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Review

Nano-technological advancements in multimodal diagnosis and treatment

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Abstract: Nanomedicine, which is blazing new trails in modern healthcare, exploits the distinctive attributes of nanoparticles (NPs) to reimagine diagnosis and therapy. With imaging, this "magic ammunition" enhances the capability of MRI, a noninvasive, high-resolution technology that can pinpoint cancers with astonishing precision. Unlike traditional treatments, which muddle the distinction between diseased and healthy tissue, gold nanoparticles (AuNPs) directly target tumors, using increased permeability and retention (EPR) effects for crystal-clear, early-stage detection. While gadolinium (Gd (III)) T1 agents are the most widely used in clinical practice, iron oxide T2 agents have fallen out of favor due to failing to perform. Beyond imaging, NPs offer a "one-two punch" in the treatment of complicated disorders by enabling photothermal (PTT), photodynamic (PDT), and multimodal therapies (MMT), as well as nano drug delivery systems (NDDS). This nanotechnology-based technique personalizes medicines for diseases such as tuberculosis (TB) and cardiovascular disease (CVD), focusing on damaged tissues to improve therapeutic response. In tuberculosis, NPs circumvent medication resistance by delivering medicines directly to infected cells, thus avoiding biological barriers that impede standard treatments. Similarly, in CVD, NPs target arterial plaques to improve treatment accuracy while reducing systemic adverse effects. Furthermore, the genomic "blueprint" is evolving parallel to nanotechnology, with India's "Genome India" initiative mapping 10,000 genomes, lighting the path for genetic chips suited for specific disorders and

moment tests. The convergence of nanotechnology and genomes is an arcade modification, bringing accuracy and personalization to the forefront of cancer and tuberculosis treatment.

Keywords: nanotechnology; theranostics; diagnosis; regenerative medicine; nano-particles

1. Introduction

1.1. Nanotechnology

Nanotechnology is the manipulation of matter at the nanoscale (1−100 nanometers), which results in the creation of nanomaterials that are useful in a variety of fields, including electronics, sports, and healthcare. Due to the special qualities that nanomaterials, such as their chemical, electrical, optical, biological, and magnetic qualities, have demonstrated, this field has changed dramatically over the last three decades and has grown to be an important area of study [1]. Furthermore, a new field of study called nano-biotechnology has evolved, combining the study of molecular biology and nanotechnology with an emphasis on nanoscale structures and functional materials [2]. This publication attempts to investigate the transformative impact of nanotechnology in the fields of diagnosis, therapy, and imaging, with the following focal areas as the objective:

a. Enhanced Tissue Regeneration for Diagnosis and Treatment: We anticipated how incorporating reactive nanomaterial into hydrogels, such as poly (2-hydroxyethyl methacrylate) (PHEMA), promotes keratinocyte and fibroblast proliferation, allowing for better skin regeneration in diagnostic and therapeutic applications.
b. Microfluidic Innovations in Identification and Therapy: We assess the transformative impact of microfluidic technology on diagnostics and drug development, emphasizing its ability to enable precise diagnosis and effective treatment employing biocompatible PDMS polymers and cell-compatible hydrogel layers.
c. Organ-on-a-Chip for Personalized Diagnosis and Treatment: We discuss the effectiveness of Organ-on-a-Chip (OoC) devices in mimicking physiological tissue structures, allowing for personalized diagnosis and treatment while reducing reliance on animal testing and emphasizing the Lung-on-a-Chip model for insights into respiratory disease mechanisms.
d. Developments in Diagnostic Imaging Techniques: We investigate the incorporation of nanotechnology into imaging modalities, specifically how nanoscale contrast agents and nanoparticles increase early illness detection, hence improving both diagnosis and treatment outcomes.
e. Refinement of Drug Delivery Systems (DDS): Our evaluation focuses on the advances made in controlled drug delivery system to address the drug absorption and excretion, similar to the refinement achieved through the photothermal therapy (PTT) a revolutionary cancer treatment that transforms light energy into thermal energy to ablate tumor cells—the expansion of thermally responsive materials has paved the way for photothermal-responsive DDS, significantly enhancing diagnostic precision and therapeutic success [3].

Further, carbon nanotubes, dendrimers, nanoparticles, nanoprobes, quantum dots, nano-diamonds, and nanowires are examples of traditional nanostructures applied to nanotechnology. These nanostructures have unusual physicochemical features that allow them to efficiently cross tiny barriers

due to their small size. They can be classed according to specific qualities, such as optoelectrical properties in metals, catalytic capabilities in ceramics, and multiple uses in semiconductors [4]. Furthermore, nanostructures are classified into several categories, including lipid, ceramic, polymeric, and semiconductor nanomaterials. We go into further length about such nanotechnology subcategories below:

1.1.1. Carbon nanotubes (CNTs)

The continuous graphite sheets that make up carbon nanotubes (CNTs) have closed fullerene caps on the ends of each tube. Among all natural materials, they have the highest mechanical strength and effectively absorb electromagnetic radiation. CNTs have catalytic qualities and are efficient heat conductors [5]. Purity, length, diameter, surface area, and amorphous structure all affect their properties. CNTs are cylindrical and part of the fullerene nanotube family. Because of their capacity to cross cell membranes and deliver small organic molecules, including proteins, medicines, and nucleic acids, to specific locations, they are employed in the healthcare industry. Functionalized CNTs can attach to proteins smaller than 80 kDa either covalently or non-covalently, allowing easier endocytosis and subsequent cellular uptake. CNTs are heated by infrared lasers for therapeutic reasons, which may allow them to target and eliminate cancer cells without damaging nearby tissue and are employed in X-ray imaging [6]. Gold-coated, surface-modified CNTs have emerged as optical nanotheranostic probes, allowing for targeted Raman imaging via NIR laser excitation. However, their clinical value is restricted due to delayed biodegradation and the hazards of free radical-induced lipid peroxidation, which may cause cell damage and organ inflammation.

1.1.2. Dendrimers

The three-dimensional globular shape of dendrimers, which arises from many branching layers, is a characteristic of these naturally biodegradable nanopolymers [7]. They are well suited for gene and drug delivery applications in healthcare because of their tiny size (1–10 nm) and lipophilic nature, which allow effective cell membrane penetration. In particular, dendrimers have demonstrated potential for systemic delivery of biochemical compounds that can pass the blood-brain barrier (BBB) and reach brain tumors. Dendrimers made up of branching units covalently attached to the core, having functional groups at the ends of these branches, and a core comprising an atom or multifunctional molecule. The covalent attachment of medicines to dendrimers is facilitated by surface modification using materials like polyethylene glycol (PEG), which increases the dendrimers' usefulness in targeted drug delivery systems [8].

1.1.3. Liposomes

Originally identified in 1965, liposomes are spherical structures that are usually composed of an internal aqueous core and an amphipathic phospholipid bilayer [9]. Liposomes' distinct core-shell nanostructure makes them ideal for loading a wide variety of therapeutic medicines, including both hydrophobic and hydrophilic compounds. This adaptability is critical for medical applications, as different diseases require different drug delivery mechanisms to be successfully treated. Because the phospholipid bilayer is amphipathic, liposomes can imitate cell membranes, thus increasing their

biocompatibility and lowering their toxicity. Liposomes' dual capability, i.e., their hydrophilic and hydrophobic improves the solubility, stability, and bioavailability of medications by making them an excellent vehicle for a variety of medicinal substances [10]. Drug delivery focuses on decreasing the adverse effects and enhancing the therapeutic efficacy by encasing medications within liposomes. By employing the lipophilic bilayer, hydrophobic medications, for instance, which are sometimes challenging to administer due to limited water solubility, are administered successfully to improve patient outcomes.

One prominent instance of liposomal medication delivery is the chemotherapy agent doxorubicin (DOX), formulated as Doxil [11]. All encapsulated DOX medications in Doxil are found as aggregated or crystalline (DOX) $_2SO_4$ salt in the aqueous phase of the core. A transmembrane ammonium ion gradient, which propels DOX loading into the aqueous core, facilitates this encapsulation [12]. DOX in liposomal form is a safer and more efficient cancer treatment alternative since it substantially lowers cardiotoxicity as compared to the free drug [10]. To minimize harm to healthy tissues, DOX is administered directly to tumor cells by encapsulating it within liposomes.

1.1.4. Polymeric micelles

Amphiphilic block copolymers, which naturally assemble into core-shell structures in water, are the building blocks for polymeric micelles and nanostructures - liposomes, dendrimers and polymeric nanoparticles. With their well-organized architecture, these nanoscale entities enhance drug delivery by improving solubility, stability, and targeted accumulation in tumor tissues. Micelles and various polymeric nanostructures can respond to endogenous stimuli, such as pH shifts, redox changes, enzymes, and exogenous factors like temperature, light, and magnetic fields, enabling the controlled release of active pharmaceutical ingredients (APIs) at specific sites [13]. The enhanced permeability and retention (EPR) effect further amplifies this specific targeting, ensuring drugs accumulate where needed while minimizing side effects. With their biocompatibility, biodegradability, and ability to be adapted for active targeting and stimuli-responsiveness, micelles and nanostructures shows the decisive innovations in advancing cancer therapy and drug delivery systems [13]. Hydrophobic medicines such as camptothecin, docetaxel, and paclitaxel are placed into the hydrophobic core, and the hydrophilic shell stabilizes the hydrophobic core and makes the system soluble in water. Polymeric micelles, typically with a diameter of a wavelength of 100 nm, have a restricted pattern of size distribution to impede their rapid excretion by the kidneys. This allows the enhanced permeability and retention (EPR) effect to drive their accumulation in tumor tissues. Moreover, their polymeric coating lessens non-specific interactions with biological components, improving the stability and bioavailability of hydrophobic medicines and improving their transport. There are two methods for creating polymeric micelles, which include direct dissolving of the polymer using a solvent followed by dialysis and one block precipitation after the addition of a solvent. As a clinical application to treat proliferative vitreoretinopathy (PVR), dasatinib was encapsulated within nanoparticles made from PEG-b-PC as micellization, as reported in the work of Li et al. [14]. The nanoparticles, which had a restricted distribution and a size of 55 nm, did not cause any harm to ARPE-19 cells [15]. When compared to free medications, this micellar formulation dramatically reduced cell attachment, migration, and proliferation. After proper administration, polymeric micelles usually make their way to the tissues of the back of the eye via the transcleral pathway [16]. Researchers are creating paclitaxel-loaded micelles that are specially and exactly made to target cancer stem cells with the

intent to treat triple-negative breast cancer (TNBC). To promote their accumulation in tumor tissue, the micelles are conjugated with folic acid, a ligand for receptors that are overexpressed in many TNBC cells. This formulation displayed positive results in clinical trials, lowering tumor metastasis and recurrence while minimizing the toxicity usually associated with paclitaxel.

Together, these developments have opened new avenues for creative applications in biological sensors, dental, medical imaging, cosmetics, textiles, and environmental cleanup. This represents a major step forward in the broad application of nanotechnology in contemporary technology and healthcare as shown in Table 1.

Table 1. From imaging to therapy: nanoparticles' extended applications in medicine.

Author	Year	Nanoparticle used	Disease diagnosis	Treatment
Guerquin-Kern et al.	2005	Not specified	Cellular analysis	SIMS microscopy
Michalski, A et al.	2011	Not specified	Proteomics	Q exertive mass spectrometer
Zhang, Y. et al.	2011	64Cu-labeled monoclonal antibody (NOTA)	CD105 expression	Positron emission tomography imaging
Chan et al.	2013	Dual-intelligent functionalized gold nanoparticles	Cancer	Imaging and therapy
Chen, F et al.	2014	TRC105(Fab)-conjugated, dual-labeled mesoporous silica nanoparticles	Tumor vasculature	PET/NIRF imaging
Park et al.	2014	Localized Surface Plasmon Resonance (LSPR) aptasensor	Ochratoxin A	Detection
Zong, T et al.	2014	Dual-ligand doxorubicin liposomes	Brain glioma	Improved targeting and therapeutic efficacy
Huang, K et al.	2015	Ultra-small gold nanoparticles	Cancer	Localization and penetration in cancer cells
Zhang, F et.al	2015	Not specified	Tumors	Noninvasive dynamic imaging
Sorrentino et al.	2015	Not specified	Biological samples, magnetic domains	Cryo nano-tomography
Tsoukalas et al.	2018	Metal-based imaging probe	Angiogenesis	dual-modality SPECT/MR imaging

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Author	Year	Nanoparticle used	Disease diagnosis	Treatment
Liu et al.	2019	One-dimensional Fe_2P	Tumors	Fenton agent in response to NIR II light and ultrasound
Li et al.	2020	Not specified	Philadelphia Chromosome-positive acute lymphoblastic leukemia	Chemotherapy-free regimen
Kowalewska et al.	2021	Not specified	Autism	PET and SPECT imaging
Wang et al.	2021	Nano rod arrays	Not specified	Fast and sensitive one-step Immunoassays
Zayed et al.	2023	Hybrid quantum dot-based Nano medicines	Cancer	Tumor-targeted drug delivery and cancer imaging
Ramaswamy et al.	2023	Microfluidic device (no specific nanoparticle)	Blood coagulation measurement	Point-of-care testing using electrical impedance sensing
Morelli et al.	2024	Gut-on-chip platform (No specific nanoparticle)	Responses to enterotoxins	Study of epithelial responses to enterotoxins

Since the initial investigations of nanoparticles in the 1980s, the field of nanomedicine has seen tremendous progress. Subsequent improvements were made possible by the groundbreaking discoveries of scientists such as Richard Feynman, who first proposed the idea of influencing matter at the nanoscale. Scientists like Don Eigler pushing the envelope in the early 2000s by modifying individual atoms sparked interest in the therapeutic uses of nanoparticles. These days, nanoparticles are important in numerous medicinal and diagnostic procedures. For illustration, gold nanoparticles studied extensively by researchers such as Paul Alivisatos have demonstrated immense promise in imaging and photothermal therapy. Furthermore, liposomes, which were created in the 1960s, have established themselves as common drug-delivery vehicles. Each type of nanoparticle presents unique advantages and limitations, necessitating further exploration to enhance their therapeutic applications. Table 2 summarizes the traits, advantages, disadvantages, and particular illnesses linked to each type of nanoparticle.

Table 2. Evaluation of nanoparticles: advantages, shortcomings, and clinical significance.

SL. No	Types	Specifications	Advantages	Disadvantages	Clinical Significance	Diseases Treated
1	Liposomes	Lipid bilayer-based spherical vesicles that range in size from 50 to 1000 nm.	Both hydrophilic (up to 80% payload) and hydrophobic medications are encapsulated.	-Short shelf life, often one to two years. -Hazard of hemolysis if not processed sufficiently	Delivery of chemotherapy; methods for targeted delivery	Breast Cancer, Ovarian Cancer
2	Dendrimers	High-branched macromolecules, ranging in size from 1 to 10 nm	-Potential for high functionalization (up to 128 surface groups) -Regulated medication release patterns.	-Higher quantities (> 10 μM) may be cytotoxic. -Biological fluid aggregation risk; possible immunogenicity	siRNA delivery for cancer gene silencing.	Prostate cancer and colorectal cancer
3	Gold Nanoparticles	Gold particles that are nanosized, usually between 1 and 100 nm	-Low toxicity and biocompatibility (< 1 μg/mL) -Resilient optical scattering and absorption characteristics	-Tissue penetration is confined, typically less than 10 μm. -Elevated quantities (> 50 μg/mL) may be cytotoxic. -Oxidative stress risk; can build up in the spleen and liver.	Photothermal treatment; diagnostic imaging agents.	Prostate and breast cancer

Continued on next page

SL. No	Types	Specifications	Advantages	Disadvantages	Clinical Significance	Diseases Treated
4	Iron Oxide Nanoparticles	5−100 nm magnetic nanoparticles are utilized in the administration of medications and surveillance.	With magnetic subjects, it may concentrate on particular tissues.	-The possibility of cytotoxicity at greater concentrations (> 100 μg/mL) -Oxidative stress risk; might affect blood parameters.	MRI contrast agents; tumor-specific medication delivery	Liver cancer and Glioblastoma Multiforme
5	Polymeric Nanoparticles	Biodegradable polymer nanoparticles, usually ranging in size from 100 to 500 nm.	Medication release under control (up to weeks)	-Drug release profile variability. -Difficult breakdown pathways; risk of irritation caused by polymers.	Delivery of vaccinations and anticancer drugs.	Infectious diseases (like HIV) and melanoma
6	Nanoshells	Usually between 50 and 150 nm, core-shell nanoparticles serve in photothermal therapy.	It works well for localized heating (increasing by less than 10 °C).	-The risk for surrounding tissues to become overheated; -long-term impacts on healthy cells; severe immunological reaction; thermal harm to non-target organs.	Localized tumor management; imaging for diagnosis.	Breast cancer, head and neck cancer

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SL. No	Types	Specifications	Advantages	Disadvantages	Clinical Significance	Diseases Treated
7	Carbon nanotubes	cylindrical nanostructures with exceptional characteristics that range in diameter from 1 to 100 nm.	Surface area is high (up to 2630 m^2/g).	-Hazards associated with inhalation (> 2.5 μm). -The possibility for triggering oxidative stress and the risk of lung damage.	Targeted medication administration in imaging and cancer treatment.	Cardiovascular Disease, Prostate Cancer.
8	Hydrogel Nanoparticles	Cross-linked polymers that can accommodate plenty of liquid, usually between 100 and 500 nm.	Mimics the features of actual tissue; regulated release of the medication (days to weeks).	Mechanical instability, the possibility of gel disintegration within the body, and polymer-induced inflammation.	Prolonged medication delivery and tissue transplantation.	Regenerative medicine, Diabetes Complications

2. Nanotechnology in multimodal diagnosis

2.1. Imaging techniques

The development of light, electron, and scanning probe microscopes has been continuously fueled by biology, resulting in the creation of new microscopy instruments. We can now see biological objects in several dimensions, at different scales and resolutions, and with chemical or molecular specificity thanks to advanced imaging techniques, which also make the molecular architecture inside cells visible. Ernst Abbe is a pivotal historical figure in this discipline, having established the resolution limits owing to diffraction through his work on wave-based optical theory [17]. Super-resolution optical imaging techniques have been made possible by advances in photochemistry and instrumentation, notwithstanding this constraint, allowing for detailed visibility at the nanoscale. In scanning probe microscopy, a sharp probe is raster-scanned across a surface to obtain a magnified view of the specimen's topography, in contrast to light and electron microscopy, which uses lens-based optical systems to create enlarged images. AFMs, or atomic force microscopes, are important scanning probe instruments that work on a different premise than traditional microscopes to create images [18]. With AFM, particular chemical and biophysical interactions between the surface of the material and a functionalized scanning tip can be probed. Near-field scanning optical microscopy (NSOM), which scans a tiny probe positioned less than a wavelength of light from the material, is another significant scanning probe technique [19]. By means of a tiny opening in the probe tip, light may be transmitted, enabling NSOM to transcend the diffraction limit of conventional optical microscopes. All imaging techniques require contrast; in actuality, a handful of the most advanced strategies entail creative methods to make structures more apparent with the goal of being successful. In this respect, MALDI-IMS improves mass spectrometric signals through matrix-assisted ionization, allowing the detection of biomolecules in the 10−100 μm range. Moreover, fluorescence microscopy utilizes dyes, which emanate light in the 400–700 nm range to illuminate biological components. The possible spatial resolution is, of course, a crucial component of any imaging modality, and in this regard, a wide variety of approaches are available that encompass resolutions from the nano to the macro length scales. Specific imaging methods with nanoscale spatial resolutions are needed to view the molecular constituents and substructures of cells. This volume focuses on such Nano imaging approaches.

We have sectioned this paper, each encompassing a distinct modality to improve our grasp of imaging techniques in nanotechnology. The positive effects of these modern imaging technologies are rendered explicit by this methodical approach. When it comes to investigating gold nanoparticles in living cells at Harvard University, soft X-ray microscopy proves essential for visualizing nanostructures. Researchers at Johns Hopkins University can better understand drug delivery systems by examining the spatial distribution of lipid nanoparticles in tissue samples using imaging mass spectrometry, especially MALDI-IMS. The Scripps Research Institute's use of cryo-electron microscopy (Cryo-EM), which helps visualize protein nanostructures, has transformed structural biology. Namely, at Memorial Sloan Kettering Cancer Center, the inclusion of iron oxide nanoparticles in Positron Emission Tomography (PET) allows for narrowed imaging of tumors, boosting sensitivity in cancer treatment. At universities, such as Stanford University, photoacoustic imaging (PAI), which has applications in NIR-absorbing nanoparticles, is improving tumor detection methods. Fortunately, fluorescence microscopy continues to be essential for researching cellular processes, especially when it comes to detecting quantum dots at MIT.

2.1.1. Soft X-ray microscopy

Soft X-rays with energies between 250 eV and 2 keV or wavelengths between 0.6 and 5 nm are used in soft X-ray microscopy [20]. This method may provide high-contrast images of organic materials without staining or labeling, which makes it especially helpful for imaging biological specimens. Three-dimensional (3D) imaging is made possible by the development of soft X-ray microscopy into soft X-ray tomography (SXT) [21]. SXT can image whole eukaryotic cells at resolutions of 30–50 nm in a state close to native [22]. Scientists can learn more about the intricate structures found inside cells by compiling pictures from various perspectives and reassembling them into a three-dimensional model.

2.1.2. Imaging mass spectrometry

The chemical specificity of mass spectrometry and the spatial resolution of imaging techniques are combined in imaging mass spectrometry [23]. Imaging mass spectrometry (IMS) comes in a variety of forms, but two stand out in particular. Secondary ion mass spectrometry (SIMS) technique ejects secondary ions from the sample surface by subjecting it to a concentrated primary ion beam. A mass spectrometer is then used to examine these secondary ions, yielding comprehensive information regarding the composition of the material [24]. Visualizing the distribution of individual molecules on the surface of the sample is achievable due to the lateral resolution, which can reach tens of nanometers. High spatial resolution tissue sample analysis using SIMS, can identify particular biomarkers linked to malignant cells (Figure 1). For example, lipid patterns suggestive of prostate cancer tissues have been found using SIMS [25]. The capacity to chart these chemical distributions at the nanoscale facilitates comprehension of the course and nature of cancer.

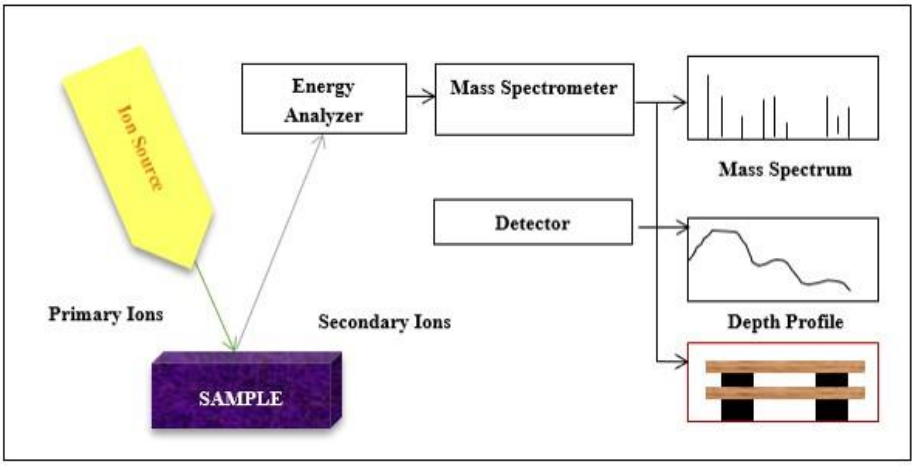

Figure 1. Secondary ion mass spectrometry (SIMS).

2.1.3. The matrix-assisted laser desorption/ionization system (MALDI) IMS

After taking the sample, the surface was coated with a matrix material, and molecules were ionized through it using a laser in MALDI IMS [26]. A mass spectrometer is then used to analyze the

ions. When analyzing the distribution of big macromolecules in a sample, such as proteins and lipids, MALDI IMS is especially helpful and provides instances of each working in the diagnosis of nano-examples in medicine. When examining brain tissues and locating proteins linked to neurodegenerative illnesses like Parkinson's or Alzheimer's, MALDI IMS is helpful [26]. By mapping the distribution of proteins like beta-amyloid in Alzheimer's disease brains, researchers can gain microscopic insights into the pathophysiology and course of the disease.

2.1.4. Cryo-electron microscopy (Cryo-EM)

Therefore, the purpose of determining the three-dimensional (3D) structures of biological molecules and complexes at almost atomic resolution, Cryo-electron microscopy, or Cryo-EM, is a crucial technology. Using this technique, the material is quickly frozen to −150°C, which successfully traps it in vitreous ice [27]. Through the process of immobilizing the sample in its glassy condition, Cryo-EM preserves its original structure, allowing for a more lucid view of biological specimens. Phase contrast is the principal imaging technique used in Cryo-EM, with amplitude contrast providing supplementary information. By weakening low-resolution features while they are in focus, the Contrast Transfer Function (CTF) alters images [28]. Defocusing the electron beam is essential to improve phase contrast. Larger windows are required for picture extraction due to the complexity of CTF correction caused by increased defocus (Figure 2).

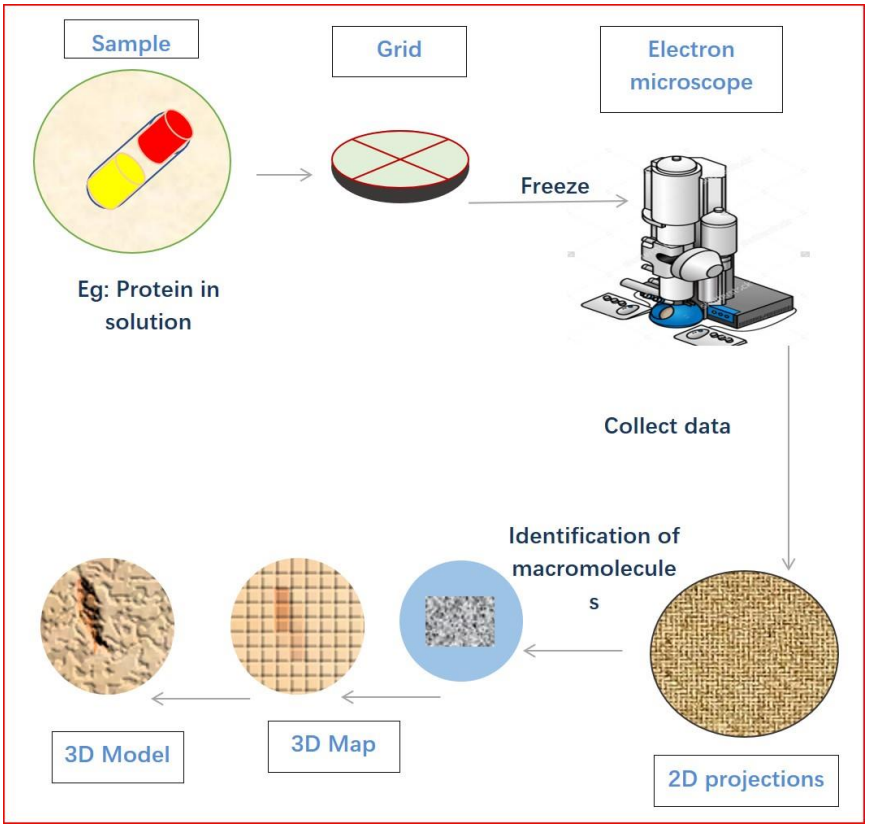

Figure 2. The schematically illustrated experiment of the cryo-EM.

High-resolution 3D reconstructions are achieved using computational reconstruction, which

adjusts for CTF effects using different defocus values (usually between −800 nm and −2 μm under focus). Because of the demanding technological requirements of high-performance TEMs, the establishment of a new Cryo-EM facility requires careful planning [29]. A steady, vibration-free floor, a reasonable ceiling height (about 4−5 meters), little electromagnetic interference, and accurate temperature regulation are a few of these. Setting up money for necessary auxiliary equipment is just as important as infrastructure. This comprises dears for storing specimens, robots for verification that prepare specimens, carbon coaters that improve the quality of imaging, and plasma glow-discharge devices that treat specimens. The accurate sample preparation and imaging procedures that are essential to Cryo-EM research and diagnostics are supported by these instruments, which are invaluable. For instance, the University of Glasgow's SCMI laboratory is housed in the Sir Michael Stoker building, which was constructed with advanced Cryo-TEMs in mind [30]. This facility is an example of how customized infrastructure improves the capacity for state-of-the-art biomedical research. By providing previously unobtainable structural elucidation of disease-associated proteins, cryo-electron microscopy (Cryo-EM) has revolutionized the healthcare industry and set the stage for targeted medication and vaccine development. The technology's major role in facilitating the quick visualization of the SARS-CoV-2 spike protein is an example of how it accelerated the creation of targeted vaccines, leading to a prompt, worldwide response to COVID-19. The ability of cryo-EM to identify pathological protein conformations, such as tau and amyloid fibrils, at near-atomic resolution has become a bedrock for structure-guided drug design in the area of neurodegenerative diseases consisting of Alzheimer's, opening up previously untapped medicinal product routes.

2.1.5. Positron emission tomography (PET) with nanoprobes

During the last ten years, there has been a lot of interest in the application of nanotechnology to nuclear imaging, specifically in positron emission tomography (PET) and single-photon emission computed tomography (SPECT) [31]. A prime example of this dual-modality capacity is radiolabeled iron oxide nanoparticles (IONPs), which can signal for both PET and magnetic resonance imaging (MRI) [32]. Especially in the field of cancer theranostics, PET has become a potent tool in the medicinal uses of nanomaterials. The *in vivo* bio-distribution is greatly influenced by their physicochemical characteristics, which include composition, surface charge, PEGylation, ligand conjugation, and size/shape. To affect the pharmacokinetics, intra-tumoral penetration, tumor bioavailability, and cargo delivery mechanisms of nanoparticulate medicines, optimization of these variables is now under progress. When it comes to investigating intricate biological pathways and *in vivo* ADME (absorption, distribution, metabolism, and excretion) profiles of nanoparticles, a vital step in overcoming clinical translation barriers, PET's sensitivity, whole-body imaging capability, non-invasiveness, and quantitative capabilities make it the perfect tool. Real-time monitoring of treatment outcomes is made possible by PET's role in image-guided therapy and drug delivery applications utilizing nanomaterials.

For example, image-guided DOX delivery employs antibody-conjugated mesoporous silica nanoparticles (MSNPs) in 4T1 breast cancer models and CD105-specific PET imaging [33]. These MSNPs with a size of about 80 nm, created using a soft-template technique, were functionalized with NOTA for 64Cu chelation and conjugated with TRC105 antibody using PEG linkers [34]. A rapid and sustained accumulation of nanoconjugates (5.9 ± 0.4% ID/g at 5 hours post-injection) was observed at the tumor location by serial PET scans. This accumulation was due to the combined effects of the

enhanced permeability and retention (EPR) impact and specific binding to CD105, which is overexpressed in the tumor vasculature. An additional indication of the effectiveness of surface-functionalized MSNs in targeted theranostics was their improved CD105-targeted DOX delivery. This strategy can be modified to develop multimodal imaging agents or to target various biomarkers and tumor types.

2.1.6. Photoacoustic imaging (PAI)

The foundation of photo-acoustic imaging, often known as PAI, is the use of incident laser pulses to cause rapid thermo-elastic expansion and contraction in soft biological tissues [35]. Ultrasonic waves are emitted because of this localized thermal expansion and are subsequently reconstructed in two or more dimensions to depict the internal spatial distribution of optical absorption [36]. In medical imaging, pulsed lasers are commonly employed as sources of excitation. Because of the extremely low optical-to-acoustic conversion efficiency in PA imaging ($< 10^{-4}$ for liquids), high-power sources are needed [37]. Low-duty cycle pulsed lasers have high peak output values at comparatively low average power. This offers the benefits of a low heating effect (at repetition rates commonly utilized in conventional PAI) and powerful photo-acoustic waves in biological tissues [38].

High frequency pulsed laser signals are easily distinguished from low frequency ambient noise. This circumvents the actual reliance on thermal diffusion and the chopping frequency, also known as the boundary condition, in the event that the excitation sources are continuous. The chosen wavelength is dictated by the optical absorption coefficients of the individuals under investigation, taking into account the optical absorption and scattering coefficients of the matrix where the items are located. Usually, the visible or near-infrared spectral areas are the wavelengths that are chosen. The ideal laser energy per pulse for computed tomography (PACT or PAT) is between mJ and sub-Joule, whereas for microscopy (PAM), endoscopy (PAE), and photo-acoustic flowmetry (PAF), it should be between μJ and sub-mJ [39,40]. The beam's (TM00 mode) spatial dispersion is significant, particularly when considering optical resolution for PAM [41]. In real-time imaging, a pulse repetition rate of at least kHz is required to achieve good resolution and power utilization efficiency. Since they offer a high power output across a range of wavelengths, from the ultraviolet (UV) to the near-infrared (NIR), solid state lasers, such as Q-switched Nd: YAG lasers, are commonly employed in PAI [42]. Dyed lasers and pulsed optical parametric oscillators (OPOs) are most suited for multi-spectral PA imaging since they can continually adjust output wavelengths over a specified range. Due to their low cost, high-power pulsed lasers, diodes, or diode modules have received particular interest in PA measurements over the past 20 years.

2.1.7. Fluorescence microscopy

By using some substances' luminous qualities, fluorescence microscopy is a method used in materials science and biology to see structures and particles. This technique uses fluorescent dyes or proteins that, when activated by light of a certain wavelength, emit light of a specified wavelength to mark particular molecules, particles, or structures. After that, this released light is detected and utilized to produce specific and sensitive images that are extremely detailed. Excitation light, or light of a specific wavelength that illuminates the sample, is the crucial principle. This allows the fluorescent labels within the sample to absorb photons and get activated. Afterward, these labels release

longer-wavelength light, or emission light, which is subsequently detected by a detector to create an image. By precisely identifying particles and biological structures, fluorescence microscopy allows for sensitive and quantitative imaging. To trace uptake and possible translocation within living organisms, this approach has been widely used to localize fluorescently labeled microplastic particles during exposure investigations. Nanoplastics, which are smaller, present a problem since they usually fall below the diffraction limit of approximately 200 nm, but individual microplastics can be resolved using standard fluorescence microscopy because of their size. The scale of nanoplastics makes current methods such as wide-field epifluorescence microscopy and laser-scanning confocal microscopy insufficient for their resolution. This limitation is resolved by sub-diffraction resolution achieved with stimulated emission depletion (STED) microscopy [43]. Two lasers are used in this method to scan a sample simultaneously: a donut-shaped depletion laser and a circular excitation laser [44]. The emission spot size is successfully reduced, and resolution is increased beyond the diffraction limit by an order of magnitude thanks to the depletion laser's suppression of fluorescence emission from the excitation spot's periphery. Dyes that can effectively undergo stimulated emission and have good photostability are needed to minimize photobleaching, as dye compatibility is a limiting factor in STED microscopy. Several fluorescent dyes that are suitable for STED microscopy are available despite this restriction, including DY-520XL, SeTau 405, and STAR 440 SXP [45]. In fluorescent approaches, photobleaching presents a challenge, especially when exposed to bright light under experimental settings. Even while dyes like Atto 647N are well known for being photostable, stimulated emission processes in STED microscopy may cause more photobleaching than in traditional techniques [46]. Furthermore, compared to laser-scanning confocal or wide-field microscopy, the ability of STED microscopy to observe dynamics and capture many z-slices is sample-dependent and somewhat limited. High numerical aperture objectives limit image depth and are therefore best suited for microscopically analyzing cells, tissue sections, small anatomical structures, or microbes. These objectives are also required to achieve nanoscale resolution using STED microscopy.

2.2. Lab-on-a-chip technologies

By consolidating many scientific protocols into a small device, lab-on-a-chip (LOC) technologies allow the processing of small volumes of biological material at a single pace. The identification of disease biomarkers—signatures of pathogenic activity, biological processes, pharmacological reactions, or the existence of a disease—requires the use of these technologies. DNA, proteins, lipids, metabolites, and mRNA profiles are just a few of the molecular signals found in urine, blood, and tissues that can be used to identify biomarkers. Because of its portability, integrated processing capabilities, immediate evaluation, and lower reagent usage, microfluidics technology improves disease diagnosis. Because prompt and precise diagnosis is critical in point-of-care (POC) settings, these qualities are crucial. Effective illness screening, diagnosis, prognosis, and therapy monitoring are supported by microfluidic devices, which enable high-sensitivity biomarker identification. The creation of multiple microfluidic instruments makes it possible to employ small amounts of biological fluids for immediate time disease surveillance and on-chip POC diagnostics. We have also included another subsection of lab-on-a-chip technology beneath, comprised of:

2.2.1. Microfluidics

Characterized by a variety of micro-domain effects, microfluidic technology is an area that is quickly developing. System sizes typically range from 10 to 100 μm. It is widely employed in several fields, from micro-arrays to cellular biophysics. Often referred to as "lab-on-a-chip" technology, microfluidics is the configuration of miniature devices that accurately regulate the physicochemical reactions of solvents [47]. High mass transfer and high analytical throughput are achieved by these chips' small size, which enhances surface area. Glass, poly(methyl methacrylate) (PDMS), poly(cyclic olefin), poly(methyl methacrylate) (PMMA), paper-based substrates, and hybrid materials are used to create microfluidic platforms [48]. Regarding performance, biocompatibility, and fabrication, each material type provides unique benefits that are suited to various diagnostic requirements.

The microfluidic chip's microchannels are linked together to produce the intended effects. By allowing inputs and outputs to pass through the chip, these microchannels function as a bridge connecting the large and small worlds. Microfluidic technology makes use of a pump and a chip to help identify behavioral changes in microfluids. Microfluidic channels on the chip enable blending and physicochemical reactions among other fluid processing operations. Among the many benefits of microfluidics, chips are their capacity to do multiple jobs at once and their low time and reagent usage. Because of its smaller size and larger surface area, the chip accelerates reactions. These chips are employed in a number of biomedical processes, including pregnancy testing, peptide analysis, tissue engineering, medical diagnostics, DNA purification, and PCR activity. Microfluidic devices can be quickly prototyped using laser ablation. In order to evaporate a polymer at the focal point, thermally, photo-degradationally, or both, the polymer is exposed to a high-intensity laser beam. Generally, a pulsed laser is used, in which the number of passes, laser intensity, wavelength, and material type are used to determine how much material is ablated by each shot. The polymer's absorption at the excimer wavelength influences this process, resulting in microchannels with rough, rippled surfaces.

2.2.2. PDMS/Polydimethylsiloxane paper-based device

With respect to its exceptional elastic modulus, biocompatibility, and great optical purity, polydimethylsiloxane (PDMS), a silicone-based elastomer, is a fundamental component in microfluidic systems. It is a formidable contender for live-cell imaging, real-time monitoring, and diagnostic applications where accuracy counts owing to its substantial oxygen permeability and minimal autofluorescence. In light of its inert nature and ability to create precisely regulated microchannels formed by soft lithography, PDMS can be utilized in developing complex microfluidic networks that are essential to fluid control at the micro scale, guaranteeing diagnostic reliability. Its naturally hydrophobic surface, which can be manipulated by surface coatings or plasma treatment, offers a customized interface optimal for multiple biomolecular tests, especially those involving the identification of proteins, DNA, and disease biomarkers. In a comparable vein compressing cellulose or nitrocellulose fibers creates a capillary-active paper substrate, an incredibly thin substance that can propel fluid flow without assistance, rendering it perfect for low-cost, cleanroom manufacturing [49]. In addition to immobilizing DNA and protein biomarkers, this substrate's macroporous structure provides a high surface-to-volume ratio, enabling quick and efficient detection. The fabrication of hydrophobic barriers and careful two-dimensional cutting are the main methods for creating two- and

three-dimensional fluid flow configurations in microfluidic paper devices, whether they are horizontal or vertical. We have illustrated the same in Figure 3. The powers of such substrates have been reinforced by discoveries in colorimetric detection methods for proteins, glucose, and nucleic acids associated with infectious illnesses. Dextran, for example, was added to an ELISA-compatible PDMS chip created by Yu et al. to boost PDMS hydrophilicity and enable covalent protein immobilization [50]. The simultaneous detection of biomarkers for hepatitis B (HBsAg), bronchial asthma, and immunoglobulin G (IgG) linked to neuromyelitis optica was made possible by this improved chip design [51,52]. With an unpredictable range spanning five orders of magnitude and a highly sensitive detection limit of 100 pg/mL, the approach offers enhanced sensitivity using 3,3′,5,5′-Tetramethylbenzidine (TMB) as a substrate in conjunction with HRP-labeled secondary antibodies [53].

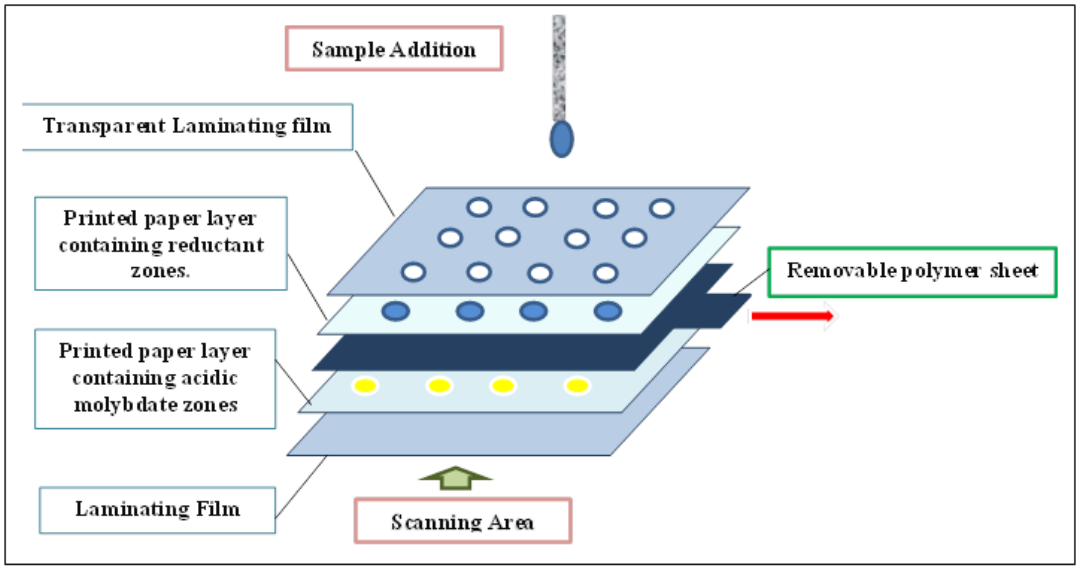

Figure 3. PDMS Schematic working.

2.2.3. Microfluidic hydrogel chip

Since polydimethylsiloxane (PDMS) polymer is transparent, biocompatible, very precise, and allows high throughput, it is commonly used for microfluidic chip designing [54]. Cell adhesion and attachment, which are essential for biological processes, including cell proliferation and differentiation, are rarely facilitated by PDMS, which presents a problem. A hydrogel layer that is compatible with cells can be applied to microfluidic channels within a PDMS-layered device as a solution to this problem. Inspired by the extracellular matrix, hydrogels are made of a three-dimensional network of natural or artificial polymers. PEG diacrylate (PEG-DA) and polyethylene glycol (PEG) are examples of synthetic polymers, whereas agarose, alginate, collagen, dextran, fibrin, and laminin are examples of natural polymers. Devices using microfluidic hydrogels commonly use PEG and its derivatives [55]. Cell/drug encapsulation and cellular barrier formation are the major applications for hydrogels.

Angiogenesis and cell migration can be studied with the help of these 3D hydrogel-based microfluidic chips. For instance, Shen et al. used conventional soft lithographic techniques to create a microfluidic system that was constructed in PDMS [56]. In order to investigate angiogenesis utilizing

cell culture experiments, this device featured hydrogel-incorporating chambers positioned in between surface-accessible microchannels. The platform was composed of three channels and four gel areas that could be accessed separately. The two side channels were used as condition and control channels, respectively, while cells were grown in the middle channel. To encourage cell attachment, extracellular matrix (ECM) proteins were coated inside the channels. Furthermore, to encourage 3D capillary morphogenesis into the hydrogel, the channels were coated with poly-D-lysine (PDL) hydrobromide. This technology replicated several elements of the in vivo milieu by enabling the application of pharmacological and biophysical stimuli to many cell types interacting over less than one millimeter.

2.2.4. Droplet-based microfluidics

Using multifunctional microspherical magnetic and pH-responsive transporters created using droplet-based microfluidics, Maher et al. described the use of a microfluidic chip in the treatment of colorectal cancer [57]. The treatment of colorectal cancer benefited greatly from the use of this pH-sensitive medication delivery system. A synergistic treatment method was used in the engineering of the microfluidic chip by including pH-sensitive magnetic microspherical carriers. Anticancer medications that have a combination synergistic effect on colorectal cancer, including 5-fluorouracil and curcumin, were selected [58]. In this work, magnetic bacterial iron oxide nanowires and permeable silicon nanoparticles were loaded with chemotherapy agents [59]. Then, utilizing droplet-based microfluidics, the medications were encapsulated into polymeric microspheres. These microspheres were created using a succinate polymer produced from hypromellose acetic acid, which is soluble at the pH of the colon and rectum but insoluble in the acidic stomach environment.

Moreover, microfluidic droplets can be used as enzymatic amplification microfluidic bioreactors, boosting the sensitivity of fluorescence detection. For instance, Joensson et al. reported a technique that uses enzyme amplification inside tiny droplets inside a microfluidic device to identify and analyze low-abundance cell-surface indicators [60]. Biotinylated antibodies were used to label cells for cell-surface biomarkers, which were attached to streptavidin-coupled β-galactosidase [61]. The device was designed to detect low-abundance biomarkers CCR5 and CD19 from single human monocytic (U937) cells. A fluorogenic substrate (fluorescein-di-β-d-galactopyranoside, FDG) was mixed with the enzyme-labeled cell stream, and laser-induced fluorescence was used to measure each droplet's fluorescence [62].

2.2.5. Organ-on-a-chip

The term "organ-on-a-chip" (OoC) refers to a 3D in vitro tissue model that is micro-engineered and consists of micro-compartments connected by several microfluidic channels [63]. In addition to being utilized for biochemical analysis, it mimics the physiological milieu of any given organ. Predicting a medicine's effects prior to clinical trials is crucial in drug research. OoC bridges the gap between preclinical testing and human trials, decreasing costs and increasing throughput in a process that is more straightforward and less time-consuming than traditional approaches. Positive cost impact was indicated by Franzen et al.'s estimate of a drop of 10–26% in R&D expenses per new medicine [64]. Optical fiber communication (OoC) facilitates effective communication between different tissues and offers various advantages, such as accurate physical condition control, improved microenvironment control, and more. While removing catabolic metabolites, it can also create oxygen and nutrients. Its

application might be restricted to surface effects, though, including medication adsorption on the inner lining and less mixed laminar flow. Personalized medicine and drug research intend to replace animal testing with microfluidics-based OoC, or microphysiological systems, thanks to recent breakthroughs [65]. The uses of microfluidic devices for biological analysis and cell culture date back three decades to the inception of OoC. Reproducing complete tissues or organs at their original scale is not the goal of OoC, in contrast to tissue engineering. Rather, for the purposes of disease modeling and drug screening, it imitates important organotypic cellular architecture and functions, 3D extracellular matrix (ECM), pharmacological variables, and biophysical cues at a lower scale [66].

Huh's prior work from the Takayama group served as the foundation for the 2010 lung-on-a-chip model published by the Harvard Medical School's Ingber group [67]. The fields of biology and engineering both paid close attention to this invention, which is credited with sparking the quick advancement of OoC technology. Lung alveolar and capillary cells are co-cultured in the Lung-on-a-Chip model on both sides of a porous membrane in microfluidic channels [68]. With this configuration, scientists may investigate breathing mechanisms at the interface between the alveoli and capillaries in human lungs and track the impact of the environment on lung cells in vitro. It offers a biomimetic model for comprehending the pathogenic processes behind several respiratory illnesses, including COVID-19 [69]. Numerous other single-organ chips, such as liver chips, kidney chips, pancreas chips, heart chips, intestinal and gut chips, blood-brain barrier (BBB) chips, and bone and bone marrow chips, have been successfully developed since the lung-on-a-chip was developed. These assays using single-organ chips are useful for analyzing adverse medication reactions and tracking the course of diseases.

It is commonly recognized that neuronal and cell-cell interactions are major players in the functioning of brain tissue. Because the brain and its tissues are so complex, 2D models like Petri dishes and culture flasks are useless because they can't accurately replicate the brain's physiological environment [70]. Researchers are now developing a brain-on-a-chip platform, which could allow for the enhanced study of brain physiological parameters on a miniaturized platform made possible by a multistep lithography approach to get around this constraint. According to Jahromi et al., the creation of microchannels with varying dimensions has facilitated the examination of brain tissues [71]. A different study that used an in vitro cell culture method was previously published; in this study, the soma and axons were physically separated, allowing only the axons to pass through the microchannels. Neuroscientists could use this method to investigate the characteristics of the axon, as well as to ascertain how medications operate on the axonal portion and examine how axon regeneration occurs following axotomy. It is important to remember that microchannels have the potential to damage cells by putting shear stress on tissues or cells. Cell injury and flow characteristics disruption could result from air bubbles trapped beneath the microchannels [72]. When creating these kinds of three-dimensional devices, a sandwich design is typically chosen. In this design, brain cells are grown in the lower layer, and endothelial cells are produced in the upper layer. A porous membrane that serves as the blood-brain barrier (BBB) separates the two layers

With the help of commercial products like TissUse's HUMIMICTM chip, organ-on-a-chip (OoC) technology has made tremendous strides [73]. Inter-organ connections may be studied thanks to this platform, which enables researchers to culture many organ compartments at once. Additionally, TissUse provides service contracts designed specifically for the development of unique organ chip platforms. By utilizing TissUse's unique fast prototyping techniques, these platforms play a crucial role in diagnosing medication toxicity and simulating human diseases. The OrganoPlate® from Mimetas is another noteworthy product. Research groups have been using it extensively to create vascularized barrier tissues in microfluidic environments. This modification helps with a number of research projects that try to comprehend cellular illness mechanisms and therapeutic impacts. The OrganoReady Caco-2TM, a ready-to-use intestinal barrier chip with biomimetic Caco-2 epithelial tubules, is another product offered by Mimetas [74]. Testing intestinal disorders, drug toxicity, and transport pathways are some of the areas in which this chip proves to be most useful. To support extensive research on intestinal health, Emulate Inc. provides intestine chips that are comparable to this one. Through the controlled microfluidic environment provided by these chips, researchers can investigate medication interactions, toxicity profiles, and disease pathogenesis. Research in the biological sciences and pharmaceutical development is more accurate and efficient due to these platforms' adaptability. Imaging techniques have significance in nanotechnology because they facilitate high-resolution visualization and evaluation of tiny objects and biological specimens. These fresh methods improve our grasp of material properties and interactions at the nanoscale, which is important for usage in medical diagnostics and materials science. Table 3 presents an exhaustive examination of numerous imaging techniques, documenting their time frame of installation, energy or wavelength utilized, sample setup processes, imaging modalities, resolution, drawbacks and benefits, and pertinent implications.

Table 3. High-resolution nanotechnology imaging: methods and applications.

Imaging technique	Year of deployment	Energy/ Wavelength used	Sample preparation	Imaging modality	Resolution	Pros	Cons	Applications
Soft X-Ray microscopy	1972	250 eV−2 keV (0.6−5 nm)	No staining/labeling required	3D imaging with soft X-ray tomography (SXT)	30−50 nm	Non-invasive and ideal for scanning nanostructures.	Limited to thin specimens; requires a specialized apparatus.	Imaging biological specimens

Continued on next page

Imaging technique	Year of deployment	Energy/ Wavelength used	Sample preparation	Imaging modality	Resolution	Pros	Cons	Applications
Secondary ion mass Spectrometry (SIMS)	1949	Concentrated primary ion beam	Not specified	Chemical composition analysis	Tens of nanometers	Excellent sensitivity at the nanoscale, detects surface changes in nanoparticles.	Samples can be damaged, and profiling depth is constraine.	Identifying biomarkers in cancer tissues
Matrix-assisted laser desorption/ionization (MALDI) IMS	1958	Laser ionization	Coating with matrix material	Analysis of macromolecule distribution	10−50 micrometers	Low-abundance biomolecules can be detected with minimal sample preparation compared to previous approaches, keeping the original structure.	Matrix effects can bias the results.	Locating proteins in neurodegenerative diseases
Cryo-electron microscopy (Cryo-EM)	1974	Electron beam, rapid freezing to −150°C	Rapid freezing to −150°C	3D structure determination	Near atomic resolution (~2−4 Å)	Visualizes ceramic nanoparticles in their original states with minimum modification; Offers near-atomic resolution	Complex preparation, high cost, possible artifacts.	Imaging biological molecules and complexes

Continued on next page

Imaging technique	Year of deployment	Energy/ Wavelength used	Sample preparation	Imaging modality	Resolution	Pros	Cons	Applications
Positron emission tomography (PET) with nanoprobes	2007	Radiolabeled iron oxide nanoparticles (IONPs)	Surface functionalization with antibodies	Dual-modality PET/MRI	Millimeter scale	Synergistic imaging capabilities	Limited spatial resolution (~1−5 mm) raises concerns about being exposed to radiation and its consequences.	Image-guided drug delivery and therapy, cancer theranostics
Photoacoustic imaging (PAI)	1880	Incident laser pulses (400−2000 nm, often NIR)	No special preparation needed	Internal spatial distribution of optical absorption	50−500 micrometers	Non-invasive; great sensitivity for identifying nanoparticles and high tissue contrast.	The penetration depth is often less than 2 cm, and tissue composition can alter functionality.	Soft biological tissue imaging, medical imaging
Fluorescence microscopy	1845	Specific excitation (UV to NIR) and emission wavelengths	Use of fluorescent dyes/proteins	Detailed imaging using fluorescent labels	Sub-diffra-ction (~20−50 nm with STED)	Capable of high-throughput screening applications in drug discover	Photo-bleaching and potential toxicity of fluorescent dyes; requires careful optimization of labeling techniques	Localizing micro- and nanoplastics, tracing uptake in organisms

Lab-on-chip technologies are a game changer in nanomedicine, allowing experts to alter, analyze, and manipulate fluids at unprecedented scales. These methods, which use microscopic channels and specialized materials, offer new opportunities for revolutionary diagnostics, targeted drug delivery, and complex tissue modeling. Table 4 summarizes a variety of microfluidic techniques, covering their basic theories, key purposes, material variations, and inherent advantages and bounds.

Table 4. Lab-on-a-chip technologies.

Technology type	Core principle	Primary Uses	Material variants	Advantages	Disadvantage
Microfluidics	Combining tiny channels to precisely regulate fluid processes	Disease diagnostics, DNA purification, peptide analysis	PDMS, PMMA, paper-based substrates	Minimizes reagent usage, accelerates analysis, high sensitivity	-Small compounds (< 1 kDa) can be absorbed by PDMS and impact the results. -Fabrication can take hours or days, affecting turnaround time.
Microfluidic hydrogel chips	The use of hydrogels to improve biological interactions and replicate extracellular matrix.	Tissue modeling, cell behavior studies, drug screening	PEG-DA, agarose, collagen	Enhanced cell adhesion, 3D tissue reconstruction, better drug delivery	-Mechanical strength can be restricted (typically less than 1 MPa). -Hydrogel characteristics might differ by up to ± 20%, affecting predictability.
Droplet-based microfluidics	Use of microscopic droplets for controlled biochemical reactions and drug delivery.	Cancer therapy, enzymatic amplification, biomarker detection	pH-sensitive microspheres, polymeric microspheres	Precise drug delivery, high sensitivity, improved detection capabilities	-Ideal for small-scale applications (< 10 μL per droplet). -Environmental factors (temperature and flow rate) can influence droplet formation (variability of ± 5%). -Improper droplet isolation can lead to contamination (less than 1% risk).

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Technology type	Core principle	Primary Uses	Material variants	Advantages	Disadvantage
Organ-on-a-Chip (OoC)	3D micro-engineered systems simulating organ functions for advanced biomedical research.	Drug development, disease modeling, personalized medicine	Polycarbonate, glass, PDMS	Mimics organ physiology, reduces costs, offers alternatives to animal testing	-The assembly design prevents wider adoption (fabrication times can exceed one week). -Inadequate replication of organ systems (for example, immunological responses may not be correctly modelled). Fluid dynamics can impact transport and reaction kinetics at nanoscale (< 100 nm).

3. Nanotechnology in multimodal treatment

Multimodal treatment refers to the fusion of varied treatment modalities to maximize therapeutic advantage. For instance, in colorectal cancer, performing tumor excision, administering chemotherapy to target residual cancer cells, utilizing immunotherapy to activate the immune system, and applying radiation to shrink remaining tumors often results in improved patient prognosis. The above method streamlines multiple formats while largely relying on nanotechnology. Targeted medicine delivery made possible by engineered nanoparticles increases bioavailability while reducing side effects. Nanomaterials are used in photothermal and photodynamic therapies to produce heat or reactive oxygen species, which destroy cancer cells only while protecting healthy tissues. While regenerative medicine relies on nanotechnology to create scaffolds that promote tissue regeneration, gene therapy uses nanocarriers to safely deliver genetic material to specific cells. As a result, the following subtopics offer an exhaustive examination of this topic:

a. Drug delivery treatment: Drug delivery systems (DDSs), which rely on active drug metabolites, have been used for a long time to treat a variety of illnesses [75]. Certain medications are inert until they undergo physiological changes, and the mode of administration determines how effective they are. Traditional drug delivery techniques, such as injectable, mucosal, nasal, inhaled, and oral, frequently resulted in uneven dispersion, poor absorption, and unintentional harm to healthy tissues. Due to issues including mucosal barriers, enzymatic breakdown, pH differences, and off-target effects, these techniques resulted in early excretion and longer treatment periods for diseases. Controlled-release drug delivery systems were created as a solution to these problems. By precisely controlling drug release through the use of innovative techniques, these sophisticated DDSs improve the efficacy of medications. Drugs are targeted to particular areas, such as tissues, cells, or organs, using strategies such as osmotic pumps, hydrogels, erodible and degradable materials, reservoirs, and matrices. Drug distribution using nanostructures can occur via passive or self-delivery mechanisms. The hydrophobic effect is the main mechanism by which medications are integrated into the interior cavity of the nanostructure in passive delivery. The hydrophobic environment encapsulating the low drug content allows the medicine to be released in the intended amount when targeted to specific areas. To facilitate convenient distribution, medicines are conjugated directly to the carrier nanostructure in self-delivery. This is where timing matters; if the medication separates from the carrier too soon, it won't travel to the target site, which will lower its bioactivity and effectiveness. Active and passive drug targeting are two categories of targeting that involve the use of nanomaterials or nano-formulations. The process of active targeting links components, like peptides and antibodies, to the system that delivers drugs so that the medications bind to receptor structures at the target site. Passive targeting involves adhesion or bonding of the drug carrier complex to the intended site, which is mediated by biochemical site, pH levels, humidity, and morphology. The bloodstream carries the medication carrier complex. Cell surface antigens or proteins, as well as receptors on cell membranes and lipid components of cell membranes, are primary targets.

b. Photo thermal and photodynamic therapy: The "photothermal effect" (PTE) is the name given to the phenomena of turning light into heat. In this procedure, a quantum system that absorbs light instead of emitting it experiences preferential thermal relaxation. At certain resonances, such as localized surface plasmon resonance (LSPR) in noble metal nanoparticles,

nanoparticles (NPs) absorb light far more powerfully than molecules [76]. Laser sources matched to the absorption peaks (λmax) of these NPs can be used to target them. However, when exposed to radiation within their absorption range, NPs with broad, strong absorption spectra, such as carbon nanotubes or copper sulfide, also show notable PTE. Nanoparticle (NP)-mediated photothermal treatment (PTT) has demonstrated enhanced therapeutic outcomes; nevertheless, it is restricted by superficial light penetration, potentially resulting in partial tumor ablation [77]. Photodynamic treatment (PDT) and PTT together can improve therapeutic results. By enhancing PDT drug distribution into cells and elevating oxygen levels in tumor tissues, which enhance local blood flow, the heating action of PTT increases PDT efficacy. Furthermore, heat-shock proteins' ability to protect during PTT may be hindered by reactive oxygen species (ROS) produced by PDT [78]. Due to the different activation wavelengths of each therapy, the combination of PTT and PDT frequently necessitates consecutive laser irradiation, which makes the treatment procedure more difficult. One laser wavelength can be used to activate dual-mode PTT and PDT agents, which are the result of research efforts. For these techniques to provide synergistic effects, extended irradiation intervals (over 5 minutes) and relatively high laser power (> 1 W/cm^2) are necessary, even though they streamline the treatment process and yield better results than single-mode therapies. Therefore, to maximize therapeutic efficacy and minimize laser-induced toxicities, simultaneous PTT and PDT with low NIR power density and shorter irradiation periods are required.

Since their dense, localized surface plasmon resonance (LSPR) bands have drawn so much attention, noble metals, including gold (Au), silver (Ag), copper (Cu), and platinum (Pt), are researched in detail [79]. In less than a picosecond (ps), these nanoparticles (NPs) undergo nonradiative relaxation of their plasmon oscillations through electron-electron and electron-lattice phonon collisions when exposed to light at their LSPR wavelengths. A slower (~ 100 ps) phonon-phonon relaxation ensues, which heats the nanoparticles and transfers heat to the surrounding medium. It has proven possible to distribute macromolecules intracellularly through the PTE by employing membrane-disruption strategies. Targeting antibodies in combination with photothermal NPs has been shown to improve bacterial membrane adherence, especially against methicillin-resistant *Staphylococcus aureus* (MRSA) strains [80]. Although they work well *in vitro*, solvents like plasma dissipate heat; thus, their practical antibacterial usage is limited to high concentrations required for efficient heating in large quantities. This is a concern for safety because it may cause NP accumulation or necessitate high laser intensity. An FDA-approved contrast agent, indocyanine green (ICG), is well-known for producing reactive oxygen species (ROS) when exposed to near-infrared (NIR) laser light. It also has photothermal effects [81]. ICG has shown good light-to-heat conversion efficiency as a PTT agent for the treatment of cancer. ICG was investigated for the treatment of metastatic breast cancer in a pilot clinical study conducted in 2011 [82]. Eight 05-nm laser irradiation at 1 W/cm² was administered to ten patients with advanced-stage illness after they received local injections of ICG and glycated chitosan. With few side effects that were mostly restricted to localized thermal injury, the treatment produced a 62.5% objective response rate and a 75% clinical benefit response rate.

c. **Gene therapy:** In an effort to improve or restore biological processes, gene therapy is an experimental technique that alters genes or gene expression to treat or prevent diseases. Because it directly modifies genetic material, it differs from conventional pharmacological therapy. There are three main methods for editing genes. Zinc Finger Nucleases, or ZFNs, alter genes by creating

double-strand breaks in particular DNA locations. These breaks are then repaired to fix mutations [83]. ZFNs and transcription activator-like effector nucleases (TALENs) are similar in that they both cause double-strand breaks at certain sites to facilitate gene editing [84]. The flexible CRISPR/Cas9 system is capable of precisely introducing double-strand breaks in DNA, enabling both in vivo and in vitro gene repair [85] (Figure 4). These methods rely on cellular repair mechanisms to heal the breaks: homologous recombination (HDR), which employs a DNA template for precise repairs, and non-homologous end joining (NHEJ), which can result in insertions or deletions. Trials involving gene therapy are now 67.4% focused on indications related to cancer. Gene therapies have a difficult time getting to cancer cells inside solid tumors. Among these is a Dense extracellular matrix, which is a physical barrier that is mainly made of collagen that is secreted by fibroblasts linked with malignancy. High interstitial fluid pressure (IFP) is raised in tumors as a result of leaky blood arteries, insufficient lymphatic drainage, and fast tumor growth, which makes medication distribution more difficult. Large molecular weight, these gene therapies and other high molecular weight treatments have trouble breaking through these obstacles.

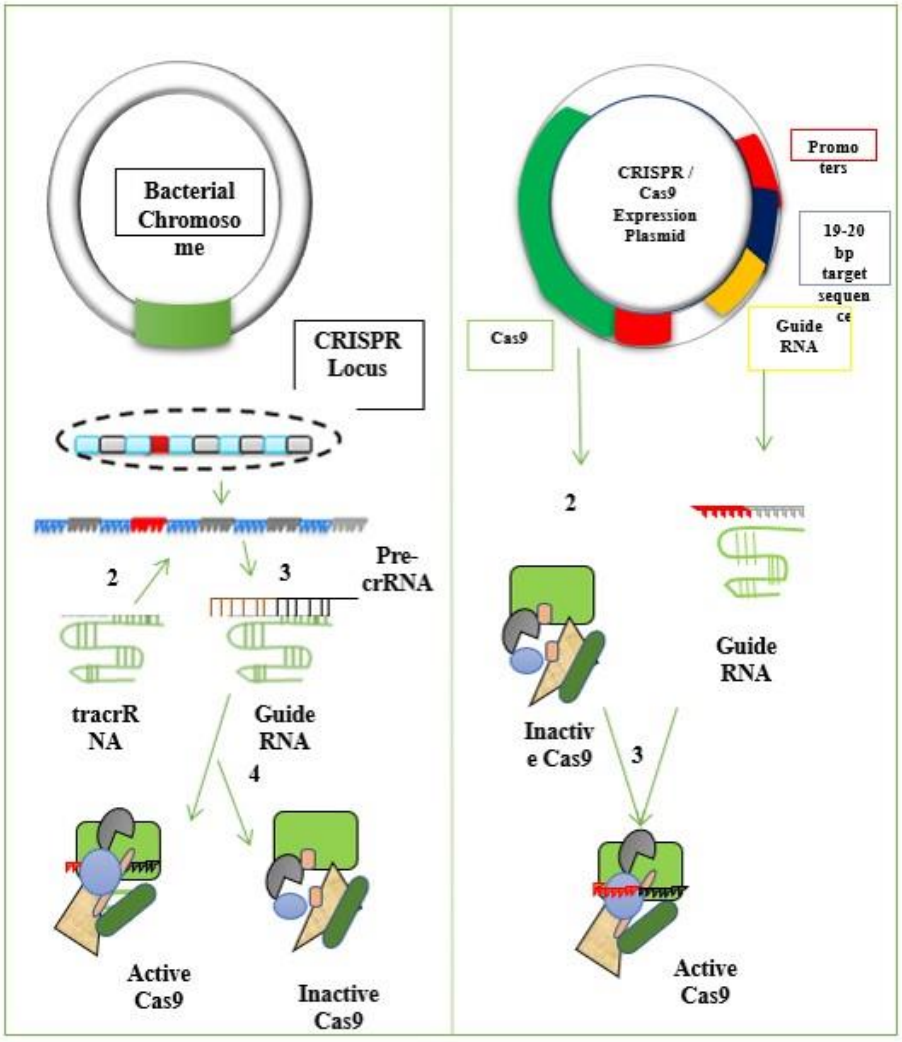

Figure 4. CRISPR/Cas9 system.

In several animal models of Alzheimer's disease (AD), gene therapy has demonstrated promise in raising enzyme activity and balancing low levels of bioactive chemicals. Promising improvements in AD pathology have been reported in initial clinical trials utilizing ex vivo gene therapy targeting nerve growth factor (NGF) [86]. Compared to small-molecule treatments for enzyme up-regulation, this strategy has advantages like improved safety, selective gene expression, and possibly higher efficacy [87]. Apolipoprotein E (APOE), neurotrophins (BDNF and NGF), Aβ-generating proteins (BACE1 and APP), and Aβ degradation enzymes (endothelin-converting enzyme, cathepsin B, and neprilysin) for siRNA-mediated gene silencing are among the many gene therapy targets presented by the diverse etiologies of AD [88].

d. **Regenerative medicine:** Scientists are investigating alternative methods to restore organ function as a result of the growing gap between organ donation and transplantation. Regenerative medicine, as defined by the US National Institutes of Health, is "the act of generating new, living tissues to replace or repair organ or tissue function lost as a result of illness, aging, trauma, or birth defects" [89]. The process of repairing fractured bone is sluggish and frequently unfinished. Moreover, autografts and allografts, which have comparable potential, have historically been utilized to promote bone repair. However, problems including poor availability, risk of infection (for allografts), morbidity at the donor site, blood loss, and difficulty in harvesting limit their utilization. Osteoinductive, minimally antigenic, biomechanically stable, and free of pathogens are the characteristics of the perfect bone graft [90]. Since they can provide mechanical support without eliciting strong immunological reactions, bioinert materials have attracted attention. The use of bioactive materials as implants for broken bone regeneration is currently receiving more attention in an effort to promote implant-bone integration. Advances in tissue engineering and nanotechnology present promising prospects for bone replacement and regeneration. The function of nano applications in regenerative medicine is explained below:

HA nanoparticles and chitosan, two commonly utilized materials, have their biological qualities improved by the inclusion of metallic nanoparticles [91]. For example, big improvements in protein adsorption and antibacterial characteristics have been demonstrated in bone tissue engineering using chitosan–nano-HA copper zinc alloy nanoparticles. Depending on the n beta TCP ratio, higher osteoconductivity, and improved mechanical properties have been seen for poly(lactic acid) (PLL) scaffolds coated with n beta TCP [92]. Beta tricalcium phosphate (n beta TCP) coatings on scaffolds with nanostructures enhance their biological characteristics. Active bone developments in rat calvaria have been demonstrated to be promoted by adding nano-HA to gelatin, which also increases matrix mineralization and alkaline phosphatase activity. Furthermore, it is thought that antibacterial medications combined with nanoparticulate elements for bone regeneration work better than conventional therapies, especially for osteomyelitis, which has been present for a long time. Plasma-treated electrospun poly(L-lactic acid) (PLLA)-co-poly(ε-caprolactone) gelatin-based scaffolds have demonstrated enhanced wound healing by significantly boosting cell proliferation and adhesion because gelatin has several integrin-binding sites [93]. Multiple wound healing mediators combined with biodegradable scaffolds appear to be promising for regulated medication delivery in the regeneration of skin tissue. According to a recent study, encasing several epidermal induction factors (EIF) in core-shell nanofibers can enhance cell proliferation by allowing numerous growth factors to be released simultaneously. The architecture of rosette nanotubes (RNTs), a type of nanomaterial, resembles those of keratin and collagen found naturally in skin [94]. Reactive

nanotubes (RNTs) have the potential to be the next generation of skin wound healing materials due to their ability to self-assemble and solidify when exposed to body temperatures. The pro-active nature of RNTs is highlighted by the fact that their incorporation into poly (2-hydroxyethyl methacrylate) (PHEMA), a hydrogel that is frequently used for skin regeneration, has been shown to enhance keratinocyte and fibroblast proliferation. A highly sulfated semisynthetic polysaccharide called pentosan polysulfate (PPS) can be added to the growth media to enhance the differentiation of chondrocytes from mesenchymal progenitor cells. [95]. PPS, both soluble and binding, promotes chondrocyte development of human mesenchymal stem cells (hMSCs) in polyethylene glycol (PEG)-hyaluronic acid (HA) media; the bound form is more effective in this regard. For intervertebral disk regeneration, this makes PPS with PEG-HA a competitive option. Biazar et al. cover several biologic nerve conduits and synthetic nerve guides in an incisive review [96]. Cirillo et al. have conducted further investigation into aligned electrospun nanofibers for nerve regeneration [97]. Via the use of PC-12 pheochromocytoma nerve cells, they synthesized an improved PCL membrane via electrospun technique and assessed its biological response. As a topological cue that promotes neurite formation and nerve regeneration, their results demonstrate that perfectly aligned PCL nanofibers containing gelatin offer greater contact guidance for nerve cells as compared to randomly oriented fibers. Heart rupture, a serious consequence of myocardial infarction, can arise from the restricted growth and regeneration potential of cardiac myocytes, which frequently cause scarring after ischemic injury. This can be decreased using techniques that improve regeneration and decrease myocyte death. Using scaffolds with a high capacity to transfer oxygen during the pretreatment of human mesenchymal stem cells (hMSCs) can encourage the differentiation of cardiomyocytes [98]. The potential for improving MSC differentiation into cardiomyocytes and for promoting cardiac regeneration has been demonstrated by electrospun hemoglobin/gelatin/fibrinogen nanofibers cross-linked with phytic acid. The remarkable cell adhesion, proliferation, and cytocompatibility of biomaterials made of poly(glycerol sebacate) (PGS) core polymers and gelatin shell polymers further validate their promise for cardiac tissue engineering.

4. Nanoparticles in DDS

Nanoparticles in Drug distribution Systems (DDS) represent the vanguard of pharmaceutical innovation, using nanoparticle capabilities to accomplish targeted distribution and controlled release of medicinal drugs. These complex structures are expertly designed to improve drug stability, solubility, and bioavailability while avoiding negative side effects and reducing dose frequency. DDS can considerably improve the pharmacokinetics and pharmacodynamics of pharmaceutical drugs by utilizing the unique physicochemical properties of nanoparticles, paving the path for more effective and efficient results from therapy. In the following sections, we go further into the operational processes of DDS, using biopolymers such as chitosan, alginate, xanthan gum (XG), and gold nanoparticles, exposing their primary functions in this transformative sector.

a. *Chitosan:* Since chitosan can function in tight epithelial junctions and has mucoadhesive qualities, it can be used in sustained drug release systems across a variety of epithelia, including the buccal, intestinal, nasal, ocular, and pulmonary [99]. To give ceftazidime topically, Silva et al. prepared an isotonic 0.75% w/w hydroxypropyl methylcellulose (HPMC) solution that contained hyaluronic acid nanoparticles, sodium tripolyphosphate, and chitosan [100]. The mucoadhesion

of these nanoparticles allowed them to interact with the ocular mucosa and prolong the release of antibiotics without causing cytotoxicity, which suggests that ocular medication delivery may be enhanced. Carbamazepine (CBZ) was delivered intranasally using carboxymethyl chitosan nanoparticles, which decreased systemic exposure and increased brain drug levels (Liu et al.) [101]. With excellent drug loading and encapsulation efficiency, these nanoparticles showed efficacious brain delivery. For transport to the gut, Jain investigated the release of 5-fluorouracil (5-FU) from hyaluronic acid-coated chitosan nanoparticles [102]. By preventing 5-FU from being released too soon in the stomach and small intestine, the nanoparticles may enhance the effectiveness of colon cancer treatment by lowering systemic toxicity.

b. *Alginate:* Because of its terminal carboxyl groups, alginate, a biopolymeric material, has anionic mucoadhesive characteristics that make it more powerful than neutral and cationic polymers. In order to raise serum insulin levels and decrease serum glucose levels in diabetic rats, Patil and Devarajan created insulin-containing alginate nanoparticles with nicotinamide acting as a permeation enhancer [103]. Nicotinamide nanoparticles given sublingually (5 IU/kg) demonstrated high bioavailability (> 80%) and pharmacological availability (> 100%). Compared to subcutaneous injection at 1 IU/kg, this method achieved a pharmacological potency of 20.2% and bioavailability of 24.1% in a streptozotocin-induced diabetic mouse model, demonstrating promise [104]. Haque et al. created alginate nanoparticles for the intranasal administration of venlafaxine (VLF) for the treatment of depression [105]. Alginate nanoparticles demonstrated larger blood-brain ratios of VLF concentration in comparison to intranasal VLF and intravenously delivered VLF solution, suggesting superior direct delivery of VLF to the brain. These nanoparticles have the potential to improve depression treatment by effectively delivering medication to the brain.

c. *Xanthan gum (XG):* A high molecular weight heteropolysaccharide with exceptional bioadhesive qualities, xanthan gum (XG) is made by the bacterium Xanthomonas campestris [106]. Xanthan gum is a popular pharmaceutical excipient because of its non-toxic and non-irritating properties. In order to cure sialorrhea, Laffleur and Michalek created a carrier that delivers tannin into the buccal mucosa by thiolating xanthan gum with L-cysteine. Compared to native xanthan gum, thiolation of xanthan gum increased its adherence to the buccal mucosa. Tannic acid works to astringe and dry the oral mucosa, while xanthan gum thiolate promotes increased salivary absorption [107]. This technology has the potential to be a useful tool for treating sialorrhea sufferers by decreasing salivary flow.

d. *Gold nanoparticles:* Gold is well-acknowledged, biocompatible, and inert. Gold nanoparticles (AuNPs) have become more well-known with the development of nanotechnology because of their distinct physicochemical characteristics. AuNPs vary in size from 1 to 100 nm. When AuNPs were originally discovered in 1857, Michael Faraday noted their distinctive behavior in solutions and their red color. In recent developments, targeted agents like as anti-epidermal growth factor receptor antibodies have been functionalized on AuNPs to promote apoptosis [108]. Furthermore, demonstrating their promise in targeted therapeutics, pH-responsive AuNPs that can release nitric oxide in acidic environments have been created. Drug delivery systems (DDSs) find gold nanoparticles (AuNPs) to be an attractive platform due to their ability to be fabricated with different core sizes. According to Chan et al., cellular uptake is influenced by the size of the nanoparticles [109]. According to their research, AuNPs with Herceptin coatings of between 2 and 100 nm are examined for breast cell internalization caused by the ErbB2 receptor [110]. Particles between 20 and 50 nm

in size had the best absorption rates and are promoted as apoptosis. Furthermore, in vivo research on passive tumor targeting using AuNPs between 10 and 100 nm showed that bigger AuNPs stayed close to the vasculature, whereas smaller AuNPs quickly diffused into the tumor matrix.

5. Theranostics: integration of diagnosis and treatment

"Theranostics", a combination of the words "therapeutics" and "diagnostics", is a forward-thinking methodology that combines therapeutic and diagnostic procedures into a single plan. The phrase was first coined by John Funkhouser to refer to the process of developing personalized treatments based on patient-specific diagnostic data [111]. This method integrates medication distribution, treatment monitoring, and diagnostic imaging under one framework to produce more tailored and efficient therapies. Despite being a relatively new concept, theranostics has been used for many years. The use of radioiodine, which has been used to identify and treat thyroid gland malignancies since the 1940s, is one prominent example. The ability to provide more exact and customized therapeutic interventions based on diagnostic data is one way that theranostics is advancing medicine.

5.1. Nanotheranostics-assisted chemotherapy

Hyaluronic acid (HA), folic acid (FA), and liposomes are examples of organic molecules derived from natural sources that are particularly useful in theranostics, a field that combines therapeutic and diagnostic capabilities [112]. Liposomes are very adaptable carriers because they can encapsulate hydrophilic and hydrophobic chemicals, improving the transport and preservation of medicinal substances. Because of its propensity to bind precisely to CD44, a molecule that is overexpressed in many malignancies, HA is used in nano-carriers to provide targeted therapy and tumor progression monitoring. Because of its affinity for folate receptors, which are frequently over-expressed in malignant tumors and enable targeted drug administration and imaging, FA is used. Antigen-antibody interactions also allow for precise targeting of particular cancer cell types, as seen in the application of these interactions in nanoscale metal-organic frameworks.

As there are several issues due to nanoparticle's biological heterogeneity and diverse clinical and epidemiological behaviors, human triple negative breast carcinoma (TNBC) lacks a tumor-specific targeted therapy. Hence to overcome these obstacles, as a clinical experimentation trial, Shetake et al. 2024 [113] developed a nano-inducer of ferroptosis for targeted chemotherapy of human TNBC. They developed a TNBC-specific targeted nano-delivery agent comprising of cRGD labeled magneto-liposome (T-LMD) and co-encapsulated with acid-coated iron oxide nanoparticles (MN-OA) and DOX in the liposome bilayer and core, respectively. Surprisingly, T-LMD was observed with increased uptake and induction of ferroptotic cell death in MDA-MB-231, a TNBC model cell line. Similarly, to address the limitations of antitumor efficacy of insufficient intracellular ferrous iron needed for ferroptosis and inadequate anti-cancer immune response, Zhu et al. 2024 [114] designed a multi-mode nano-platform (MP-FA@R-F NPs), which exhibited a synergistic effect of ferroptosis, apoptosis and induced immune response for enhanced antitumor therapy. MP-FA@R-F NPs target folate receptors, which were overexpressed on the tumor cells to promote intracellular uptake. Further, the obstacles related to intracellular oxidative stress remain the primary cause of evading tumor apoptosis. In this regard, Cui et al. 2024 [115] developed a chitosan

oligosaccharide-functionalized nano-prodrug (pH-GSH-H_2O_2-GGT) for cascade chemotherapy through oxidative stress amplification as a precise synergistic chemotherapy. The disulfide bond-conjugated DOX prodrug was constructed as a GSH-scavenger. Concurrently, the nano-prodrug was found to enhance strong oxidative stress and tumor cell apoptosis. Collectively, the developed nano-prodrug showed potential for synergistic tumor therapy. Since chemotherapy drugs have many limitations, including low aqueous solubility, inadequate targeting, and noticeable side effects, which contribute to the low effectiveness of chemotherapy, Pei et al. 2024 [116] developed a hyaluronic acid (HA) modified mesoporous carbon nitride–copper oxide nano-drug delivery system (PCNCuO HA@AZD) loaded with osimertinib (AZD) for effective chemotherapy/chemodynamic combination therapy of non-small cell lung cancer (NSCLC). The released copper (II) ions could generate ·OH by reacting with H_2O_2 through Fenton reaction, triggering the apoptosis of the tumor cells, thereby achieving chemodynamic therapy (CDT).

5.2. Nanotheranostics-assisted radiotherapy

Iikuni and colleagues developed a radiotheranostic agent using an ureidosulfonamide (US) scaffold labeled with 111In and 90Y to target carbonic anhydrase-IX (CA-IX), a biomarker commonly overexpressed in hypoxic tumor cells [117]. The 111In (γ-emitter) enables single photon emission computed tomography (SPECT) imaging, while 90Y (β-emitter) provides radiotherapy. This dual-labeled US scaffold effectively targets CA-IX-expressing HT-29 cells under hypoxic conditions, resulting in enhanced absorption of the radiotheranostic agents. In contrast, MDA-MB-231 cells, which have low CA-IX expression, show significantly reduced absorption. In vivo studies confirmed that the 111In and 90Y-labeled US accumulated significantly in HT-29 tumor-bearing mice, leading to delayed tumor growth compared to controls treated with saline [117].

Clinical trials incorporating the radiotracer Indium-111 (111In), demonstrating its diagnostic and therapeutic roles, are as follows.

a. 111In-Panitumumab for Nodal Staging in Head and Neck Cancer: A Phase I trial to examine the security and efficiency of 111In-panitumumab for staging malignant lymph nodes in head and neck cancer, with the goal of improving pre- and intraoperative localization.
b. [111In]In-CP04 for Medullary Thyroid Cancer: This Phase I trial looks into the use of 111In-CP04, a cholecystokinin-2 receptor ligand, in progressing or metastatic medullary thyroid cancer for diagnostic imaging and targeted therapy.
c. The Phase 0 study of 111In-CHX-Aδ-trastuzumab in HER2-positive breast, lung, and bladder malignancies evaluates HER2 lesion imaging, dosimetry, and biodistribution.

5.3. Photodynamic theranostics using direct irradiation

TPCI (Tetra-(p-carboxyphenyl) porphyrin Iodine), a small molecule photosensitizer with real-time monitoring and photodynamic treatment (PDT) capabilities, was created by Gao and coworkers [118]. TPCI, which has a high singlet oxygen quantum yield (98.6%), fluoresces weakly at first but kills cancer cells when exposed to moderate radiation. It subsequently moves to the nucleus, where it triggers the real-time monitoring of the cell death mechanism known as aggregation-induced emission (AIE). Studies conducted *in vivo* on tumor-bearing mice validated its efficacy in photodynamic theranostics. Furthermore, Li et al. 2015 [119] also developed cyclometalated iridium (III) complexes (1–3)

containing bis-*N*-heterocyclic carbene (NHC) ligands as theranostic and photodynamic agents targeting mitochondria. As observed in experimental and clinical trials, the developed complexes were more cytotoxic than cisplatin against the cancer cells and penetrated into human cervical carcinoma (HeLa) cells quickly and efficiently. All developed complexes showed and proved 3 orders of magnitude higher cytotoxicity upon irradiation at 365 nm, which are so far the highest photocytotoxic responses reported for iridium complexes.

Ricardo et al. 2024 [120] developed and tested low-cost Casiopeina III-ia zinc oxide nanoparticles (Cas III-ia-ZnO NPs) ZnO NPs exhibited fluorescence with emission at different wavelengths depending on their agglomeration and enabling real-time tracking bio-distribution along with apoptotic activity. In this developed system, ZnO NPs served as a sensitizer, generating reactive oxygen species (ROS) *in situ*, which also showed effective results while being tested on the HeLa and MDA-MB-231 cell lines when impregnated with CasIII-ia. As a result, test treatment of Cas-ZnO NPs eliminated cancer cells when combined with PDT.

5.4. siRNA therapy with nanotheranostics assistance

Using quantum dots (QDs) and small interfering RNAs (siRNAs), Kim et al. created an aptamer-based theranostic platform for targeted gene therapy and diagnosis [121]. Aptamo-QLs is the name of their platform, which targets triple-negative breast cancer cells (MDA-MB-231) by inserting anti-EGF receptor aptamer-lipid conjugates into QD-lipid nano-carriers [122]. Fluorescein isothiocyanate fluorescence has shown that this method enables high absorption of aptamo-QLs by cancer cells. Aptamo-QL-treated tumors showed increased red QD fluorescence in in vivo investigations using MDA-MB-231 xenografts as compared to QL-only groups. When two therapeutic siRNAs were co-delivered with aptamo-QLs, tumor growth inhibition was greatly increased, and recurrence was decreased. Recently, Li et al. 2024 designed a layer-by-layer self-assembly to load Cu2-xS nanoparticle (NP) cores with the siRNA of the*PD-L1* immune escape-related gene and wrapped a silk fibroin shell to form a multifunctional copper-based silk fibroin nanoplatform, denoted as CuS-PEI-siRNA-SFNs [123]. CuS-PEI-siRNA-SFNs induced cuproptosis and exerted an antitumor effect *via multiple* mechanisms, including photothermal therapy (PTT), chemodynamic therapy (CDT), and immune activation. The experimental results showed that CuS-PEI-siRNA-SFNs efficiently accumulated in the tumor tissues of 4T1 tumor-bearing inhibited primary tumor and lung metastasis and displayed excellent biosafety and antitumor activity. Furthermore, very recently, Zhang et al. 2024 developed a sequential release nanomaterial system for the co-delivery of hypoxia-induced tirapazamine and HIF-1α siRNA in cancer therapy [124]. This temporal sequential system designated as TPZ@MSN/HIF-1α siRNA@PDA@GOx (MTRPG) in which mesoporous silica nanoparticles served as a core to load hypoxia-induced chemotherapy drug tirapazamine (TPZ) and gene-targeted nucleic acid drug HIF-1α siRNA, polydopamine (PDA) as acid-responsive coating as well as to realize photothermal therapy, and glucose oxidase (GOx) as the outer layer to achieve starvation therapy and construct strong hypoxia to activate TPZ. Results demonstrated that released HIF-1α siRNA interfered with the up-regulated HIF-1α induced by the hypoxia condition and then caused hypoxia tolerance in tumors, reduced its expression, and achieved synergistic killing of cancer tumor cells with chemotherapy, thus possessing a promising future in clinical application.
Clinical evaluation of siRNA nanoparticles in fighting cancer:

a. Targeting VEGF in Metastatic Melanoma: A clinical trial used siRNA nanoparticles directed at the VEGF gene to reduce tumor angiogenesis in patients with metastatic melanoma. By inhibiting VEGF, the therapy hoped to reduce blood vessel development, slowing tumor growth and metastasis.
b. Viral Infection Management: Reseachers looked at siRNA nanoparticles that target the HCV genome in patients with chronic hepatitis C. The goal was to break down viral RNA, which successfully lowered viral load and improved liver function in infected individuals.
c. Combination Therapy in NSCLC: A trial combining siRNA nanoparticles with chemotherapy to target drug resistance genes in non-small cell lung cancer, thus boosting overall curative performance.
d. Addressing KRAS Mutations in Pancreatic Cancer: Researchers produced siRNA nanoparticles that target the KRAS oncogene, which needs to be frequently mutated in pancreatic cancer. They focused on delivering siRNA to pancreatic cancer cells in order to quiet the KRAS gene and limit tumor growth. It sought to evaluate the safety, biodistribution, and preliminary efficacy of this geared method in patients with advanced pancreatic cancer by combining gene-silencing precision with the targeted delivery capabilities of nanotheranostics.

6. Future prospects

Owing to consistent developments in nanotechnology, the field of multimodal diagnostics and therapies is about to undergo a revolutionary transformation. Modern imaging techniques, such as cryo-electron microscopy (Cryo-EM) and soft X-ray tomography, promise to deliver unparalleled high-resolution insights into cellular architecture. These advancements have implications for improving our understanding of material interactions at the molecular level, especially in departments like pathology and radiology, where precise imaging is critical for proper diagnosis. Thermo Fisher Scientific, for example, provides advanced Cryo-EM technologies that allow for thorough structural study of biomolecules, which aids research into cancer and neurological illnesses. In addition, methods such as Atomic Force Microscopy (AFM) and Near-field Scanning Optical Microscopy (NSOM) will allow for systematic investigation of nanoscale events, setting a tone for novel diagnostic applications. In cancer therapies, Bruker offers AFM technologies that enable tumor microenvironment research, which improves our capacity to detect disease pathways early on, allowing for timely intervention and individualized treatment strategies. As the therapeutic upfront, the combination of RNA interference (RNAi) and nanoparticles is predicted to usher in a new era of centered therapy techniques, especially for complicated illnesses such as Alzheimer's disease (AD) [125]. This collaboration will enable exact gene targeting and better medication delivery mechanisms, dramatically increasing therapeutic options. The use of liposomes, such as Doxil, to encapsulate doxorubicin (DOX) demonstrates how nanotechnology might reduce side effects while targeting cancer cells, a breakthrough that will be important in oncology departments. Companies such as Janssen Pharmaceuticals are pioneering liposomal formulations to improve the therapeutic index of chemotherapeutic drugs. Nanoparticles meant with therapeutic drugs such as dasatinib have proven to be powerful friends in the fight against proliferative vitreoretinopathy (PVR) [126]. This is especially important in the area of ophthalmology, where innovative businesses like Novartis are leading the development of nanoparticle-based drug delivery systems to improve the management of retinal illnesses such as diabetic retinopathy and retinal vein occlusion. As we look to the future, researchers

need to address the serious issues regarding the biocompatibility and optimization of these new technologies. By tackling these important queries, we will be able to fully tap into the revolutionary power of nanotechnology, setting the way for an overhaul in diagnostic and therapeutic tactics within the medical mil

7. Conclusion

As we enter a new age in cancer care, the rapid growth of theranostics powered by nanotechnology exposes a transformative path in detection and therapy. We found nanotechnology's extraordinary capabilities, highlighting its vital roles in increasing tissue regeneration, pioneering microfluidic advances, inventing Organ-on-a-Chip technologies, refining diagnostic imaging, and changing drug delivery systems. Despite these great advances, we must be cautious about the safety characteristics of nanoparticles. While they hold significant promise, thorough safety evaluations are required for their successful clinical use. Metal-based nanosystems, including liposomes, nanoparticles (NPs), and metal-organic frameworks (MOFs), are at the forefront of targeted drug delivery, phototherapy, and immunotherapy. Furthermore, the combination of nano and micromaterials with physical stimuli has greatly increased imaging techniques, improving MRI contrast and photoacoustic resolution. With the worldwide cancer burden anticipated to climb to about 30 million new cases per year by 2040, it is vital to address manufacturing, quality control, and safety concerns to protect patient health. To traverse this region properly, significant safety precautions must be prioritized. Long-term biodistribution studies are vital for determining the organ-specific accumulation and potential toxicity of nanoparticles, including gold nanoparticles. Immunotoxicity tests will detect inflammatory responses, whereas genotoxicity tests are required to assess DNA damage risks. Exploring biodegradable materials has the potential to reduce environmental impact, emphasizing the importance of sustainable nanotechnology processes. Looking ahead, nanotheranostics holds great promise. Corporations such as BIND Therapeutics and Nanotherapeutics, Inc. are leading the way for novel targeted nanoparticle medicines. In Europe, Curetis Naamloze Vennootschap (N.V.) and Nanobiotix are making substantial advances in molecular diagnosis and cancer therapy. Moreover, advances in personalized medicine from Japan and China demonstrate a worldwide commitment to improving cancer treatment. MedImmune, an AstraZeneca company, is also actively developing nanotheranostic systems for targeted drug administration and imaging.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

Authors report no conflict of interest in preparation of the manuscript.

Author contributions

Conceptualization, D.B. and T.A.; methodology, D.B., T.A., S.S. and M.B..; software, D.B., T.A. and S.S..; validation, D.B. and T.A.; formal analysis, D.B. and S.S..; investigation, D.B., T.A. and M.B.; resources,D.B. and T.A..; data curation, D.B., T.A., S.S. and M.B..; writing—original draft preparation, D.B., T.A.; writing—review and editing, D.B., T.A., S.S. and M.B.; visualization, D.B. and S.S.; supervision, D.B., S.S. and M.B. project administration, D.B.; All authors have read and agreed to the published version of the manuscript.

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