PPEGMA- <i>b</i> -P(DPA- <i>co</i> -HEMA)- C6e)	Antimicrobial and photodynamic therapy under acidic conditions (pH 6)	Liu et al. [45]
10.	Colon cancer	Theranostic fluorescent emitting bacteria	Salmonella strains	Bacterial fluorescence imaging of tumor used in compaction with immunotherapy	Woong et al. [46]
11.	Orthopedic implants	pH responsive drug depots in titanium nanotubes	Ampicillin loaded in titanium nanotubes	Release of drug from switchable PMMA gates, when pH drops due to bacterial growth	Chen et al. [47]
12.	Bacterial biofilm infected tissues	pH response based antimicrobial photosensitive nanosheets	MnO <sub>2</sub> nanosheets loaded with chlorine e6	Detection of bacterial infected tissues by fluorescence and treatment by photodynamic therapy	Xui et al. [48]
13.	CNS	Bipolymeric nanogels	HEC and linseed oil-based polyol	Drug delivery to CNS by crossing BBB	Vashist et al. [49]
14.	Cancer cells	Gold-silica nanoparticles	Silica nanoparticles coated with gold nanoparticles and monoclonal antibody of anti-CTLA-4	Mitochondrial imaging by enhanced upconversion luminescent, followed by photodynamic therapy and activating immune response to provide anticancer therapy	Lin et al. [50]
15.	Cancer tumors	Bacterial based nanoheaters and imaging agents	Bacterial OMV packing melamin and other cargo proteins including fluorescent proteins and optoacoustic chromoproteins	Optoacoustic based imaging for diagnosis and therapeutic treatment of tumor by photothermal therapy	Gujrati et al. [51]
16.	Diabetes wound healing	Bioactive antibacterial dressing material	Polyethylene glycol diacrylate (PEGDA) with bioactive glass nanoparticles containing copper and sodium alginate	Silica based hydrogel scaffold to enhance wound healing in diabetes by increasing HIF1 $\alpha$ -VEGF expression	Li et al. [52]

(continued)

Table 3.1 (continued)

S. no.	Targeted disease	Therapeutic agents	Active agent	Platform implemented	References
17.	Tuberculosis meningitis	Aptamer based therapeutic molecules	G-quadruplex-(G4)-forming aptamer MS10-Trunc	Diagnosis and treatment of <i>Mycobacterium tuberculosis</i> (Mtb) MS-specific single-stranded DNA using G-quadruplex aptamers against them	Dhiman et al. [53]
18.	<i>Staphylococcus aureus</i> infections	Fluorescent vancomycin based nanoparticles	Vancomycin-silicon based nanoparticles	Diagnosis gram positive bacterial infections like <i>S. aureus</i> and effectively treat at lower concentrations than free vancomycin	Zhai et al. [54]

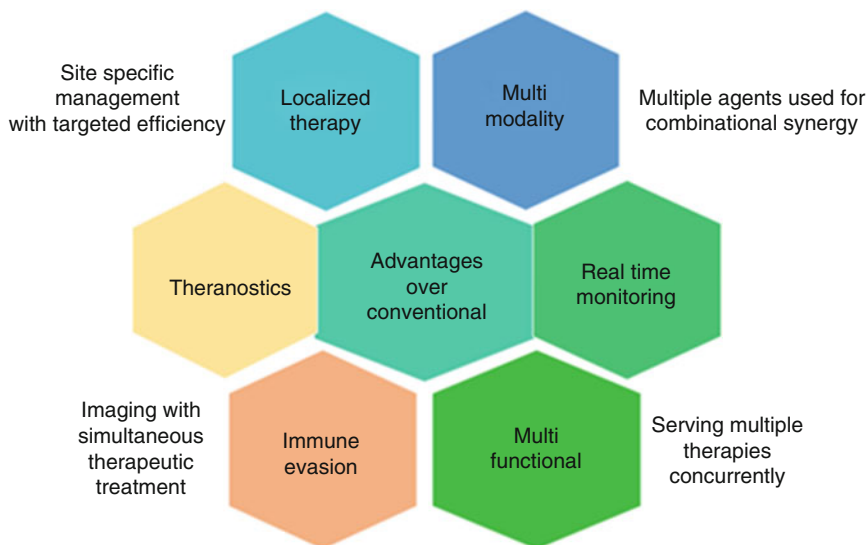
[40], while using sonotherapy to treat MDR bacterial infections has introduced a completely new treatment model [43].

Nanoparticles have great potential for various modifications, making them more preferable over others available for a wide range of clinical treatments. Photothermal therapy, photodynamic therapy, along with drug release and real-time imaging of the affected area, have been broad areas of research for the last two decades. Continuous efforts are being made to bring them to the hospitals, as they are much more cost-effective and have higher therapeutic efficacy with minimum side effects over other therapies in use. Gold–silica nanoparticles [50], antibiotic nanoaggregates [44], pH-dependent antimicrobial nanoparticles [45, 47, 48], bacterial based theranostics [42, 46, 51] are a few examples of a wide range of diseases. They provide therapy and diagnosis, in much more targeted and effective ways.

One among the stated, bacterial therapy is another mode of therapeutic treatment prevalent in research labs these days. To provide imaging and on-demand therapeutic efficacy, this mode of theranostics is being explored extensively as the delivery model for eventually targeting multidrug-resistant bacterial biofilms and infections, colon cancer models, etc. [42, 46, 51]. Nucleic acid-based sensors and delivery probes are a new mode of theranostics, which are the latest and least explored so far. Such sensitive point-of-care sensors have an enormous amount of opportunities for modification as they are more selective, sensitive, accuracy, and efficacy. Also, by activation, silencing, trigger, and blockade, drug delivery can be manipulated as per the requirement. For example, using DNA based active agents can help us sense the desired gene sequences, and then treat effectively [39, 53].

### **3.5 Advantages of Smart Theranostics Agents Over Conventional Therapy**

The design strategy of theranostics depends mainly upon targeting selective areas, providing imaging capabilities, and delivering specific cargo with minimal side effects. Nanotheranostics present new opportunities to upgrade the safety and efficacy of conventional therapeutics and have shown promising applications in diseases like cancer, cardiovascular diseases, inflammatory diseases, and pathogenic infections. Nanoparticles exhibit superior theranostic performance, owing to their exceptional properties for efficient drug loading, easy surface functionalization, biocompatibility, prolonged circulation time, high fluorescence, and photostability for bio-imaging. Some of the most widely utilized nanomaterials are magnetic nanoparticles, gold nanoparticles, carbon nanomaterials, polymers, dendrimers, and liposomes. The advantages of such theranostic systems are described below: Few of the advantages of smart theranostics have been shown in Fig. 3.3.



**Fig. 3.3** A schematic of the advantages of smart theranostic agents over the conventional therapies

### 3.5.1 Localized Therapy

Localized therapy refers to the treatment that is directed towards particular cells, tissue, or organ. This may include surgery, topical treatment, laser therapy, cryo-therapy, and radiation therapy. The delivery of therapeutic nanoparticles can be done by active (utilizing site-specific biomolecules) or passive mode (using enhanced permeation effect). In the case of neurodegenerative disorders, one of the major challenges has been the transportation of drugs to cross the blood–brain barrier (BBB). For Alzheimer’s, liposomal nanoparticles have shown successful crossing of BBB [3]. Dual functional PEG-PLGA nanoparticles present targeted delivery towards the amyloid plaque [55]. Generation of the long-lasting anti-coagulant surface over a freshly formed clot via thrombin targeted bivalirudin-functionalized perfluorocarbon nanoparticle provides site-specific management of acute thrombosis [56].

### 3.5.2 Multimodality

Combination therapy exploits the synergistic effect of more than one drug, which improves the efficiency of drugs and reduces side effects. This approach allows multiple agents to be delivered sequentially or simultaneously. For example, platinum-based lipid-coated nanoparticles encapsulated with mi-RNA is an excellent example of chemotherapy combined with gene therapy [57]. A tri-synergistic

approach (phototherapy–photodynamic therapy–chemotherapy) has been developed to overcome the chemotherapy resistance in tumor-bearing mice for breast cancer treatment [58]. AIE-based (aggregation-induced emission) based nanostructures and the derived systems show outstanding results in detection, differentiation, and killing various microbes. They also demonstrate the excellent application in image-guided PDI (photodynamic inactivation) for pathogenic infections [59].

### ***3.5.3 Simultaneous Diagnosis and Therapy***

Distinctive properties of plasmonic nanoparticles have been exploited for simultaneous diagnosis and therapy of different diseases. Electron rich surface of gold nanostructures makes them excellent contrast imaging agents. When combined with photothermal therapy, such a system can perform both functions. In theranostics, therapy with fluorescent imaging therapy has been utilized with metal nanoparticles like gold, iron oxide, silver nanoparticles, and quantum dot-based nanoparticles [60].

### ***3.5.4 Multifunctionality***

Bioactive molecules absorb near-infrared (NIR) region light, which has been a reliable tool for visualization, detection, and treatment of cancer [61]. Studies have shown the use of gold nanoparticles (AuNPs) as nanocarriers for drug delivery, photosensitizers for cancer diagnostics, and plasmonic photothermal (PPT)/photodynamic (PDT) therapy [62]. Similarly NIR active graphene sheet and quantum dot have been utilized for imaging, PDT and PTT [10].

### ***3.5.5 Real-Time Monitoring***

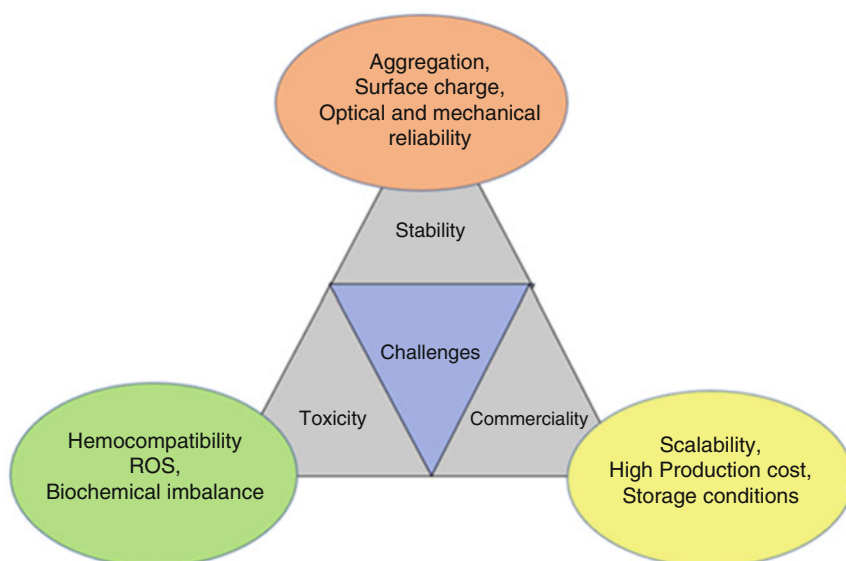
Upcoming treatment modalities in research insist on real-time monitoring of the position of malignancies in case of cancer or different parameters in case of other diseases. Sun et al. developed a vascular endothelial growth factor (VEGF) loaded-IR800 and conjugated with graphene oxide (GO-IR800-VEGF) nanoparticles for imaging angiogenesis of ischemic muscle in a mice hind limb model. This system was able to deliver VEGF to the injury site, and its efficiency was analyzed by positron emission tomography (PET) [63].

### 3.5.6 Immune-Evasion

Functionalization of nanotherapeutics with recognized antigens to increase their retention time is another emerging strategy. Specially designed formulations can induce biomimetic and bioinspired mechanisms. For example, PLGA coated with RBC membranes mimics both the surface and shape of the red blood cell membrane. In such molecules, both physical and chemical biomimicry act in synergy, mechanism that enhancing detoxification of nanoparticles. It further improves its survival rate in a mouse model of sepsis, resisting cellular uptake [64].

## 3.6 Challenges for Responsible Development

Development in theranostics is not immune to different challenges and limitations which demand further research. Nanomedicine offers numerous advantages over conventional drugs such as localized therapy, lower dosage, higher bioavailability, and an ability to dovetail diagnostics with a therapeutic agent. Even though this increasingly evolving field presents a promising alternative to conventional modalities of treatment, issues of stability, toxicity, and commerciality shown in Fig. 3.4 need to be addressed before considering them superior to existing drugs.



**Fig. 3.4** Overview of the major challenges being faced in the development strategies of smart theranostics



### 3.6.1 Toxicity

When using materials for biological applications, they are imperative to be biocompatible. Nanoparticle-associated toxicity can arise from various properties such as chemical makeup, morphology, size, charge, and surface modifications. Even though gold and platinum are inert, a cytotoxic cationic surfactant, CTAB (N-cetyltrimethylammonium bromide), which is used as a soft template in the synthesis of gold nanorods, needs to be replaced with other polymers such as polyethylene glycol (PEG) [65]. Similarly, with appropriate surface modifications, the cytotoxicity of other nanoparticles can also be mitigated.

The size of nanoparticles has a complex relationship with toxicity. Toxicity can be induced by interaction with cell membrane proteins and reactive oxygen species (ROS) generation. It has been reported that  $\text{TiO}_2$  nanoparticles with less than 10 nm and greater than 30 nm induce similar levels of ROS per surface area. However, ROS generation drastically increased upon the increasing size from 10 to 30 nm [66]. Nanoparticles of smaller size have a higher specific surface area. Hence, they interact more with cell membrane proteins, increasing their cellular uptake. The shape of the nanostructures also affects biocompatibility. Rod-shaped  $\text{Fe}_2\text{O}_3$  and  $\text{CeO}_2$  nanoparticles are stated as more cytotoxic than spherical nanoparticles [67, 68].

Moreover, the particles with higher positive surface charges are more toxic as they interact with negatively charged surface proteins. This leads to higher bioavailability due to enhanced endocytosis and a higher number of particles per cell remain cytotoxic. In literature, positively charged ZnO nanoparticles were found to be more cytotoxic than the negatively charged [69]. Different surface modifications can manipulate the surface charge. In one study [70], modifying surface of  $\text{Fe}_3\text{O}_4$  nanoparticles with negatively charged oleic acid decreased their cytotoxicity.

Nanoparticles confer low toxic effects as compared to free drug treatment or traditional chemotherapy. However, toxicity from nanoparticle treatment should be at the level where the efficacy is maximum compared to toxic effects.

Some nanoparticles may be nephrotoxic like Gadolinium, whereas inorganic nanoparticles might cause cytokine fluctuation in the body. Silica and silver nanoparticles in mice have shown Alzheimer-like symptoms, which might affect the nervous system. Copper oxide has shown dose-dependent toxicity as well as DNA fragmentation in neural cells due to reactive oxygen species (ROS) that weakens the brain process of learning and memory storage. Iron nanoparticles have also shown (Alzheimer's disease) AD-like symptoms [71].

Toxicity due to various factors discourages the clinical applications of nanoparticles in theranostics. Because the mechanisms that cause cytotoxicity are poorly understood, current research is focused on identifying biochemical and molecular mechanisms underlying the toxicity of nanoparticles.

### 3.6.2 *Stability*

Nanoparticles are more reactive and less stable due to higher surface energy. The stability of nanoparticles can be defined in relation to shape, aggregation, size, and surface chemistry [72]. Aggregation of nanoparticles is caused due to interactions at short distances. Plasmonic nanoparticles such as gold and platinum illustrate the changes due to aggregation well. Due to peculiar optical properties of these materials, they exhibit a color change of solution leading to differed absorbance. Their clustering can be characterized by spectroscopy and electron microscopy. Aggregation can be prevented by reducing the probability of collision of particles. Methods to decrease the collision include reducing the storage time, the concentration of nanoparticles in the solution, or performing chemical changes like surface modifications, and adjusting pH.

The stability of nanoparticles in terms of shape and size can be defined as retaining the morphology, size of individual nanoparticles as well as size distribution during storage and experiments. As nanomaterial's chemical, mechanical, and optical properties strongly depend on size and shape, changes in these components lead to the deterioration of desired performance. The dimensionality of the nanoparticles can be preserved by maintaining the homogeneity of the original solution [73] and adding stabilizing agents.

### 3.6.3 *Commerciality*

The valuation of nanomedicine industry is estimated to reach approximately \$334 billion by 2025. However, at present, the field is still in infancy and needs major transformation in terms of R&D and investments. For the translation of emerging healthcare technologies into the industry, factors such as superiority as compared to existing products, scalability, and costs are considered of paramount importance. The laboratory synthesis methods of nanomaterials consist of multiple steps, many of which involve the addition of reagents in stoichiometric proportions. After synthesis, purification methods and optimized parameters change from laboratory to industry.

Nanomaterials are synthesized by top to bottom approach in industries instead of the bottom to top approach in small scale synthesis. It enables enterprises to bypass the complex process of trace solvent removal. After scale-up, the desired properties of the material might be lost. For example, in the production of nanoparticles using the emulsion method, upon increasing impeller speed and agitation time, particle entrapment efficiency remained unchanged while nanoparticle size decreased [74].

Cost is also a significant factor in considering nanomedicine as an alternative to drug formulations. Nanotherapeutics are significantly expensive due to complex manufacturing processes, extremely lengthy, and more cost involved in the validation of concepts, difficult navigation through clinical trials, and sporadic

involvement of Big Pharma as compared to small and medium enterprises. For example, an anticancer drug Doxorubicin costs 8–9 times less than its nanoformulation, Doxil. However, it does not significantly increase the life span of patients. Some people justify the higher costs of nanoformulations due to their reduced side effects [75]. In the upcoming time, the increased involvement of large pharmaceutical companies and market-driven research can help overcome the current challenges faced by this industry.

### 3.7 Future Perspective

Currently, disease diagnosis and treatment encompass a single-target approach. Utilizing “multi-target combination” conjugated with multifaceted nanoparticles based multimode imaging system to treat complex disease will significantly improve treatment efficacy averting disease progression. Nucleic-acid based sensors and delivery probes are evolving as a mode of CNS and deep tissue disease theranostics expressing an enormous amount of opportunities in enhancing selectivity, sensitivity, and accuracy for POC devices. In-depth understanding of toxicity study synchronized nanoparticle modification will help in improving degradation and excretion rate. Further, finding ways to manufacture cost-effective miniaturization of nanodevices will aid a surgically placed prophylactic tool with routine monitoring and easy moderation. Also, intervening research will see new method optimization techniques, universally applicable diagnostic assay, improved universal safety guidelines, stability, and storage parameter advancements. Filling loopholes, these aspects can improve the current nanosystems leveraging human-based technologies.

### 3.8 Conclusion

The point-of-care in theranostic has been proved to be a dynamic domain where rapid advancement in precision and accuracy is liberating research and market value. Disease management has circumference early diagnosis, standardized therapy, and periodic monitoring. Delay in diagnosing, initiation of first-line therapy, and inability in detecting disease recurrence threaten life. Nanotheranostics concept of combining diagnosis and therapy in single multifunctional advent has evident “personalized medicine” allowing patient readout with maximum proximity and minimal intervention at individual latitude. The synergistic approach of nanotechnology actuated disease-specific molecular signatures has opened the possibilities of detailed visualization. Nanoparticles engineered surface modification and target functionalization have revealed the opportunity to transcend the immune system and increased molecules circulation time. Targeting specific biomarkers via active or passive mode further provides scope of enhanced cellular uptake of the active moiety reducing multidrug resistance and adverse side effects that would otherwise deter

efficient recovery. However, long term toxicity and stability remain a major challenge when adhering to better biocompatibility. Hence, health care offers nanotheranostic to explore with future advances in biomarker discoveries, diagnostics, drug-delivery systems, and biologics.

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