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Exosome-driven biohybrid nanorobots: bridging nature and nanotechnology in biomedical innovation

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Exosome- and extracellular vesicle (EV)-based biohybrid nanorobots represent a cutting-edge approach in nanomedicine, combining the natural targeting, immune tolerance, and molecular transport capabilities of EVs with the functional versatility of engineered nanomaterials. These hybrid systems can be designed for guided or autonomous navigation, enabling site-specific drug delivery with minimal cytotoxicity. Recent advances have integrated magnetic, photothermal, or drug-loaded components into EVs, transforming them into innovative nanoscale delivery systems. As naturally secreted vesicles from most cell types, EVs facilitate intercellular communication and are increasingly recognized for their clinical potential in treating conditions like Crohn's disease, type 1 diabetes, and COVID-19. Biohybrid EV nanorobots offer enhanced biodistribution, stability, and cellular uptake compared to traditional nanoparticles. Key design challenges include ensuring reproducibility, size control, and functional stability. This next-generation drug delivery platform holds promise for overcoming limitations of conventional systems while advancing targeted therapy and personalized medicine.

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1. Introduction

Nanorobotic systems engineered at the nanoscale using nanomaterials and nanotechnologies have emerged as a transformative frontier in biomedical science. Over the past few decades, integrating nanorobotics into clinical applications has significantly advanced the field of precision medicine. Medical robotic systems, particularly in macroscale interventions, combine patient-specific data (*e.g.*, medical imaging and laboratory test results) with generalized anatomical and statistical models to enhance the performance of diagnostic, surgical, and rehabilitative tasks.[1] In surgical settings, robots have demonstrated advantages such as enhanced dexterity, tremor filtration, improved ergonomics, and access to anatomically constrained sites (Fig. 1A), thus supporting the progression of minimally invasive procedures.

At the nanoscale, nanorobots are uniquely suited for biomedical applications because their dimensions match many biological structures, including proteins, viruses, and cellular organelles. This compatibility facilitates their use in real-time biosensing, disease diagnostics, and targeted drug delivery tasks. Nanorobots can be engineered to deliver pharmacological or mechanical interventions at precise intravascular or intracellular locations, offering new paradigms in personalized therapy.[2] They also promise future applications in cancer cell eradication, vaccine enhancement, and guided molecular transport.

Extracellular vesicles (EVs) represent a heterogeneous population of membrane-bound vesicles secreted by cells, broadly classified into exosomes, microvesicles, and apoptotic bodies. Among them, exosomes are a well-defined subtype, typically ranging from 30–150 nm in size, formed through the endosomal pathway involving multivesicular bodies (MVBs). Exosomes are enriched with bioactive molecules, including proteins (*e.g.*, receptors, transcription factors, enzymes), lipids, and nucleic acids (*e.g.*, DNA, mRNA, miRNA), which reflect the

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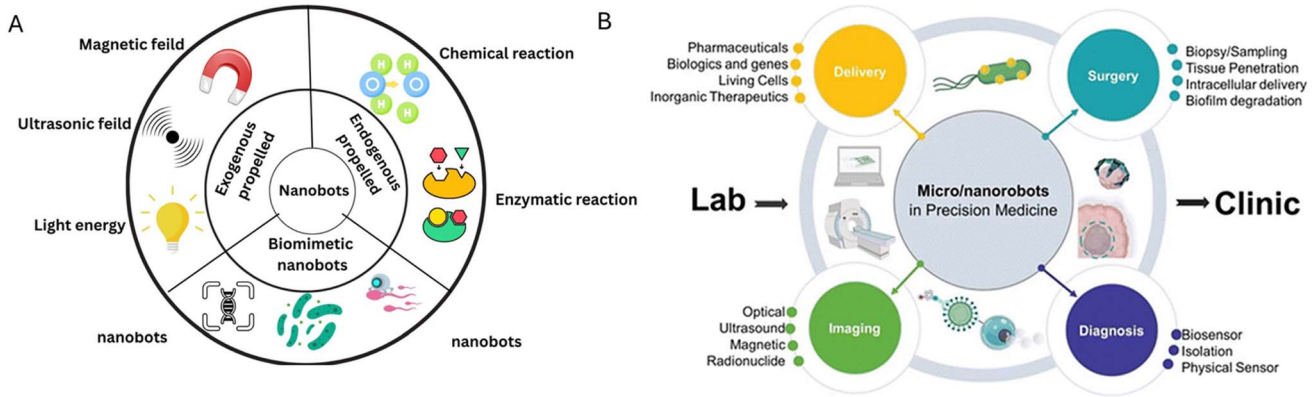

Fig. 1 (A) Schematic diagram of nanobots based on their movement (external *vs.* internal propulsion) and biomimetic design. Propulsion mechanisms of nanorobots: internal propulsion relies on chemically driven or biologically powered movement, whereas external propulsion utilizes applied fields (magnetic, ultrasound, or light). In biomedical contexts, external propulsion offers precise spatial control, while internal propulsion provides autonomous navigation in complex biological environments. Each type serves unique roles in medical and scientific fields, offering advanced solutions for targeted and efficient interventions. (B) Current trends of micro/nanorobotics in precision medicine. Reprinted from ref. 5 under Creative Commons Attribution 4.0 International License.

physiological status of their originating cells.[3] These vesicles are key mediators of intercellular communication and play essential roles in healthy and pathological contexts. In contrast, microvesicles (100–1000 nm) bud directly from the plasma membrane, while apoptotic bodies (>1000 nm) originate from cell fragmentation. These distinct origins lead to differences in molecular cargo: exosomes are enriched in endosomal proteins (*e.g.*, tetraspanins, Alix, TSG101) and specific RNAs, while microvesicles and apoptotic bodies often carry cytoskeletal and nuclear components. Given these differences, exosomes provide a unique biological platform for engineering biohybrid nanorobots due to their defined biogenesis, nanoscale size, and cargo specificity.

Current advances in exosome isolation, including sequential ultracentrifugation, ultrafiltration, and immunoaffinity capture, enable their characterisation as biocompatible, non-immunogenic nanocarriers. These properties position exosomes as promising vehicles for targeted delivery and disease diagnosis, particularly when hybridized with synthetic nanomaterials.[4] By leveraging EVs' unique biological traits and engineered nanostructure programmability, researchers are now exploring biohybrid nanorobots as next-generation theranostic tools in precision medicine (Fig. 1B).

In this review, we explore the emerging field of exosome-based nanorobots, highlighting their potential as transformative tools in precision medicine. By combining the natural targeting ability and biocompatibility of exosomes with advanced nanotechnology and genetic engineering, researchers are developing biohybrid systems capable of targeted drug delivery, gene therapy, diagnostics, and regenerative applications.

2. Exosome and extracellular vesicle

Exosomes and other EVs are nanoscale membrane-bound particles secreted by virtually all cell types into the extracellular space. These vesicles are increasingly recognized as pivotal mediators of intercellular communication, facilitating the transfer of bioactive molecules such as proteins, lipids, metabolites, and nucleic acids between cells. Exosomes, typically ranging from 30–150 nm in size, originate from the endosomal pathway *via* inward budding of multivesicular bodies (MVBs), while other classes of EVs such as microvesicles (100–1000 nm) and apoptotic bodies (>1000 nm) are formed through direct budding from the plasma membrane or apoptotic processes, respectively.[6,7]

Initially thought to function solely in cellular waste disposal,[8] exosomes have been identified as critical players in physiological and pathological contexts. They are implicated in immune modulation,[9] stem cell maintenance,[10] neural signaling,[11] and tissue regeneration,[12] while also playing roles in the progression of diseases such as cancer,[13] neurodegeneration,[14] cardiovascular disorders,[15] and inflammation.[16] The heterogeneity of EVs in size, density, and molecular composition complicates their classification and functional analysis. While exosomes are considered to arise from the endosomal system, microvesicles form by outward budding of the plasma membrane, and apoptotic bodies emerge during programmed cell death.[17,18] Despite their shared structural features such as a lipid bilayer envelope these subtypes differ significantly in molecular content and biogenesis pathways, often leading to challenges in isolation and characterization.

Clinically, exosomes have garnered intense interest as diagnostic biomarkers and therapeutic carriers. Their endogenous origin confers biocompatibility and reduced immunogenicity, and their lipid bilayer structure protects encapsulated nucleic acids and proteins from enzymatic degradation. Furthermore, their small size and membrane composition allow them to cross physiological barriers, including the blood–brain barrier, making them attractive vectors for central nervous system (CNS)-targeted therapies.[19,20] Efforts in exosome engineering are focused on improving cargo loading efficiency, enhancing target specificity, and overcoming challenges related to off-target effects and innate bio-distribution. One of the

significant hurdles for clinical translation is the lack of precise targeting, as exosomes may distribute non-specifically throughout the body, potentially leading to unintended immunogenic responses due to cellular impurities from their origin.[21] A deeper understanding of exosome biology and biogenesis is essential to address these limitations, particularly for developing next-generation biohybrid nanorobots that integrate exosomal features with synthetic functional materials.

2.1 Biological composition of exosome

Exosomes are nanoscale vesicles encapsulated by a lipid bilayer membrane and are rich in diverse biomolecular cargo, including proteins, nucleic acids, and metabolites.[22] The molecular contents of exosomes largely reflect the type and physiological condition of their parent or donor cells, making them highly dynamic and cell-type-specific messengers[23] Fig. 2 illustrates the structural and compositional complexity of exosomes. The lipid bilayer of exosomes is composed of sphingomyelin, cholesterol, and ceramides components that not only provide membrane rigidity but also play essential roles in cargo sorting, exosome formation, release, and intercellular signaling.[24] This unique lipid composition contributes to the exosomes' structural integrity and bioactivity, particularly when functioning as natural nanocarriers in biomedical applications.

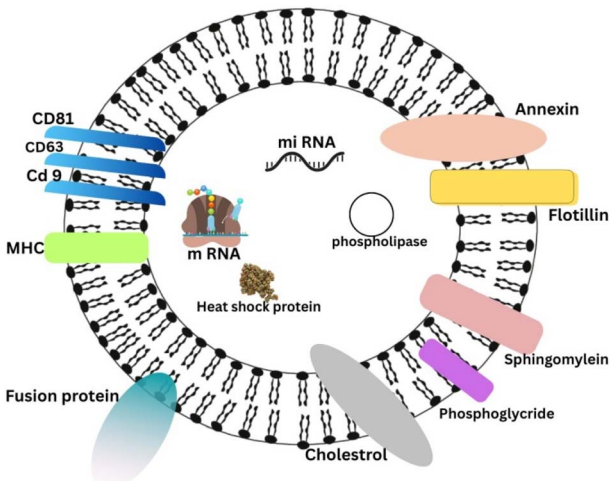

Fig. 2 This schematic represents a biological exosome, a nanoscale vesicle released from cells that carries a complex mixture of biomolecules. Exosomes are enriched in cholesterol, sphingomyelin, and phosphoglycerides, which confer stability in circulation. Their membranes display characteristic tetraspanin proteins (CD9, CD63, CD81), major histocompatibility complexes (MHC), and scaffolding proteins such as annexins and flotillins, which play roles in membrane trafficking, immune modulation, and intercellular communication. Internally, exosomes encapsulate a diverse molecular cargo, including mRNA, microRNA (miRNA), proteins (*e.g.*, heat shock proteins), and enzymes (*e.g.*, phospholipases), enabling them to transfer functional biomolecules across cells. This natural architecture provides the biological blueprint for engineering exosome-based nanocarriers and biohybrid nanorobots, with potential applications in targeted therapy, diagnostics, and regenerative medicine.

Regarding nucleic acids, exosomes harbor a broad array, including genomic DNA, messenger RNA (mRNA), and multiple classes of non-coding RNAs. MicroRNAs (miRNAs) are the most abundant and well-characterized, playing pivotal roles in regulating gene expression and intercellular communication.[25] These miRNAs involve various biological processes, including exocytosis, hematopoiesis, angiogenesis, and immune regulation. Beyond miRNAs, exosomes also contain other non-coding RNA species such as ribosomal RNA (rRNA), long non-coding RNA (lncRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), and piwi-interacting RNA (piRNA). These RNA molecules contribute to various cellular processes and are particularly implicated in regulating oncogenic pathways and tumor progression.[26] Exosomes' rich and multifunctional biological composition highlights their potential as diagnostic biomarkers and biohybrid nanorobotic platforms. Understanding these molecular features is essential for engineering exosome-based delivery systems with high precision and therapeutic efficacy.

2.2 Exosome biogenesis incorporated in multiverse bodies

Exosome biogenesis is intricately linked to the endosomal system, particularly through the formation of intraluminal vesicles (ILVs) within MVBs, which are advanced late endosomal compartments. These MVBs contain multiple ILVs enriched with proteins, lipids, and cytosolic components that reflect their cellular origin. When MVBs fuse with the plasma membrane, ILVs are secreted into the extracellular space as exosomes.[27,7] MVBs have dual fates: they may undergo exocytosis by fusing with the plasma membrane to release exosomes, or they may enter a degradative pathway *via* lysosomal fusion, or autophagosome-lysosome fusion.[28] The heterogeneity of MVBs suggests a functional distinction between secretory and degradative subpopulations, although the extent of this differentiation remains under investigation.[29]

Fig. 3 schematically illustrates this process in a tumor cell: extracellular materials are first internalized through endocytosis, forming early endosomes. These mature into MVBs containing ILVs the precursors of exosomes. MVBs release exosomes through fusion with the plasma membrane upon maturation or are degraded *via* lysosomal pathways. Tetraspanins and lipid components also play essential roles in the membrane remodeling processes crucial for exosome generation and release. A central player in exosome biogenesis is the Endosomal Sorting Complex Required for Transport (ESCRT) machinery, a multi-protein system that drives membrane invagination and cargo sorting. This includes ESCRT-0, -I, -II, and -III, along with the ATPase Vps4.[30] The ESCRT pathway typically functions through ubiquitin-dependent sorting, recognizing polyubiquitinated proteins *via* key adaptors such as Hrs, STAM1, and TSG101. However, there is evidence of both ubiquitin-dependent and -independent mechanisms for cargo incorporation. For instance, while MHC-II proteins are enriched *via* ubiquitination signals,[31] their non-ubiquitinated forms have also been observed in exosomes, suggesting alternative routes for cargo loading.[32,33]

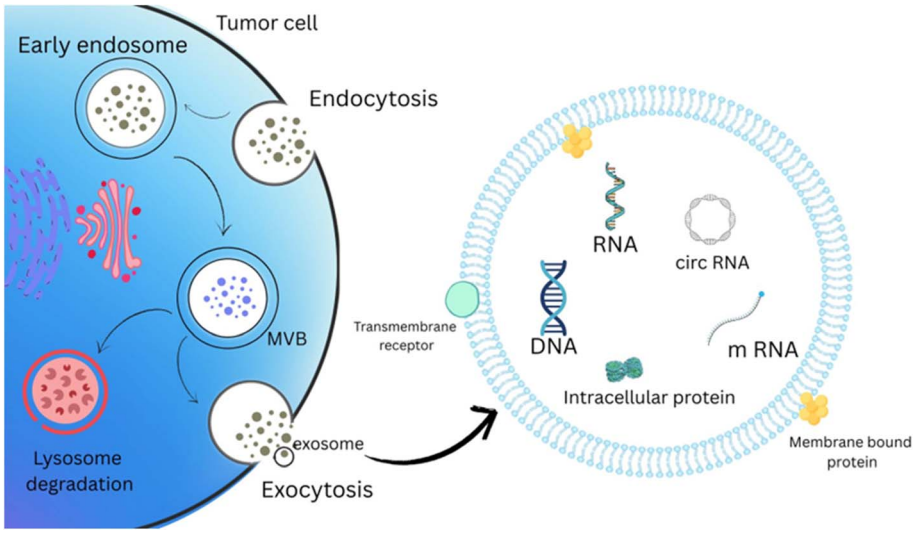

Fig. 3 This image illustrates the biogenesis and function of exosomes, particularly in a tumor cell.

Exosome secretion is heavily regulated by the Rab GTPase family members of the Ras superfamily involved in intracellular vesicle transport and membrane trafficking. Rab27a influences exosome size and release, while Rab27b contributes to MVB positioning within the cell.[34] Rab11 and Rab35 are involved in endosome recycling and vesicle docking. Notably, Rab35 mediates exosomal proteolipid protein (PLP) release in oligodendrocytes by promoting MVB-plasma membrane fusion.[5] Understanding the complex interplay of these molecular regulators is fundamental to harnessing exosomes in biohybrid nanorobotic systems for targeted therapeutic delivery, as engineering efforts often rely on modulating MVB trafficking and exosome loading pathways.

Table 1 describes the different therapeutic agents. The exosome content of proteins, lipids, and potentially nucleic acids can be manipulated to increase their therapeutic potential

Table 1 Genetically modified exosome as drug delivery

Therapeutic agent	Targeting molecule	Delivery method	Target cell types	Therapeutic purpose	References
BACE1 siRNA	RVG peptide	LAMP-2B	Neuronal cells (*e.g.*, Neuro-2a)	Potential treatment for Alzheimer's disease	36
miRNA-124	RVG peptide	LAMP-2B	Cortical neural precursor cells	Enhances neural regeneration post-ischemia	36
KRAS siRNA	iRGD peptide	LAMP-2B	Adenocarcinoma, lung epithelial cells (A549)	Targets mutated KRAS gene in cancers	37
DOX (doxorubicin)	iRGD peptide	LAMP-2B	Breast cancer cells	Facilitates specific DOX delivery for cancer therapy	37
SOX2 siRNA	Tlyp-1 peptide	LAMP-2B	Lung cancer cells, stem-like A549 cells	Gene-based treatment for malignancies	38
miRNA-140	CAP peptide	LAMP-2B	Chondrocytes	Slows down progression of osteoarthritis	39
KGN	E7 peptide	LAMP-2B	Synovial mesenchymal stem cells (SF-MSCs)	Aids in cartilage regeneration	40
Imatinib, BCR-ABL siRNA	IL-3	LAMP-2B	Leukemia cells (*e.g.*, LAMA84, K562)	Inhibits growth of leukemia cells	41
5-Fluorouracil	zHER affibody	LAMP-2B	Colorectal carcinoma cells (HCT-116)	Enhances chemotherapy and reduces drug resistance	42
Anti-miRNA-21	DARPins	LAMP-2B	HER2+ breast cancer cells (SKBR3)	RNA interference therapy for HER2-positive tumors	42
Tpd50 siRNA	DARPin	LAMP-2B	HER2+ breast cancer cells	Silencing HER2-related genes in cancer	43
MSC-derived exosomes	IMTP peptide	LAMP-2B	Cardiomyocytes (H9c2)	Heart tissue repair after damage	44
miRNA-let7a	GE11 peptide	LAMP-2B	Breast cancer cells (HCC70)	Targets tumors expressing EGFR	45

significantly. Thus, therapeutic particles could be introduced to exosomes or exosome mimetics through engineering parental cells or direct loading. Parent cell. Indirect engineering of exosomes is when the parent cells are modified, either genetically or by another means, to excrete exosomes that have the desired properties. In this process, functional molecules such as small-molecule therapeutic drugs or nucleic acids are introduced into the parent cell's cytoplasm at high concentrations before exosome formation, which leads to the encapsulation of the functional molecules within the lumen of the exosomes during biogenesis. Indirect engineering can also modify exosomes to display functional molecules on their surface through positional modification of the parent cell. In contrast, direct engineering of exosomes takes place after exosomes are isolated. This engineering includes loading therapeutic agents such as small-molecule drugs, proteins, or even nanomaterials into exosomes through membrane permeabilization. Generally, direct engineering is less complicated than modifying parent cells, and it is much more widely used for developing novel DDS. Direct engineering uses a variety of chemical and mechanical methods, such as electroporation, sonication, extrusion, freeze-thaw, membrane permeabilization with surfactant molecules (saponin, Triton), and dialysis,[35] since the former integrate directly onto the exosome lipid bilayer without having to cross it. Active-cargo loading is based on implementing chemical or physical methods that alter exosomes' permeability.

Understanding EV biogenesis is not only fundamental for appreciating their natural biological roles but also crucial for their integration into advanced nanorobotic systems. The intrinsic features of exosomes, such as their membrane composition, capacity for cargo loading, and natural targeting ability to provide a biological blueprint that inspires nanorobotic design. Thus, the transition from cellular exosome production to engineering biohybrid nanorobots is practical and necessary, bridging natural vesicular biology with artificial propulsion and control strategies.

3. Nanorobotics, basic principles and design

Nanorobotics is dedicated to the research and development of artificial machines with a maximum size on the micron scale for a wide range of real-world applications. This emerging research field has received ever-increasing attention, especially after molecular machines were selected as the topic of the Nobel Prize in Chemistry 2016. This interdisciplinary field focuses on designing and engineering nanoscale devices capable of performing specific tasks in biological or artificial environments. These nanomachines typically range from 1 to 100 nanometers in size and are composed of materials such as metals, polymers, or biomolecules. Designed at the molecular or cellular scale, nanorobots are increasingly envisioned as tools in targeted diagnostics, drug delivery, and minimally invasive therapies.[46] The construction of nanorobots follows two primary strategies: bottom-up self-assembly using molecular recognition and top-down fabrication using lithographic techniques. Functional nanorobots must integrate features that support sensing, propulsion, navigation, and environmental interaction. Intelligent nanorobots are often responsive to various stimuli including pH, temperature, magnetic fields, or biomolecular signals, enabling site-specific delivery with reduced off-target effects. Propulsion may be biologically or externally guided using acoustic, magnetic, or optical forces as shown in Fig. 4.[47]

Surface modifications play a crucial role in achieving target specificity. For example, functionalizing nanorobots with ligands or antibodies enables them to recognize and bind to disease-specific markers. Drug encapsulation within a payload chamber further supports controlled release at target sites. A significant innovation in nanorobotics is the development of theranostic nanorobots platforms that combine therapeutic and diagnostic capabilities. Despite their immense potential, the field of nanorobotics remains in its infancy, facing key challenges such as precision navigation in biological fluids, biocompatibility, scalability, and high development costs.

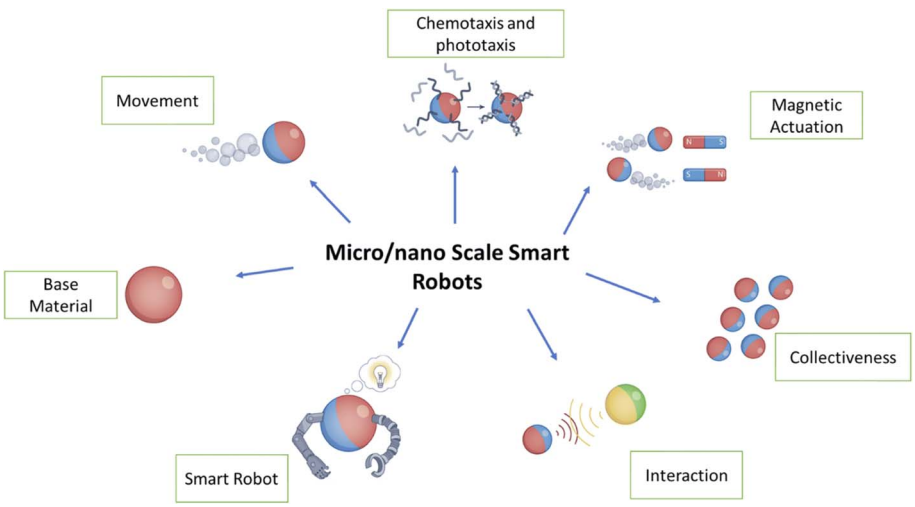

Fig. 4 Micro- and nano-robots constructed using micro- and nano-scale materials, Reprinted from ref. 47, Copyright 2022, Nature.

3.1 Biohybrid nanorobots with nanotechnology

Biohybrid nanorobots represent a convergence of biological building blocks and engineered nanomaterials to create multifunctional systems capable of active navigation and targeted therapy. Exosomes (extracellular vesicles, EVs), with their natural stability, targeting ability, and cargo-carrying capacity, provide a unique biological scaffold for constructing such nanorobots. Unlike purely synthetic nanostructures, exosome-based hybrids combine biocompatibility with design flexibility, offering translational potential for precision medicine. In this section, we highlight their integration with diverse design and control strategies.[48] Nanorobots draw energy from magnetic fields and ultrasonic sound, and a nanorobot could interface with a piezoelectric effect-based membrane that could capture ultrasonic oscillations from the water and transform it into electrical power, as shown in Fig. 5.[49] Synthetic parts provide structural integrity, controllability, and functional flexibility for propulsion, imaging, and delivering drugs and other moieties. The result is a biohybrid nanorobot that can operate sophisticated processes, including controlled site-specific drug delivery, biosensing and detection, detoxification, and repairing organelles or performing surgical-like functions at the cellular level. A common approach involves coating synthetic nanoparticles with cell membranes or embedding them within bio-derived vesicles, allowing the hybrid system to evade immune detection and mimic natural cellular behavior. Enzymes can be incorporated to facilitate catalytic movement, while magnetic or light-responsive materials enable external control over their navigation. The synergy of biological intelligence and technological precision in biohybrid nanorobots holds enormous promise for personalised medicine, particularly in targeting hard-to-reach disease sites, minimising side effects, and enhancing therapeutic outcomes. The composition of nano robots include payload which is the hollow part of the structure which consists of a small dose of drug or a medicine.[47]

A micro camera and a nano robot can be manually controlled by the operator to navigate through the body, meaning that the operator can design the nano robot to carry out a pre-planned path. Other than that an electrodes, protruding or sticky electrodes could also be used to kill or destroy the cancerous cells that have formed inside the body by using electric current to heat the cancerous cells causing thermal damage that would lead the cancerous cells to die.[1] Laser that could burn the harmful materials such as arterial plaque, blood clot, and cancerous cells. Ultrasonic signal generators are needed if nanorobots must classify and destroy kidney stones. Next, Swimming tail because nanorobots must flow against the blood's direction, there must be some propulsion device to enter the body. It has application in biomedical, environmental, and other areas, with many benefits.[46] The positive side of nanorobots is their speed and survivability, but the negative aspects are the expensive design and development, complexity and invisibility.

For instance, Fe_3O_4-exosome hybrids enable magnetically guided navigation and simultaneous MRI tracking, while gold–exosome composites allow light-triggered release for photothermal therapy. Antibody- or peptide-modified exosomes improve receptor-specific targeting, expanding disease-specific applications. These design principles highlight the modular flexibility of exosomes as chassis for nanorobotic engineering.[49] Control strategies for exosome-based nanorobots can be broadly classified as external or internal. External approaches include magnetic steering (enabling deep tissue navigation), ultrasound-driven propulsion (non-invasive and tissue-penetrant), and near-infrared photothermal guidance (spatio-temporal control), as shown in Table 2. Internally, catalytic nanomotors exploit chemical gradients or enzymatic reactions to achieve autonomous movement in complex microenvironments. Hybrid approaches integrating magnetic and enzymatic control are also emerging, offering both long-range navigation and local adaptability. These diverse strategies underscore the feasibility of precise navigation *in vivo*, which remains a critical challenge for clinical translation.

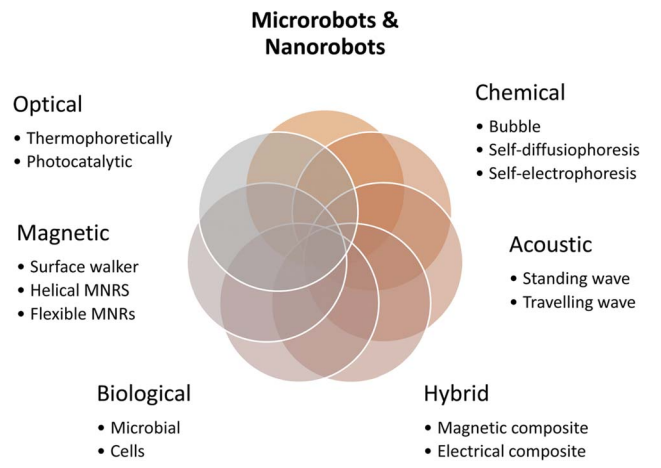

Fig. 5 Different types of Biohybrid nanorobots that can be controlled through proper actuation to accomplish specific tasks, Reprinted from ref. 49 Copyright 2024, RSC.

3.2 Engineering exosome-based nanorobots

Engineering exosome-based biohybrid nanorobots typically involves integrating the natural vesicular properties of exosomes with synthetic nanomaterials or propulsion systems to achieve enhanced functionality. Though exosomes are naturally derived, they can still be manipulated using various forms of genetic engineering. Several strategies have been reported. For instance, exosomes have been coated onto magnetic nanoparticles, enabling remote-controlled navigation across the blood–brain barrier in murine models.[50] Similarly, gold–exosome hybrid nanostructures have been fabricated to achieve light-triggered drug release with improved tumor penetration.[53] Another approach involves coupling exosomes with catalytic nanomotors, which convert chemical energy into propulsion, enhancing drug delivery efficiency in hypoxic tumor environments.[52] These studies provide experimental evidence that exosomes can serve as biologically derived building blocks for functional nanorobots, combining their natural biocompatibility and targeting capacity with externally controllable navigation systems. Such hybrid systems highlight the feasibility of

Table 2 Control mechanisms for exosome-based biohybrid nanorobots

Control mechanism	Representative system	Advantages	Limitations	Applications	References
Magnetic field	Fe_3O_4-exosome hybrids	Deep tissue penetration; MRI compatibility; remote guidance	Requires external magnetic setup	BBB crossing, targeted tumor therapy	50
Ultrasound	Acoustically propelled exosome nanorobots	Non-invasive, controllable in real time, safe in biological tissue	Limited spatial precision in complex microenvironments	Local drug delivery, clot dissolution	40
Near-infrared (NIR) light	Gold–exosome nanohybrids	Triggered release, photothermal effect, spatiotemporal control	Limited tissue penetration depth	Photothermal cancer therapy	51
Catalytic/enzymatic propulsion	Exosome–catalase nanomotors	Autonomous propulsion in hypoxic environments	Short propulsion duration, requires chemical gradients	Tumor microenvironment navigation	52

translating exosome-based nanorobots into precision nanomedicine platforms.

Genetic engineering techniques are employed to functionalize exosomes with specific targeting ligands, therapeutic cargos, and surface modifications. These engineered exosomes can be further developed into biohybrid nanorobots with advanced functionalities for targeted therapy, gene/RNA delivery, molecular diagnostics, and exosome imaging. This integrated approach enables the development of multifunctional nanorobots for precision medicine and next-generation theranostic applications, as shown in Fig. 6.

This genetic fusion greatly improves exosome targeting ability and their selective targeting of cells, especially tumor cells. There have been multiple modifications strategies for the exosome. For example, exosomes derived from HEK293 were genetically modified to express GE11 short peptide and express miRNA Let-7a.[45] The GE11 peptide targets the epidermal growth factor receptor, which is highly overexpressed in many epithelial tumors. Engineered exosomes were labeled with 1,1′-dioctadecyltetramethylindole tricarbocyanine iodide and delivered by intravenous injection in tumor-bearing mice. Imaging studies were conducted *in vitro* and *in vivo*; it was found that GE11-modified exosomes accumulated at the tumor site at three times the level in the control group, and the delivered miRNA Let-7a inhibited tumor growth. Another common approach is ligand modification of the exosome surface to facilitate targeting. As an example, exosomes originating from HEK293T cells were functionalized with lipophilic hyaluronic acid (lipHA-hEVs), serving as a targeting ligand for breast cancer cells that exhibit high levels of CD44 expression, enabling the effective delivery of doxorubicin. These lipHA-hEVs targeted doxorubicin specifically into breast cancer cells, resulting in an 89% reduction in tumor mass and a 50% improvement in animal survival.[54] In addition, receptor protein engineering is another preparation strategy that can be applied to targeted delivery of exosomes. Recently a method was developed in HEK293 cells that allowed the partitioning of reporter proteins into the exosomes so that the exosomes could be messenger RNA (mRNA) or protein-based vaccines.[55]

3.3 EV-derived nanorobots

Nanorobots derived from EVs are a new class of biologically inspired nanodevices designed by considering the natural properties of EVs, *i.e.*, exosomes and microvesicles for precise biomedical applications. When researchers develop EV derived nanorobots, they use the native structure of EVs as a biological chassis and release functional nanomaterials into the EV interior or modulate functional nanomaterials onto the surface of the EV.[55] The construction methods can vary widely, but include surface modifications by introducing functional ligands, peptides, or antibodies that enhance tissue or cell specificity; incorporating magnetic or plasmonic nanoparticles into the EV structure that provide guided navigation and imaging functions. The loading of EVs can occur through passive loading, such as through incubation, or *via* active loading techniques, such as electroporation, sonication, or extrusion. Hybrid

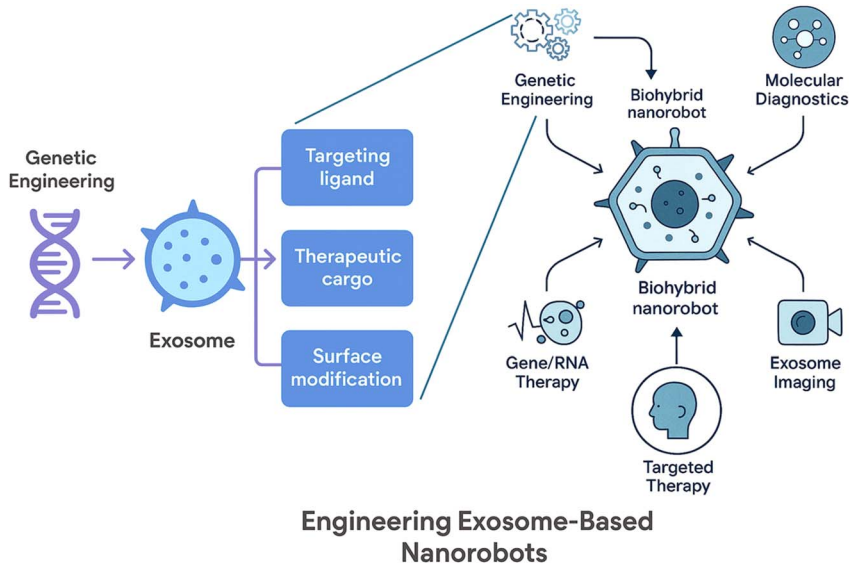

Fig. 6 Schematic representation of the engineering process and potential applications of exosome-based biohybrid nanorobots.

designs can also incorporate responsive elements that enable the nanorobots to release their cargo based upon environmental signals (*e.g.*, pH, temperature, enzymatic activity) in pathological sites.[56] Significantly, EVs from certain types of cells (*i.e.*, immune cells or tumor cells) can also acquire vesicular membrane proteins that further enhance their target specificity. Surface modification is a key strategy to enhance the therapeutic potential of exosome-based nanorobots, enabling targeted delivery, prolonged circulation, and functional integration. Two broad approaches exist: pre-modification and post-modification. Pre-modification involves engineering the parent cells prior to exosome secretion—for example, through genetic modification to express targeting ligands (*e.g.*, Lamp2b fused with integrin-specific peptides) or metabolic labeling to incorporate functional moieties into exosomal membranes.[55] This approach ensures stable incorporation of functional proteins or peptides and is more physiologically relevant but is limited by complex cell engineering and safety concerns for clinical translation. In contrast, post-modification strategies alter exosomes after isolation, using techniques such as click chemistry, lipid insertion, or electroporation-mediated surface conjugation. Post-modification offers high flexibility, scalability, and compatibility with a wide range of therapeutic cargos; however, it may compromise exosomal integrity and reproducibility. In clinical applications, pre-modification is advantageous for personalized, cell-specific targeting, whereas post-modification is often more practical for large-scale, standardized exosome therapeutics.[51]

4. Functionalities of exosome-based nanorobots

The advanced exosome-based nanorobots exemplify many potential hyperfunctions that make them useful for biomedical applications, especially as targeted therapy and diagnostics. One primary function is drug delivery small molecules, biopharmaceuticals, RNA, CRISPR-Cas systems, *etc.* That targets diseased cells with high precision, with minimal off-targets and systemic toxicity. The fact that exosomes are biologically derived allows them to fend off detection by the immune system and cross biological barriers, such as the blood–brain barrier, for potential use in treating neurological disorders and cancers as drug delivery carriers. Another essential function is the possibility of engineering these nanorobots with a targeting ligand or antibody on the surface of the exosome to specify further the delivery to a specific cell type or tumor markers. These nanorobots can even be programmed with imaging agents, such as fluorescent dyes or magnetic nanoparticles, enabling visualisation of real-time localisation and accumulation of the weapon cues in tissues. Some designs of the nanorobots possess responsiveness to stimuli, for example, CV36p or combination with transition metal nanoparticles that can monitor releases of the drugs *in situ* for a maximised localised concentration. Their capability to partake in intercellular messaging allows for new possibilities for modulating immune responses or delivering genetic material to reprogram diseased cells. These multifunctional aspects make exosome-based nanorobots an ideal technology platform for next-generation precision medicine, capable of being diagnostic, therapeutic, and monitoring in one biocompatible device.

4.1 Therapeutic cargo delivery

Exosome-based biohybrid nanorobots are emerging as highly promising vehicles for therapeutic cargo delivery due to their unique combination of biological compatibility, innate targeting ability, and capacity for engineered functionalization. These nanorobots can encapsulate therapeutic cargo and direct it to specific tissues or cells through passive distribution, such as enhanced permeability and retention (EPR) effect, and active targeting mechanisms using ligands, aptamers, or antibodies.

Table 3 Chemically modified exosome drug delivery

Therapeutic cargo	Targeting molecule	Delivery technique	Target cell types	Intended function	References
Curcumin-SPION	Neuropilin-1-specific peptide	Click chemistry	Glioma cells (U251)	Enables both diagnosis and therapy of brain tumors	62
Paclitaxel (PTX)	AA	DSPE-PEG-AA	Mouse lung carcinoma cells (3LL-M27) with sigma receptor	Enhances drug delivery and reduces lung metastases	63
Quantum dot-based agent	RGD	DSPE-PEG-RGD	Breast cancer cells (MCF-7, integrin αvβ3-positive)	Facilitates light-induced tumor therapy (photothermal)	64
Erastin	Folate	DSPE-PEG-folate	Breast cancer cells (MDA-MB-231)	Induces ferroptosis selectively in tumor cells	59
Survivin-targeting siRNA	PSMA RNA aptamer, EGFR RNA aptamer, Folate	Cholesterol-conjugated	Breast, prostate, and colon cancer cells	Delivers RNA therapy specifically to cancer cells	59
miRNA-let7, VEGF siRNA	AS1411 aptamer	Cholesterol-conjugated	Nucleolin-expressing tumor cells (MDA-MB-231)	RNA-based treatment for nucleolin-positive cancers	59
DOX (doxorubicin)	sgc8 aptamer	Diacyl lipid-PEG_2	Leukemia cells	Provides targeted delivery for cancer treatment	60

Unlike purely synthetic microrobots, which often face immune clearance and biocompatibility limitations, exosome-driven nanorobots leverage their natural vesicular architecture to encapsulate and deliver drugs, nucleic acids, or proteins directly to diseased tissues.[51] The loading of therapeutic cargo into exosomes can be achieved through either passive methods such as incubation or diffusion or active methods like electroporation, sonication, or chemical transfection (Table 3). Once administered, the cargo can be released in response to internal biological triggers such as acidic pH, enzymatic activity, or redox conditions, or through external stimuli like magnetic fields, heat, or ultrasound.

Several strategies have been developed to engineer exosome-based nanorobots for precision delivery. For instance, surface modification with ligands or antibodies enables receptor-specific targeting, allowing exosome nanorobots to home in on cancer cells or inflamed tissues. Hybridization with magnetic nanoparticles (*e.g.*, Fe_3O_4) allows external guidance by magnetic fields, improving spatiotemporal control and ensuring cargo reaches its intended site. Similarly, gold–exosome hybrids have been designed for photothermal therapy, in which near-infrared irradiation triggers both local heating and controlled release of encapsulated drugs.[57,52]

Cargo loading strategies further expand the therapeutic scope. Exosomes have been engineered to carry small interfering RNA (siRNA), CRISPR/Cas9 components, and conventional chemotherapeutics. For example, mesenchymal stem cell (MSC)-derived exosomes loaded with siRNA have demonstrated efficient blood–brain barrier penetration and suppression of neurodegenerative pathways in preclinical models. Likewise, exosome-based nanorobots carrying paclitaxel or doxorubicin have shown improved accumulation at tumor sites compared to free drugs, reducing systemic toxicity.[58] Inaddition to that, exosomes share structural similarities with synthetic lipid-based vesicles, such as liposomes, but exhibit several unique advantages that position them as superior therapeutic carriers. Both systems consist of a lipid bilayer capable of encapsulating hydrophilic and hydrophobic cargos. However, exosomes differ in their endogenous origin, carrying intrinsic proteins (*e.g.*, tetraspanins, integrins), nucleic acids, and signaling molecules that facilitate cellular uptake and natural biodistribution.[59] Unlike liposomes, exosomes demonstrate enhanced immune evasion, stability in circulation, and innate targeting capacity derived from their parent cells. Consequently, exosomes are increasingly viewed as the preferred therapeutic nanocarrier release, particularly in applications requiring personalized or disease-specific targeting.

This spatiotemporally controlled release ensures that the therapeutic agents are delivered precisely to the intended site, thereby improving treatment outcomes while reducing side effects. While early microrobot studies provided proof-of-concept for propulsion and controlled release, the integration of these principles into exosome biohybrids represents a translational leap.[57,52] By combining natural biocompatibility with engineered navigation modules, exosome-based nanorobots present a versatile platform for targeted, minimally invasive, and personalized therapy. Ongoing preclinical studies and early clinical trials further support their potential to transform

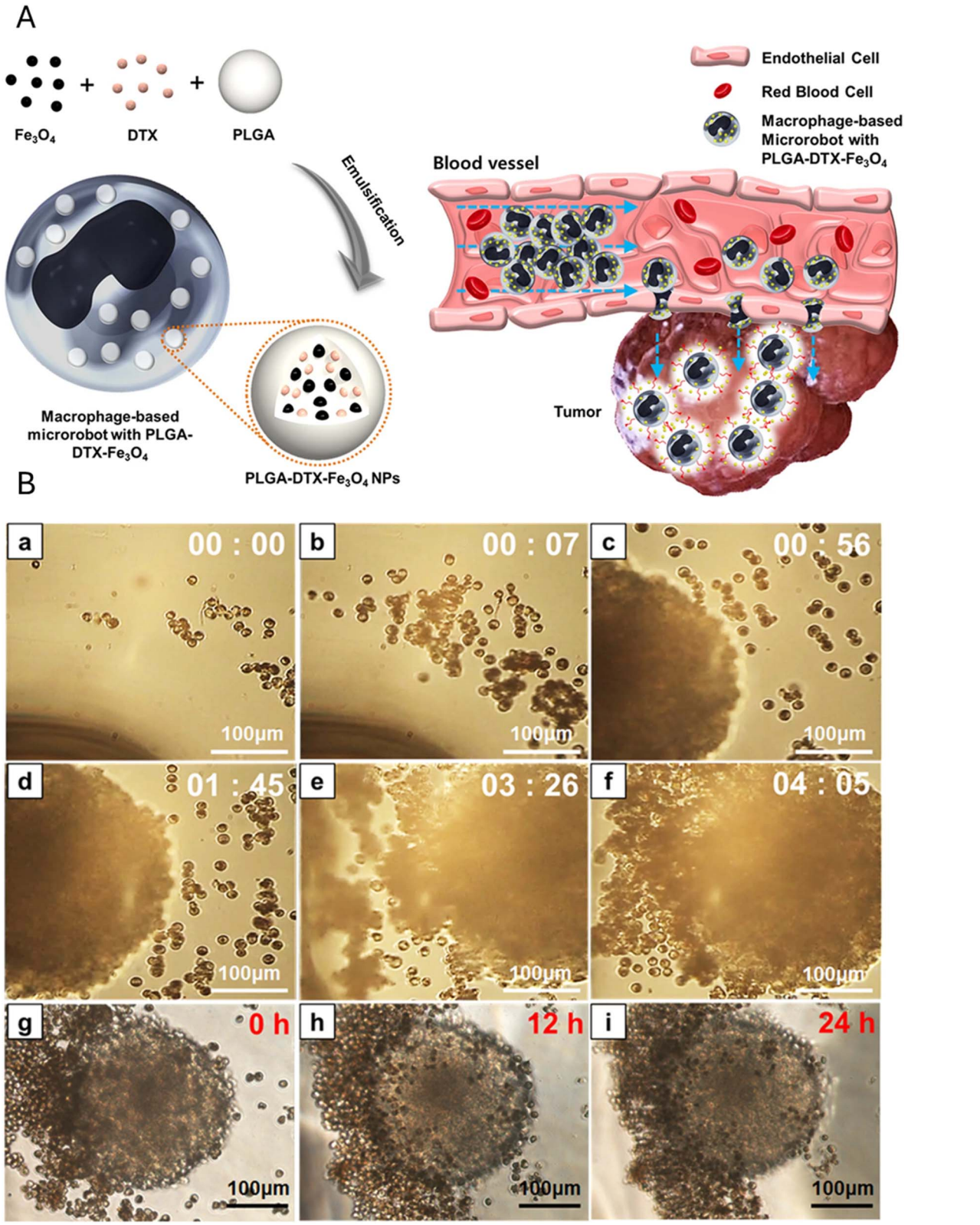

Fig. 7 (A) Macrophage-based biohybrid microrobots for active tumor therapy. Diagram of the macrophage-based microrobot with PLGA-DTX-Fe_3O_4 (left); depiction of possible tumour targeting and therapy in an *in vivo* environment (right). (B) Evaluation of (a–f) active targeting with microrobots toward tumor spheroid using external magnetic field for 10 min (scale bar 100 μm). (g–i) Tumor spheroid attachment property of the microrobots due to the intrinsic tumor infiltration characteristics of the macrophages (scale bar 100 μm). Reprinted from ref. 61. Copyright 2016, licensed under a Creative Commons Attribution 4.0 International License.

therapeutic cargo delivery across oncology, neurology, and regenerative medicine. Building on these advances, several applications of exosome-based biohybrid nanorobots have now been explored in diverse biomedical domains, ranging from oncology to neurology and regenerative medicine.

4.2 Leukocyte and erythrocyte based hybrid microrobots

Leukocyte- and erythrocyte-based hybrid microrobots have been extensively investigated because of their natural immune evasion and long circulation properties, making them attractive for proof-of-concept demonstrations.[60] Leukocytes, also known as white blood cells (WBCs), are immune system cells that help to protect the body from cancer, infectious diseases, and foreign invaders. Leukocytes have been engineered into biohybrid microrobots by taking advantage of their innate properties/functions such as chemotaxis and secretion. Macrophages are a key component of the innate immune system, with roles in development, homeostasis, illness, and other physiological processes. Macrophages are produced from monocytes, and their phenotypes and functions can be altered by manipulating environmental signals.[61]

Using mouse J774A-1 macrophages, Yasa and colleagues demonstrated macrophage-based biohybrid microrobots (so-called "immunobots") capable of combining macrophages' immunomodulatory ability with the navigable mobility of 3D-printed microswimmers for targeted immunotherapy.[61] Previously, researchers built macrophage-based microrobots to deliver anticancer medications to tumor locations (Fig. 7A and B). Recently, dual-targeting macrophage-based microrobots with controllability by inherent chemotaxis and external magnetic field were designed to perform NIR-responsive precision medication release to tumor areas in a spatiotemporally regulated manner. Furthermore, monocyte-based microrobots have been developed with chemotactic transmigrating motility comparable to that of monocytes. Neutrophils, also known as polymorphonuclear neutrophils (PMNs), are the most numerous granulocyte type and occupy 40–70% of leukocytes in the human body. They are a crucial component of the innate immune system.[51] Neutrophils with native chemotaxis have been transformed into self-guided biohybrid micromotors by phagocytosing mesoporous silica nanoparticles (MSNs), resulting in great drug-loading capacity. Neutrophil-based microrobots ("Neutrobots") were capable of actively delivering cargos into malignant gliomas *in vivo*.[51] Immunobots have the distinct benefit of avoiding phagocytosis and elimination by the mononuclear phagocyte system (MPS) and exhibiting chemotactic motility toward sick areas (such as infection, malignancy, or inflammation). As a result, immunocyte-based microrobots can autonomously target sick regions, transport therapeutic medicines, and release them locally.

Erythrocytes, known as red blood cells (RBCs), have long been regarded as an appealing endogenous cargo-carrier material for medication delivery, with researchers making considerable progress in designing erythrocyte-based carriers.[58] Incorporating magnetic iron oxide NPs (20 nm) transformed native mouse RBCs into functional micromotors capable of ultrasonic propulsion, magnetic navigation, and retention of regular erythrocyte structural and biological properties, as illustrated in Fig. 8. RBCs are the most numerous cells in the human body and have a long circulation half-life (~120 days in blood), making them ideal for creating erythrocyte microrobots to transport drugs to sick locations. Furthermore, platelets have been identified as a viable cargo-carrier material for targeted medication delivery.[60] Recently, endogenous platelet-based enzyme-powered Janus micromotors have been created by asymmetrically immobilizing urease on the partial surface of native platelets.[58] Platelets are natively selective for wounded tissues and the tumor microenvironment. Platelet-based microrobots have a longer circulation life (8–10 days) and can accumulate and distribute drugs to specific tissues.

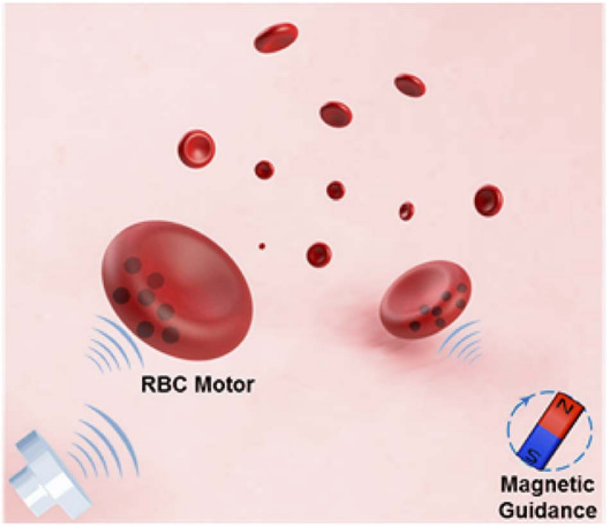

Fig. 8 Schematic illustration of magnetically navigated, ultrasonically propelled RBC micromotors in the whole blood. Reprinted from ref. 58. Copyright 2014, American Chemical Society.

However, as the large-scale manufacturing of immune cell- and blood cell-derived platforms faces practical barriers, including donor variability, limited expansion potential, and complex isolation protocols. To address these translational bottlenecks, recent research has shifted toward more scalable alternatives such as mesenchymal stem cell (MSC)-derived exosomes, exosome-mimetic nanovesicles generated through extrusion or microfluidics, and hybrid systems integrating exosomes with synthetic nanoparticles. These platforms combine the functional benefits of biological vesicles with greater reproducibility and scalability, thereby providing a more feasible pathway toward clinical application.

5. Applications in biomedicine

One interesting and thoroughly developed area of nanotechnological development is the creation of nanorobots, which consist of components engineered to the nanometer scale and smaller. There is a diversity of fields that could benefit from nanorobots, and many areas are being researched. Nanobiotechnology is the design of technologies such as

pharmaceuticals and mechanical devices at the nanometer scale for studying biological systems and treating pathology.[65] While these translational applications demonstrate significant promise, they also reveal key limitations in reproducibility, stability, and scalability. These challenges are discussed in the following section.

5.1 Oncology

Enhancing the treatment quality and healthcare outcomes of cancer patients and mitigating the mortality and morbidity associated with oncological diseases and their treatment is one of the identified goals of the Institute of Medicine.[66] Given the ageing population and resulting cancer diagnoses, there is a growing demand for this type of upgrade. Nanotechnology has shown unprecedented advances in the treatment of cancer. There are examples of nanoparticle technology beginning to play an increasing role in increasing sensitivity of cancer imaging tools,[67] reducing drug resistance,[68] and developing metastasis treatment.[69] Fig. 9 explains the treatment of cancer, which we will discuss below. A limitation of traditional chemotherapy continues to be the toxic effects on normal cells limiting the dose. This has improved with the advent of targeted therapies, and nanoparticle technology has improved treatment selectivity. Researchers have now developed a nanorobot capable of autonomously detecting cancerous cells and releasing therapeutic agents at the site of diseased cancerous cells. The nanorobot can be created to recognize a variety of cell surface receptors, and the released payload can also be altered based on need. The nanorobot is made using engineered DNA strands, which have been manipulated to fold into a predetermined tertiary structure. When the nanorobot recognizes a target, it undergoes a structural change gets "closed" to "open" and releases the stored therapy. Studies have also investigated the possibility of using nanorobots in tumor resection procedures to enhance intraoperative tumor margin mapping and detection. An analogous method without the use of nanorobots has been investigated and proven to be effective. The study showed that radioisotope guided sentinel lymph node dissection, followed by a radioactive colloid injection into the prostate the day before tumor resection, was more sensitive than open lymph node dissection[70] in identifying early metastases. Nanorobots could help simplify hospitals procedures, if patients do not have to be admitted a day ahead, then it will reduce the risk of falling prostatitis from an injection. It is also known that during an actual procedure, these intravascular nanorobots can differentiate precisely between cancerous and non-cancerous tissues. With the advancement of nanotechnology, there are infinite ways to improve cancer therapy; soon, the chances of every single nanorobotic system arising additional, excising applications will increase. Enhanced development of existing technology towards a proposed design can establish new norms about cancer treatment, screening, and prevention in general.

5.2 Neurosurgery

From a theoretical proposal, nanotechnology has developed into a thriving field of ideas, and it is currently the subject of active practical research and advancements. Neurosurgery is ideally positioned to gain from many of the advancements that nanotechnology offers because it frequently operates at the microscopic level. Among many other advantages, these include less invasive intracranial monitoring, better pathology detection, and medication delivery.[71] Developments in the production of microelectromechanical systems have significantly accelerated our capacity to operate on an ever-decreasing scale. These developments could enable manipulation at the individual cell level, and soon, possibly at the molecular level.[72] Nerve damage and spinal cord injury are significant concerns in the field of neurosurgery and can significantly change a patient's life. Since more than a century ago, transected nerves have been reconnected, and technique and technology have advanced. Several approaches are currently being investigated to maximise and improve nerve reconnection results, such as encouraging axon regeneration through growth factors[73] and

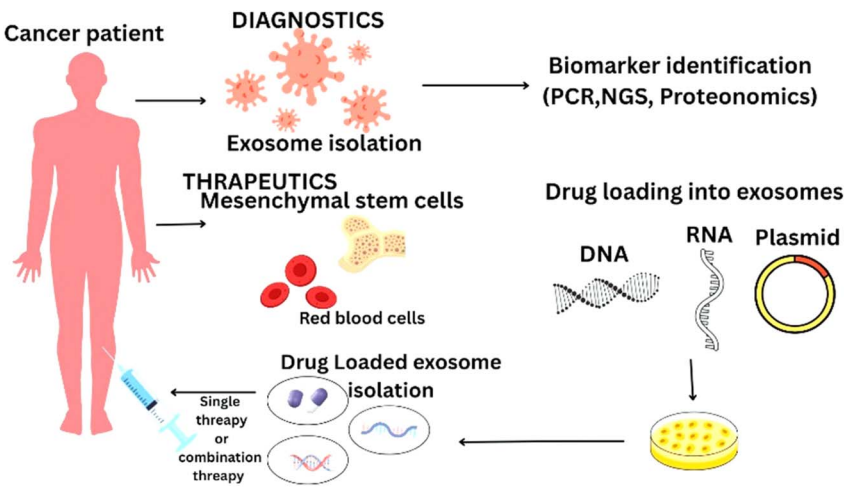

Fig. 9 The figure highlights how exosomes, nanosized vesicles naturally secreted by cells, can be harnessed from cancer patients for diagnostic and therapeutic purposes.

Table 4 Selected preclinical and clinical studies of exosome-based therapeutics

Disease/condition	Exosome source	Application	Stage	References
Parkinson's (mouse)	MSC-derived exosome nanorobots	Dopamine delivery	Preclinical	50
Glioblastoma (mouse)	Engineered exosomes	siRNA delivery	Preclinical	55
NSCLC (human)	Dendritic-cell-derived exosomes	Immunotherapy	Phase I trial	56
GvHD (human)	MSC-derived exosomes	Immunomodulation	Clinical case study	77

enriched scaffolds.[74] A crucial first step in function restoration is reestablishing connectivity to transected axons. Technical restrictions on surgery on that scale[75] limit the ability to accomplish this. In neurosurgery, treating cerebral aneurysms before rupture is one of the best strategies to reduce morbidity and death. A cerebral aneurysm rupture has a high fatality rate. Nearly half of patients pass away within 30 days, 25% within 24 hours of aneurysm rupture, and 10% before they even get to the hospital.[76] Cost-effective recommendations for cerebral aneurysm screening have not been established. Aneurysm screening or closer monitoring of an existing aneurysm may be possible with nanorobotics.

In preclinical models, exosome-functionalized nanomotors have demonstrated improved blood–brain barrier penetration and targeted delivery in Parkinson's and glioblastoma models.[50,55] Similarly, engineered exosomes carrying siRNA or chemotherapeutics have shown enhanced tumor regression in murine models of breast and ovarian cancer.[57,52] Clinically, exosome-based therapeutics are advancing into early trials (Table 4). Dendritic-cell-derived exosomes were tested in a Phase I trial in non-small cell lung cancer, showing safety and immune activation.[56] Another study demonstrated that mesenchymal stem cell-derived exosomes alleviated symptoms in patients with graft-versus-host disease.[77] More recently, exosome formulations have entered trials for Alzheimer's disease and pancreatic cancer, reflecting their expanding therapeutic scope (ClinicalTrials.gov Identifiers: NCT04388982; NCT03608631). These examples highlight the progression from experimental design to clinical feasibility, strengthening the case for exosome-inspired nanorobotics as a next-generation biomedical platform.

5.3 Blood science

The field of hematology has a well-established foundation of research supporting the promising applications of nanomedicine and nanorobotic technologies. These innovations offer significant potential for advancing diagnostics, treatment, and patient care within blood-related medical conditions. Applications for nanorobotics in hematology[78] are numerous and include everything from restoring primary hemostasis to emergency transfusions of non-blood oxygen carrying compounds. A nanorobot known as a respirocyte is one of these gadgets that is presently being designed. As it moves through the bloodstream, this robot is outfitted to perform three tasks. Initially, oxygen is collected through the respiratory system and distributed throughout the bloodstream. The second is carbon dioxide extraction from tissues and its subsequent inhalation. Lastly, it powers its operations by metabolizing circulating glucose.[79] The robot would be roughly one micron, or 1000 nanometers, in size overall. On the other hand, the contained components would be built at the nanoscale. These consist of oxygen and carbon dioxide loading rotors with a maximum diameter of 14 nm in any one dimension[80] and an onboard computer with a diameter of 58 nm. Because of its design, the respirocyte can carry 236 times as much oxygen per unit. Another area where nanorobotics may be helpful is in the hemostasis process. Hemostasis is a complex process that balances fibrinolysis and thrombosis through a few steps, promoters, and inhibitors. Hemostasis can effectively stop bleeding and encourage vessel repair when it is performed correctly. However, Physiologic hemostasis has inherent limitations, such as an average bleeding time of roughly five minutes,[81] which nanorobotics can help to overcome. Furthermore, there are risks associated with our current approaches to correcting physiologic hemostatic mechanisms that are impaired, such as thrombocytopenia. Patients receiving platelet transfusions risk contracting infections from pathogens and possibly inciting an immunological reaction.[82] The suggested nanorobot for this purpose has been referred to as an artificial mechanical. Lastly, phagocytic agents are yet another possible application for nanorobots in this field. “Microbivores” is the term used to describe these nanorobots. These robots' external surface would have many programmable binding sites for pathogens or antigens, ranging from E. Coli to HIV.[83,84] Microbivores may be able to cure septicemia within hours of administration and are thought to be up to 80 times more effective than our body's natural phagocytic processes. Developing nanorobotical capabilities to combat infection may open promising treatment options considering the increasing antibacterial resistance. The translational potential of exosome (EV)-based nanorobots is supported by growing evidence from both preclinical and clinical studies.

6. Challenges and considerations

While biohybrid nanorobots using exosomes and EVs are highly promising for targeted therapy and diagnostics, they are currently hampered by many clinical translation challenges and considerations. One of the most critical issues is the lack of standardization and scalability of the EV isolation and purification methods. Protocol options such as ultracentrifugation, size-exclusion chromatography, and microfluidics are effective methods for small-scale EV isolations. Still, they may not be as consistent, yield, or retain purity for larger applications. Also, EV stability and retention of biological function throughout

current isolation and purification, storage, and dealing with potential direct mechanical stresses associated with the purification process are challenging. Another important consideration is that EVs may be heterogeneous about size, content, and origin of production, and which affects our ability to create consistent and predictable therapeutic agents. Functionalization of EVs is also possible, however it must be performed thoughtfully to not interfere with the native bioactivity of the EVs or stimulate unwanted immune responses following use. The field is still developing regulatory frameworks for biologically derived nanodevices, which may slow clinical evaluation and ultimately patient access. Using genetically modified or patient-derived material in exosomes and EVs also has ethical and safety implications. To surmount these hurdles, the best course of action is to leverage an interdisciplinary approach that finds synergies in bioengineering, materials science, and clinical research to ensure that exosome and EV-based biohybrid nanorobots are applied safely, effectively, and reproducibly for medicine.

6.1 Biocompatibility and safety concern

Biocompatibility and safety are the most critical considerations in developing and clinically using exosome- and EV-based biohybrid nanorobots. Although exosomes and EVs are natural carriers and therefore biocompatible, their development into engineered nanorobots leads to some safety questions. A significant consideration is immunogenicity, which may be exacerbated when exosomes are derived from non-autologous materials or when exosomal surface proteins are modified from the native surface composition through synthetic materials or targeting ligands. The immune system may recognize the engineered nanomedicine as foreign, and to respond with inflammatory or clearance responses, reducing efficacy. Upon isolation, cell-specific characteristics of EV composition can also add variability to safety profiles, especially if the initial cell source harbors oncogenic or infectious factors. The second important aspect is the possibility of off-target effects. If the nanorobots show selectivity to healthy tissue when potentially delivering a toxic payload or altering the behavior of normal cells this may be an issue of toxicity. Added possible toxicity are synthetic components: magnetic nanoparticles, quantum dots, or polymer coatings – have advantages in navigating to their target or permitting imaging but could be also associated with biological toxicity, accumulation of materials in the organs most notably the liver, kidneys, or spleen, as well as generally chronic effects. In addition to their potential off target effects, the timely removal of the Nanorobots through excretion is an essential factor to avoid unintentional bioaccumulation of toxicity following their potential use to promote human health. There requires a study of the potential metabolic dysregulation and the potential toxicology of both the biological cell content of the vesicles/nanorobots and the artificial material itself. Importantly, we have not standardised toxicity testing, pharmacokinetics, or immunological considerations. Therefore, standardising the regulatory approval of nanoparticles as therapeutics from synthesis protocol to efficacy will take time. In Fig. 10, biological interactions and implications of various types of nanoparticles within the human body.

6.2 Scalability and production challenges

The large-scale production of exosome and EV-based biohybrid nanorobots has several technological and logistical challenges limiting their broader application in clinical and industrial contexts. A particularly significant challenge of creating biohybrid nanorobots centers around the isolation and purification of EVs in sufficient quantity and uniform quality. The principal methods for isolating and purifying EVs, which include differential ultracentrifugation, size-exclusion chromatography, and density gradient centrifugation, are effective for preparation in a laboratory, yet are time-consuming, labor-intensive, and unfit for high-throughput production. Each of the methods for isolating and purifying EVs also has the

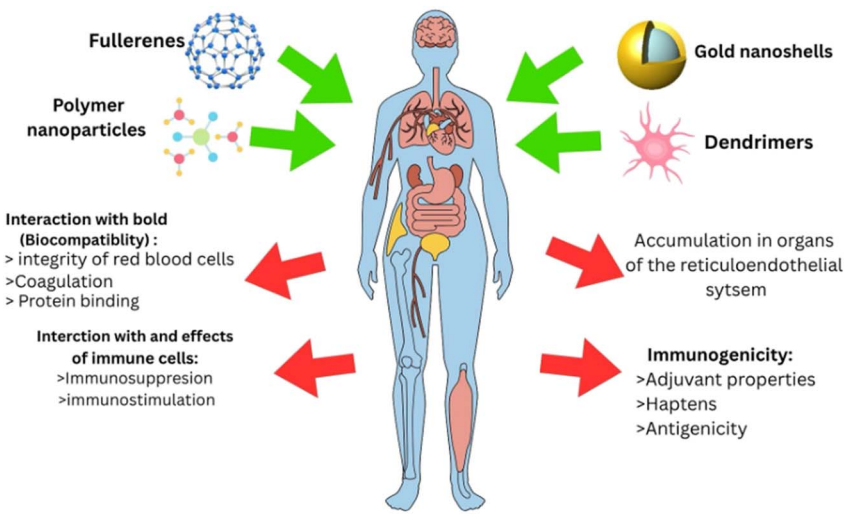

Fig. 10 This diagram comprehensively overviews how various nanoparticles interact with human physiology.

potential to disrupt the structural integrity of the vesicles or lead to contamination by unwanted proteins, nucleic acids, or other extracellular components which can affect the functionality and reproducibility of the final product. Additionally, given the inherent heterogeneity of EVs (*e.g.* cell type from which they originate, culture conditions and methods used to produce the EVs, and methods used to isolate them), efforts to reach a validated and standardized formulation of EVs will face significant barriers. Scaling up production also means keeping a tight grip on controlling the biohybrid assembly process, particularly integrating synthetic nanomaterials or functional agents on, or in, the EVs. Stabilization and bioactivity of bio and synthetic components during large-scale manufacturing adds complexity.

Additionally, defining storage and transport conditions that maintain the functionality of the biohybrid nanorobots, entirely without compromising their biocompatibility, is presently a key hurdle. Likewise, regulatory definitions around large-scale manufacture of biohybrid nanodevices are still being addressed, meaning commercialisation is limited. Overall, the challenge to achieve efficient, reproducible and cost-effective large-scale manufacture of exosome and EV-based biohybrid nanorobots is significant, and will require future bioprocess engineering innovation, as well as quality control and standardization protocols for successful clinical translation and dissemination into standard therapeutic use.

6.3 Regulatory and ethical considerations

Exosome and EV-based biohybrid nanorobots offer great potential for health applications but also present unique and complicated regulatory and ethical issues that must be considered to ensure their safe and appropriate use. Regarding regulatory moves forward, these systems combine biological materials through exosome and EVs with synthetic nanostructures, which adds complexity to the device's classification. These devices' combined biological/synthetic nature does not fit into the conventional categories of biologics, medical devices or pharmaceuticals, suggesting that the pathway to regulatory approval will be complex. To date, agencies such as the FDA and EMA have not developed a clear framework regarding biohybrid nanorobots, where rules exist regarding the manufacturing, quality control, pharmacokinetics and long-term safety of their biological component. Considering these uncertainties, it may be difficult for researchers and companies to move their technologies from the bench into clinical applications. Moreover, ethical issues are especially significant when human-derived materials, such as patient-specific exosomes, are utilized. It is essential to thoroughly vet ethical questions such as informed consent, ownership of biological materials, and data privacy, especially in personalized medicine. In the case of manipulating exosomes for targeted therapeutics especially in the case of using a genetic payload further ethical considerations arise around unintended biological outcomes, any potential germline changes, and inappropriate use in non-therapeutic applications. Beyond that, there is also an ethical responsibility to ensure equitable access to these advanced treatments to avoid increased access inequity. Lastly, it is vital to utilize appropriate transparency in disclosing the research's risks, benefits, and limitations to uphold public trust and ethical validity. Researchers should also consider the environmental impacts of manufacturing nanomaterials at scale and disposal of biological waste.

7. Prospects and emerging applications

Exosome and EV-based biohybrid nanorobots have a bright future ahead of them, and they have the potential to completely transform several fields in biotechnology, diagnostics, and medicine. Exosome-based nanorobots may deliver CRISPR-Cas9 components with high efficiency and little immunological response in targeted gene editing, an emerging application. Regenerative medicine is another rapidly expanding field. These biohybrid systems may directly deliver growth factors or vesicles derived from stem cells to injured tissues, promoting regeneration and repair. Exosome-inspired nanorobots may be used as decoys to neutralize bacterial toxins or capture viruses in treating infectious diseases. Furthermore, integration with biosensors and artificial intelligence may make real-time diagnostics and therapeutic modifications possible, opening the door for "smart" theranostic systems.

7.1 Advancements in nanorobotic technologies

The inherent bioactivity of EVs and the programmability of synthetic nanomaterials are advantageous to these hybrid systems, which combine biological materials with engineered nanocomponents. The accurate functionalization of EVs and exosomes is among the most significant developments. Researchers have improved nanorobots' capacity to identify and bind cell types or disease markers by adding targeting ligands, peptides, or antibodies to their surfaces. Furthermore, surface engineering has allowed it to incorporate imaging agents like magnetic materials, fluorescent dyes, and gold nanoparticles, enabling real-time tracking and diagnostic imaging of nanorobot movement and localization within living organisms. Another breakthrough is the development of advanced cargo loading techniques. Stimuli-responsive designs have been introduced, this allows your nanorobots to unload their drugs in response to a trigger from the environment, for example changes in pH levels, changes in temperature, changes in redox conditions, or the presence of specific enzymes. These smart nanodevices are capable of specific and timely drug delivery, increasing treatment efficacy and minimizing adverse reactions to the rest of the body. Another crucial area of development is propulsion and navigation. Active movement has been made possible by incorporating synthetic components, whereas natural EVs depend on biological transport mechanisms and passive diffusion. Propulsion systems powered by magnetic, acoustic, light-driven, and chemical energy are being integrated into biohybrid frameworks to direct nanorobots to target tissues or deep-seated tumours. Unprecedented control over the biodistribution of therapeutic agents is made possible by these externally controlled navigation systems, especially in tissues

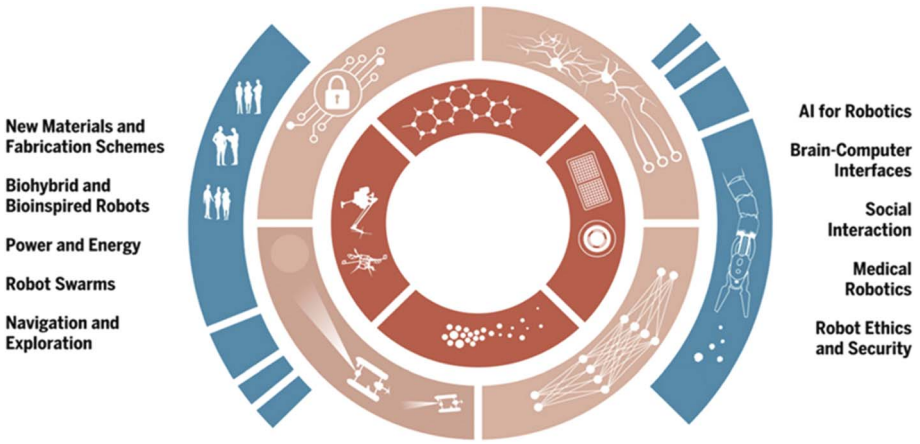

Fig. 11 Main challenges in future robotics need to be resolved (Yang *et al.*, 2018). Reprinted from ref. 49. Copyright 2024, RSC.

like the brain or tumor microenvironments that are otherwise challenging to reach.

Emerging trends include the integration and miniaturization of logic circuits and microelectronic sensors, which enable nanorobots to make decisions *in vivo*, including identifying pathological signals and taking autonomous therapeutic action. Significant advancements have also been made in the design, optimization, and behavior prediction of these nanorobots by applying artificial intelligence (AI) and machine learning algorithms. AI-assisted modeling improves treatment precision and personalization by assisting in selecting EV sources, cargo combinations, targeting ligands, and delivery routes. One of the main obstacles to the clinical translation of EV-based nanorobots is being addressed by the development of microfluidic technologies, which will allow for the automated and scalable production of these devices. These tools significantly increase consistency, yield, and quality control by continuously and carefully isolating, purifying, loading, and modifying EVs. Furthermore, combining immunotherapy and nanorobotics creates new opportunities for immune modulation and cancer treatment.

7.2 Potential disruptive innovations

It is anticipated that the design, optimization, and deployment of these biohybrids will be entirely transformed by incorporating AI and machine learning algorithms into nanorobotic systems. AI-driven models are being ushered in an era of precision and personalization that can evaluate vast datasets and forecast the most effective cargo combinations, targeting ligands, and delivery routes customized for illnesses or individual patients, as shown in Fig. 11. Creating self-regulating nanorobots, smart devices that can recognise disease-specific biomarkers and determine when and where to release their therapeutic payloads on their own, is one of the most exciting future directions. These biohybrid platforms may incorporate biosensors and molecular logic gates that interpret various biological inputs to distinguish between healthy and diseased cells with previously unheard-of accuracy. Such nanorobots might, for example, stay dormant while in circulation and only activate when tumor-specific enzymes or microenvironmental cues like low pH, oxidative stress, or inflammatory markers are present. This selective activation may significantly lower off-target effects and increase treatment effectiveness, particularly in cancer and neurodegenerative diseases.

Additionally, to externally navigate nanorobots to deep tissue sites, such as the central nervous system, which is still a significant challenge in current drug delivery systems, wireless control mechanisms such as magnetic fields, ultrasound, or near-infrared light are being investigated. Tissue engineering and regenerative medicine are two of the most fascinating fields. To improve tissue repair, lower inflammation, and encourage cellular regeneration, EV-based biohybrid nanorobots may be designed to deliver regenerative molecules or exosomes derived from stem cells straight to injured tissues. This strategy may significantly speed up recovery from ailments like degenerative joint diseases, spinal cord injuries, and myocardial infarction. Another potential use is creating customized nanorobots from a patient's cells, which ensure compatibility and reduce immunological rejection. These autologous nanorobots could carry individualized treatment plans catered to the patient's genetic and biochemical profile.

8. Conclusion

Nowadays, exosome- and EV-based biohybrid nanorobots represent a transformative advancement in nanomedicine, merging the natural targeting capabilities and biocompatibility of EVs with the functional precision of engineered nanomaterials. These hybrid systems offer promising avenues for targeted drug delivery, diagnostics, and real-time disease monitoring particularly in oncology and neurology, where precision and barrier penetration are critical. Their ability to function as theragnostic agents, deliver genetic or chemotherapeutic payloads, and interact selectively with diseased tissues opens new pathways for personalised and regenerative medicine. However, scalability, long-term safety, standardization, and regulatory approval remain. With the continued integration of biotechnology, artificial intelligence, and nanotechnology,

biohybrid nanorobots are poised to redefine the landscape of precision healthcare, offering minimally invasive, intelligent solutions for complex medical conditions.

Authors contribution

Subham Preetam: conceptualization, investigation, writing – review & original draft, visualization. Aditi: data curation, formal analysis, writing – review & editing. Jutishna Bora: software. Shailendra Thapliyal: resources. Shalini Mehta: investigation. Seema Ramniwas: visualization. Ravi Deshwal: software. Sarvesh Rustagi: resources. Nayan Talukdar: methodology. Smita Lata: supervision, writing – review & editing. Sumira Malik: supervision, project administration. All the authors wrote, read, and agreed to this version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

No primary research results have been included and no new data were generated as part of this review.

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