Review

Nano Biomed Eng

An Exploration into the Current Application Status and Prospects of Nanorobots in Orthopedic Diseases Based on Their Medical Functions

Qi Yang[1,§], Fei Yu[2,§]✉

[1] Peking University Shenzhen Hospital, Shenzhen 518036, China

[2] Shenzhen Second People's Hospital, The First Affiliated Hospital of Shenzhen University, Shenzhen 518035, China

[§] These authors contribute equal to this work.

✉ Corresponding author. E-mail: yufei89@pku.edu.cn

Received: Aug. 12, 2024; **Revised:** Sep. 6, 2024; **Accepted:** Sep. 14, 2024

Citation: Q. Yang, F. Yu. An exploration into the current application status and prospects of nanorobots in orthopedic diseases based on their medical functions. *Nano Biomedicine and Engineering*, 2024, 16(4): 542−557.

http://doi.org/10.26599/NBE.2024.9290098

Abstract

As a category of common clinical conditions, orthopedic diseases exhibit an increasing incidence in the aging population under the background of technological advancement. Although conventional treatment methods can alleviate the symptoms and improve the quality of life of patients with orthopedic diseases, their efficacy needs to be further enhanced. Nanorobots developed based on nanotechnology have presented important application prospects in the biomedical field. They are characterized by a small size and can be employed in nanoscale operation, which can change the management of orthopedic diseases through drug delivery, disease diagnosis, and disease treatment. They also play an important role in diseases related to hard (bones and cartilages) and soft (spinal cord, peripheral nerves, blood vessels, muscles, and ligaments) tissues in the bone movement system. In this review, the application status and prospects of nanorobots in the diagnosis and treatment of orthopedic diseases are explored based on their functional role in the medical field. Scientific attempts at the treatment of orthopedic diseases based on nanorobots may be made by strengthening the targeted differentiation of cells induced by nanorobots, repairing damaged cells from a genetic perspective, developing precise targeted delivery systems for therapeutic drugs, generating stimulation signals to improve the state of damaged cells, forming damaged tissue substitutes, and coordinating driving factors for physical therapy.

Keywords: nanorobots; orthopedic diseases; soft tissue diseases; hard tissue diseases; drug delivery; disease diagnosis and treatment

Introduction

In the context of the advancement of science and technology and the improvement of medical levels, the number of elderly people in China is increasing in the accelerated aging process. In this process, an increasing number of orthopedic diseases may emerge, such as osteoarthritis [1], osteoporosis [2], muscle strain [3], and other elderly diseases, which will cause pain and movement disorders in the elderly, thus seriously endangering their health. The development of transportation and the improvement of life quality have also led to highly prevalent orthopedic diseases among young individuals. The incidence of fracture [4], soft tissue (such as blood vessels, tendons, nerves, and ligaments) injury [5], bone tumors [6], and other orthopedic diseases among young individuals has also increased with each passing year. If orthopedic diseases are not treated in time, they may lead to disability [7], thus causing a heavy burden on patients and national medical care. Therefore, there is an urgent demand for effective treatment methods for these diseases.

For orthopedic diseases in the early stages, conservative methods are usually used for treatment [8], such as changing lifestyle habits or administering painkillers or protective drugs. If relevant symptoms get aggravated, surgery is usually required [9], despite the fact that surgical treatment is not a one-time solution. A relapse of orthopedic diseases may still occur in these patients, and no better treatment option can be selected under this circumstance. Therefore, the development of new treatment methods for these diseases has attracted much attention from researchers, and nanorobots have been highlighted in this process. With the development of technology, nanorobots have been widely used in drug delivery [10], disease diagnosis [11], and disease treatment [12], and their important role in the diagnosis and treatment of orthopedic diseases has also been corroborated by accumulating evidence [13]. These findings are expected to provide evidence support for the research and transformation of orthopedic disease treatment under the promotion of the “Healthy China” strategy.

Nanorobots are developed based on biological principles at the molecular level and can operate in nanoscale spaces. In the medicine and biology fields, nanorobots can simulate the physiological processes of various molecular substances in life activities, and they can be considered an artificial intelligence system capable of performing fine operation between cells, tissues, or organs. Nanorobots have a size ranging from 1 to 100 nm and can achieve autonomous positioning, recognition, screening, transportation, operation, and other processes through manually programmed processes. Additionally, they can be applied to the military security, environmental exploration, aerospace, medical, and health fields, thereby exhibiting broad application prospects [14] (Fig. 1).

The concept of nanorobots was proposed by Richard Feynman (a winner of the Nobel Prize in Physics) in 1959. As reported in There's Plenty of Room at the Bottom, Feynman believed that it would be feasible for humans to fabricate a micromachine with a molecular size that could be used at the molecular or even atomic level to achieve its biomedical applications. Under the inspiration of this concept, many scholars have made numerous scientific attempts in the fields of nanotechnology and nanorobots [15].

Nanorobots can be used in various fields (Table 1). For example, in the industrial field, nanorobots can be employed to grasp different parts through temperature and ultraviolet control and weld them as needed to manufacture nanoscale microdevices based on new manufacturing platforms [21]. Besides, nanorobots can also be used in environmental pollution control. Some investigators have developed a nanorobot with polycaprolactone/Fe_3O_4 microspheres as the skeleton, which is covered with micelle polyethyleneimine to effectively eliminate nerve agents in polluted water sources and restore our living environment [22]. For example, in the energy development field, Chen et al. designed a new near-infrared-driven intelligent nanorobot that utilized amidoxime as its claw to capture uranium, thereby improving the efficiency of selective and rapid extraction of uranium from low-abundance natural water through self-propelled nuclear reactors [23]. Moreover, nanorobots are also widely used in the biomedical field, and their targeted and precise treatment application has become an emerging field in biomedical science. The exploration

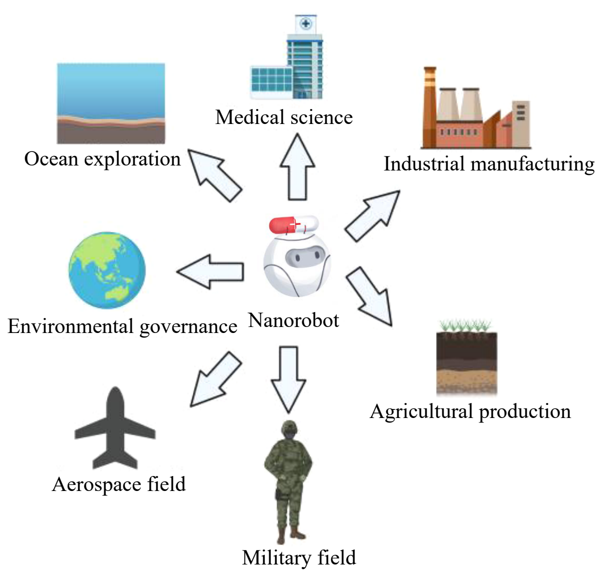

Fig. 1 Application fields of nanorobots. The robot arm in the center of the figure represents nanorobot, and the surrounding icons represent different application fields. (Note: Created with BioRender.com)

Table 1 The role of nanorobots and related technologies in different fields

Nanorobot	Structure	Function	Application prospect
Nanoparticle-DNA-assembled nanorobots that can move along a solid-state membrane surface [16]	One nanoparticle and four single-stranded DNA	Driving nanorobots to move through electroosmosis	Freight transportation; nanomanipulation
Pt/CNT micro-nanorobots manufactured based on the template electrochemical method and chemical vapor deposition [17]	Platinum as the robot anode and carbon nanotubes as the robot cathode	Based on the principle of glucose fuel cells, glucose in biological fluids is used as the driving fuel	Targeted therapy; biosensors; drug carriers
Antibacterial nanorobots (MXene@sAuNR) [18]	Ultra-small gold nanorods (sAuNR) loaded on the surface of MXene nanosheets	Causing mechanical damage to bacterial cell membranes caused by exercise	Eliminating bacterial contamination in water sources
Thermosensitive magnetic nanorobots (TM nanorobots) [19]	A pluronic triblock copolymer combined with Fe_3O_4 nanoparticles	Removing arsenic and atrazine	Monitoring the collection and treatment of toxic pollutants
Magnetic nanorobotic swarms [20]	With temperature-sensitive hydrogel as the carrier, six magnetic nanorobot assembly modes are packaged in three fields	Ensuring the stable distribution of magnetic nanomachines among the population	Nanorobot assembly in the fields of healthcare, catalysis, and 3D printing.
DNA industrial nanorobots [21]	Based on a DNA origami cross-shaped plate, three monomer plates are connected at their ends to form a trimer. The monomer plates at both ends are folded 90 degrees	Grasping different parts under temperature or ultraviolet radiation irradiation, and arranging, welding, and releasing them	Production of nanomaterials

of their preparation materials and driving methods [24] may provide a foundation for their further application in drug delivery, disease diagnosis, and disease treatment (Table 2).

The Application of Nanorobots in the Biomedical Field

In the field of biomedical science, nanorobots are particularly suitable for working in small and complex environments inside living organisms due to their small size and applicability in intelligent fine operation. Besides, they can achieve drug delivery [33], disease diagnosis [34], and disease treatment [35] through carrying substances, performing micro-operation, and targeted positioning. As shown in Table 3, nanorobots with complex multi-material three-dimensional structures were designed and manufactured by different synthesis methods, such as layer-by-layer (LbL) self-assembly , physical method (physical vapor deposition (PVD), direct laser writing (DLW) microfluidic synthesis), chemical method (template electrodeposition (TAE). These nanorobots have been validated to possess favorable biological safety, high driving controllability, and strong action execution power (Fig. 2).

Table 2 Biomedical functionalities of nanorobots in different systems

System	Nanorobot	Function
Movement system [25]	RNA-biomineral nanomachine	A bone-induced microenvironment has been generated within the bone defect, which facilitates rapid biomineralization and osteogenesis, thus achieving fibrous and extracellular mineralization of collagen scaffolds and promoting bone defect repair
Digestive system [26]	Twin-bioengine yeast micro/nanorobots (TBY-robots)	Autonomous navigation to the site of inflammation through enzyme macrophage conversion; treatment of colitis and gastric ulcer by increasing drug accumulation in the affected area
Respiratory system [27]	Algae-nanoparticle hybrid microrobots	The uptake of pulmonary macrophages allows nanorobots to stay in the lungs for a long time and treat pulmonary infections caused by Pseudomonas aeruginosa through antibiotic delivery
Urinary system [28]	Enzyme-powered bacterial outer membrane vesicle (OMV) nanorobots;	Treatment of bladder cancer through siRNA transport and immune stimulation
Reproductive system [29]	morphology-reconfigurable millirobots	Controlled release of drugs in the fallopian tubes through *in situ* programming and switching of motion modes during movement
Cardiovascular system [30]	Polymer conical nanoswimmers	By changing the functionalized position of the gold nanoshell, the reverse blood flow motion of the nanorobot can be achieved, which helps to actively target and remove blood clots in the bloodstream
Nervous system [31]	Magnetically levitated nanorobots	Detect the chemical signals of damaged nerve cells, and assess the healing, regeneration, and repair of nerve cells after injury
Immune system [32]	Magnetically actuated OVA-$CaCO_3$ -SPIO robots (OCS-robots)	Controllable active movement under a rotating magnetic field, weakening the acidic environment of tumors, achieving lysosome escape and dendritic cell antigen presentation, and utilizing cytotoxic T lymphocytes for active immunotherapy of melanoma

Table 3 Synthesis methods of nanorobots

Synthesis method	Nanorobot	Function
Self-assembly synthesis method [36]	Artificial flexible sperm-like nanorobots	It has fine self-assembly (covalently linked with high yield through biotin streptavidin protein bonds) head–tail structures and significant bidirectional motion (under the action of a precessing magnetic field) performance
Chemical synthesis methods [37]	Nanorobots with selective hollow deconstruction	Hollow structures can efficiently load substances and achieve controlled release under near-infrared light irradiation
Biological synthesis methods [38]	Self-propelled Janus nanocatalytic robots (JNCRs)	It can achieve a real-time tracking by non-invasive MRI, with a stronger tumor penetration ability; under near-infrared light irradiation and iron-mediated Fenton reactions, heat up and produce reactive oxygen species, ultimately achieving tumor treatment
4D printing method [39]	Environmentally adaptive shape-morphing microrobots (SMMRs)	It can achieve the encapsulation and controlled release of drugs for tumor treatment
Physical method [40]	Enzyme (urease)-powered liquid metal (LM) nanobots	Driven by urease, under the action of near-infrared light, it deforms and synergistically carries drugs to eliminate diseases. Through ultrasound and photoacoustic imaging, self-tracking is achieved, thus realizing the diagnosis and treatment of bladder diseases

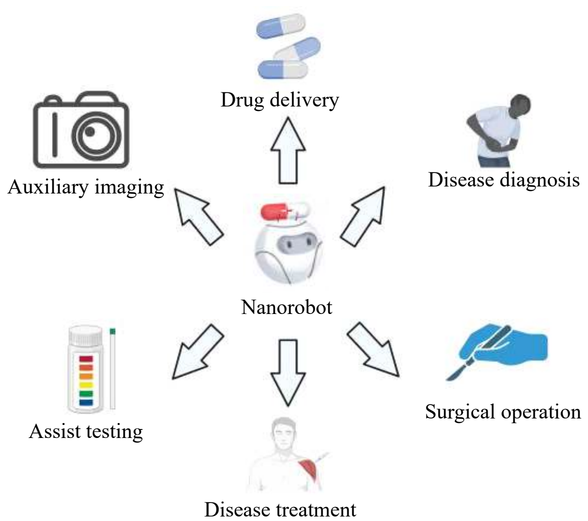

Fig. 2 Applications of nanorobots in the biomedical field. The robot in the center of the figure represents nanorobot, and the surrounding icons represent the functions of nanorobot in the medical field. (Note: Created with BioRender.com)

Nanorobots for drug delivery

Drug delivery is a system that can regulate the comprehensive or precise distribution of specific drugs in organisms from the time, space, and dosage levels. Developing new technologies to achieve timely and targeted drug delivery is beneficial to improving drug utilization, enhancing the therapeutic effect of drugs, and reducing the toxic side effects of drugs on the human body. As a technology suitable for drug delivery [41], nanorobots can deliver drugs in a targeted and precise manner, distinguish healthy organs from affected organs, and direct sufficient amounts of therapeutic drugs to affected organs without impacting healthy tissues. This not only improves the cure rate but also reduces treatment costs and time.

The biological barrier may prevent drugs from reaching specific parts of the human body, and a higher drug concentration to cross the biological barrier can lead to more side effects related to drugs. Moreover, it is difficult for the drug concentration at the lesion site to reach the therapeutic dose. Serra-Casablanca et al. developed a nanorobot using catalase as a driving force and verified its effectiveness through an *in vitro* model simulating intestinal mucus secretion. The results demonstrated that the nanorobot can overcome the barrier effect of mucus and exhibited stronger power compared with passive nanotransport particles. They concluded that this nanorobot was an effective drug carrier that could improve drug delivery efficiency by penetrating the mucus barrier [42]. Nanorobot drug delivery also plays an important role in the treatment of malignant tumors, such as medulloblastoma in children. The blood–brain barrier (BBB) is considered an obstacle to the treatment of this malignant tumor. Tylawsky et al. proposed a nano therapy targeting the endothelial tumor vascular system. They used a nanocarrier based on fucoidan to target endothelial P-selectin-induced caveolin-1-dependent cell transfer, thus selectively and actively transporting the nanocarrier into the brain tumor microenvironment. This nanocarrier exerted significant therapeutic effects on medulloblastoma and reduced bone toxicity and drug exposure to healthy brain tissue [43]. In clinical practice, bladder cancer has a poor therapeutic effect, and the conventional drug delivery system cannot effectively achieve the killing effect of drugs on cancer cells in the urine environment. Simó et al. constructed a radiolabeled mesoporous silica urease-driven nanorobot and verified its role in the cancer-origin mouse model. They found that the nanorobot can accumulate at the tumor site, with a strong tumor cell penetration ability, and the nanorobot can reduce the tumor of mice by about 90% by delivering drugs to the bladder [44]. Nanorobots can also be used as photosensitizers for research and development, enabling them to have photothermal and high-temperature induction effects. Dutta et al. constructed a spiky zinc oxide nanorobot inspired by sunflower pollen, which was modified with the photothermal agent polydopamine containing catechol and added with antibacterial and anticancer drugs, such as tetracycline and doxorubicin. The nanorobot can be driven by near-infrared radiation and effectively resist *Escherichia coli* and *Staphylococcus epidermidi*s while inducing apoptosis of melanoma cells [45]. Multicomponent nanorobots also indicate a direction for future development. Andhari et al. chemically coupled magnetic Fe_3O_4 nanoparticles with anti-epithelial cell adhesion molecule antibodies into multi-walled carbon nanotubes loaded with doxorubicin hydrochloride (an anticancer drug). Based on that, they designed a new type of multicomponent magnetic nanorobot. Driven by the external magnetic field, it can act on human colorectal cancer cells and shrink tumor spheres *in vitro*, thus confirming that nanorobots can be used as drug-delivery agents for tumor treatment in distant parts of the human body [46]. Nanorobots synthesized by enzymes and biological components have great potential in the biomedical field. Yang et al. combined chromophoric lipid vesicles extracted from thermophilic bacteria with a biomolecule motor (FOF1-ATP enzyme) to fabricate a tumor-specific programmed response biomolecule drug delivery motor nanorobot. After *in vivo* injection, it achieved tumor targeting and deposition through proton-driven and self-propulsion capabilities. Further, this nanorobot can accumulate around the tumor or penetrate the tumor by crossing physiological barriers, thus playing a role in tumor enhancement therapy [47]. The subcellular organelle structure can also affect the progression of diseases. However, intracellular regulation through nanorobots is currently a challenge in the nanotechnology field. Peng et al. constructed an autonomous nanorobot that can actively deliver mitochondrial-targeted drugs. They encapsulated mitomycin triphenylphosphonium in zeolite imidazolate frame-67 nanoparticles through simple procedures. In the presence of triphenylphosphonium cations, the nanorobot can move towards intracellular mitochondria to enhance the targeting delivery of drugs and induce mitochondrion-mediated apoptosis and mitochondrial dysregulation, ultimately playing a role in anti-cancer and inhibiting cancer cell metastasis [48].

An effective drug delivery system is also required in the treatment of orthopedic diseases. Nanorobots can carry various drugs with therapeutic concentrations, including nonsteroidal anti-inflammatory drugs (such as celecoxib), bone tissue protective drugs (such as chondroitin sulfate), and bone mass protective drugs (such as denosumab). Biochemical driving, electric and magnetic field driving, photothermal driving, and ultrasound driving (Table 4) are used to deliver drugs in a targeted manner; while the driving factors serve as targeted driving conditions and drug-controlled release conditions for nanorobots, and they can also be used as auxiliary treatment methods for orthopedic diseases. The construction materials and forms of nanorobots mainly include biomimetic and self-assembled metal materials, biological cell membrane materials, and synthetic organic compound materials. However, it is still necessary to improve the delivery and therapeutic efficiency of nanorobots in the treatment of orthopedic diseases and ultimately achieve targeted drug delivery in the skeletal system, tissue, organ, cell, and even subcellular levels. This is also a future research direction.

Nanorobots for disease diagnosis

From a medical perspective, diagnosis can be defined as the judgment of the mental and physical state of the human body. The human body has complex functions, which can be achieved by the close cooperation of various tissues and organs. Many diseases may not be induced by a single factor or system, which may hinder the accurate diagnosis of these diseases. In addition, it is also difficult to confirm the diagnosis for some diseases due to unknown causes, which is also not conducive to subsequent treatment. The emergence of nanorobots can provide clinicians with a pair of “microscopic eyes” to identify some specific symptoms. Besides, nanorobots can also provide information on body changes that cannot be observed by the naked eye, which contributes to improving the diagnostic level of clinicians [56].

The accurate diagnosis of tumors may be achieved by integrating nanorobots with tumor biomarker recognition. Wang et al. developed a logic-gated

Table 4 Nanorobots with different driving modes

Driven mode	Nanorobot	Function
Chemically driven [49]	Cysteine-arginine-glutamic acid-lysine-alanine (CREKA)-modified ceria@polydopamine nanobowls(CREKA-Ce@PDA/DOX NBs)	It catalyzes endogenous chemical fuel H_2O_2 to generate O_2 for driving, load doxorubicin and release it under acidic or near-infrared light conditions, with a stronger ability to penetrate cells and tumors, used for the treatment of solid tumors
Electrically driven [50]	Phase-modulated optoelectronic hybrid nanomotors	They can precisely control of micro/nanomotor motion directions based on transient bipolar photoelectrochemical combined electrical signals and light pulses in semiconductor particles
Magnetically driven [51]	Dual-functional laser-guided magnetic nanorobots	Driven by a torque force mixed magnetic field, they can overcome gravity movement under ultrasound imaging guidance and locally release doxorubicin under near-infrared laser irradiation for the treatment of peritoneal metastatic cancer
Ultrasonically driven [52]	High-intensity-focused ultrasound-driven nanomotors	Under the action of high-intensity focused ultrasound, the carried gambogic acid is more efficiently transported to the triple-negative breast cancer tumor, thus playing an immunotherapeutic role by mediating the iron death of tumor cells
Light driven [53]	Fuel-free carbon nitride (C_3N_4)/polypyrrole nanoparticle (PPyNP)-based smart micro/nanomotors	Micro/nanorobots move towards the light source under low light conditions, and move away from the light source under high light conditions
Temperature driven [54]	Self-assembled stomatocyte nanomotors	They can expand or shrink the opening size of the crack cell shape during temperature changes, thereby controlling the entry of hydrogen oxide and regulating the movement of nanomotors, which can be used for cargo transportation
Biologically driven [55]	Biodegradable metal–organic framework-based nanobots	Driven by biocatalytic buoyancy, they can perform directional vertical movement and accurately identify cancer cells, and the captured cells can recover metabolism after separation
Enzyme driven [44]	Radio-labeled mesoporous silica-based urease-powered nanobots	They can accumulate in tumor cells or tissues driven by urease, exhibiting a strong tumor penetration ability, and they can carry radioactive iodine to treat bladder cancer

nanorobot based on a DNA tetrahedral scaffold, which was loaded with various tumor biomarker aptamers and therapeutic substances. The nanorobot can not only accurately distinguish different tumor cell lines but also accurately kill tumor cells through the synergistic effect of effector aptamer demethylation drugs, thus achieving accurate diagnosis and treatment of various tumors [57]. Single-stranded DNA (ssDNA) can also be used for the recognition, diagnosis, and treatment of cancers. Shen et al. utilized artificial intelligence (AI) to design a nanorobot with a stable pentahedral ssDNA framework by assembling two ssDNA chains into the robot with the assistance of two functional small interfering RNAs (siRNAs). This nanorobot can achieve the recognition and diagnosis of cancers by five functions, including skeleton fixation, logical dual recognition of tumor cell membrane proteins, enzyme loading, dual microRNA (miRNA) detection, and synergistic siRNA loading. Besides, this nanorobot can also be applied to targeted therapies [58]. DNA molecular machines are also a research hotspot in the field of nanorobots, including DNA loops, walkers, tweezers, and robots. Other operational procedures at the DNA level can also be used for disease diagnosis through DNA molecular machines. Zong et al. constructed a DNA molecular machine driven by endonucleases. In this DNA molecular machine, a dual recognition probe was used to recognize specific base sequences and conduct signal transduction, and a hairpin-type probe was used to recognize bases and amplify the signal, ultimately enabling the label-free detection of DNA glycosylation enzyme activities. This system brings new strategies to biomedical research and disease diagnosis [59]. Nanorobots can also be integrated with the internet. Martins et al. proposed a human brain/cloud interface based on neural nanotube technology, which is expected to be applied to the accurate diagnosis and treatment of about 400 kinds of human brain diseases. Using this technology, three kinds of nanorobots, namely, endoneurobots, gliabots, and synaptobots, can cross the BBB, accurately navigate in a single brain cell, transfer the data in brain cells to a supercomputer through the cloud system, and detect the brain state [60]. Although it will take some time to realize this technology, its application in the diagnosis of brain diseases will gradually become a reality with the development of brain-computer interfaces and nanotechnology. The combination of single-cell technology and nanorobots has also been applied in the diagnosis of heart diseases. Hou et al. established a robotic nanomanipulation platform based on atomic force microscopy. In terms of this platform, a single probe was used to measure the force curve of myocardial cells, and a “nanotweezer” prepared with dual probes was used to perform surgery on myocardial cells. In this system, the probe served as a nanorobot, which laid a foundation for the early diagnosis of heart diseases at the single-cell level, thus conducing to subsequent diagnosis and treatment [61]. Imaging technology plays an important role in disease diagnosis. The application of imaging technology can be enhanced through a combination with the navigation function of magnetic nanorobots. Oral et al. prepared a magnetic nanorobot decorated with magnetite particles based on the contrast agent barium sulfate. This robot can accurately navigate in microfluidic channels under the action of a rotating magnetic field, thus achieving specific localization in the gastrointestinal tract *in vivo* without being affected by gastrointestinal peristalsis. This robot may improve the controllability of imaging-based disease diagnosis systems [62]. Some nanorobots designed with a special shape can more efficiently deliver imaging agents and therapeutic drugs, which is more conducive to the diagnosis and treatment of relevant diseases. Tang et al. constructed a flagellar nanorobot by polymerizing flagella using a magnetic nanoparticle head coated with anti-biotin proteins and a single biotin tip driven by a wirelessly generated rotating magnetic field. This nanorobot can be manually guided to perform complex swimming trajectories under a microscope under the influence of rotation frequency and direction, magnetic particle size, and flagellar length. Meanwhile, quantitative defocusing technology can be employed to improve the performance of nanorobots in the delivery of imaging agents and drugs [63].

The same disease may manifest in different subtypes, and orthopedic diseases also have several subtypes. However, the subtypes of orthopedic diseases have not been well defined. Patients with orthopedic diseases can be divided into different groups based on age, gender, and BMI values. Specifically, osteoarthritis can be divided into primary and secondary types (injury type, drug type, and metabolic type); lumbar disc degeneration can be divided into bulging type, protruding type, prolapsed free type, Schmorl nodule type, and transosseous protrusion type. Currently, the types of orthopedic diseases in the elderly can be defined based on imaging technology in combination with their medical history, but relevant influencing factors have not been sufficiently clarified. With the development of nanorobot technology, orthopedic diseases can be diagnosed based on some specific genes or signaling pathways related to the pathogenesis of these diseases. On that basis, more targeted therapeutic drugs may be developed, with the avoidance of serious side effects on other system organs, thus reducing the impact on the normal physiological system of these patients. Nanorobots combined with single- or double-stranded probe technology can be employed to locate or manipulate single cells. Besides, the coding mechanism can be introduced to normalize cells at the DNA, RNA, and even miRNA, long non-coding RNA (lncRNA), or circular RNA (circRNA) levels. In addition, new contrast agents can be utilized to directly display affected cells. Moreover, the comparison and analysis based on the massive data in the cloud may provide the optimal treatment plan. These presumptions may be achieved in the future, which will contribute to a higher cure rate for orthopedic diseases.

Nanorobots for disease treatment

Surgical treatment is the main method used in the field of orthopedics for the treatment of various emergencies or end-stage diseases, including open surgery and minimally invasive surgery (such as total hip or knee replacement, unicompartmental replacement, and osteotomy for the treatment of osteoarthritis; vertebral augmentation, open reduction, and internal fixation for the treatment of osteoporotic fractures; discectomy for the treatment of lumbar disc degeneration). With the advancement of medical technology, the type of surgical treatment has gradually shifted from open surgery with larger traumas to minimally invasive surgery with smaller traumas. The development of nanotechnology has increased the application of nanorobots in complex surgical procedures [64]. Their applications may be expanded to cover both internal and external treatment methods, which is more in line with the enhanced recovery after surgery (ERAS) concept in the medical field.

In such neurodegenerative diseases as Parkinson's disease and Alzheimer's disease, nanorobots can help compensate for the loss of neuronal cells. Saniotis et al. proposed a nano-cognitive device based on the concept of an “interface of endophytic bacteria”, which can be regarded as a new type of future neural prosthesis that can be connected to a neural network through a mechanism similar to that of endosomes. This device can sense action potentials and transmit them to interconnected neurons, thereby resolving neurodegenerative diseases [65]. Nanorobots can also be used for cancer treatment. Wavhale et al. reported a magnesio-Fe_3O_4-based magnetic fluorescence nanorobot, which was equipped with EpCAM antibody/transferrin, cyanine 5 NHS (Cy5) dye, fourth-generation dendritic macromolecules for multiple coupling, and glutathione coupled to one side of magnesium nanoparticles through simple surface modification and chemical conjugation. The nanorobot exhibited self-propulsion and navigation capabilities in the blood and can capture cancer cells with higher sensitivity and efficiency [66]. Nanorobots are capable of fine manipulation, largely relying on their accurate navigation function in cell structures. Yang et al. constructed an intelligent DNA nanorobot by using a barrel-shaped DNA nanostructure as a framework and a molecular reaction cascade embedded in the robot as a computing core. It can intelligently sense the concentration of thrombin in the environment and trigger autonomous coagulation in the presence of excessive thrombin. This can avoid the side effects caused by excessive thrombin and provide a new solution to the personalized and precise treatment of diseases [67]. Chemotherapy plays an important role in tumor treatment. When the therapeutic dose of drugs is reached in the conventional chemotherapy scheme, serious side effects and insufficient treatment effects on tumor cells may be induced. Yan et al. designed a bionic head/hollow tail nanorobot based on the site-selective super assembly strategy. They found that this nanorobot can effectively cross the body barrier to reach the interior of tumors and reshape the tumor microenvironment through the photothermal effect, thus inhibiting the tumor growth of three negative breast cancer bone metastasis mice [68]. Intelligent nanorobots are characterized by simple operation and precise control. Si et al. proposed a coding manipulation method to drive the movement of DNA nanoparticle assembly nanorobots by using electrophoresis and the electroosmotic effect in independently charged array nanopores. This nanorobot was constructed with one nanoparticle as the robot's body and 1-2 ssDNAs as the robot's legs. It can be employed in the capturing, releasing, jumping, and crawling procedures, providing a strategy for the implementation of nanosurgery [69]. The biocompatibility of nanorobots can also affect their therapeutic effect on diseases. Tang et al. developed an enzyme-powered bacterial outer membrane vesicle nanorobot, which was powered by urease immobilized on the bacterial outer membrane vesicle to decompose urea. This nanorobot displayed favorable biocompatibility, immunogenicity, and material loading performance. Besides, this nanorobot can protect the gene silencing tool (siRNA) from degradation by body enzymes, thus playing an important role in the treatment of *in situ* bladder tumors [28]. The morphology of nanorobots can also affect their functions. Inspired by the swimming posture of natural microorganisms, Li et al. developed a symmetrical multi-link dual-arm magnetic nanorobot, which can achieve "free swimming" under the action of the magnetic field and viscous force. Its movement speed and remote navigation ability were also optimized, which was conducive to achieving nanoscale biomedical operation [70]. The advancement of microscopy technology has also promoted the application of nanorobots in surgical procedures. Chacko et al. constructed a stimulated emission-depleted atomic force microscope and used it for biological nanomanipulation with the assistance of rapid visualization. This technology may enable the implementation of nanosurgery at the single-cell level and promote the development of physical targeting technology [71]. The advancement of medical imaging technology also promotes non-invasive medical operation technology. Aziz et al. used high-frequency ultrasound and photoacoustic imaging to obtain anatomical and molecular information *in vitro* and *ex vivo* tissues, respectively, by a magnetically driven micromotor. This micromotor can be considered a nanorobot that can enter the uterus and bladder of mice through magnetic field control and identify its own signals and endogenous signals through multispectral analyses, which was conducive to non-invasive *in vivo* medical intervention [72].

With the advancement of nanotechnology, the treatment methods for orthopedic diseases have gradually diversified. Conventional drug therapy and surgical treatment will undergo qualitative changes with the emergence of nanorobots. On that basis, nanorobots will play a more important role in the treatment of orthopedic diseases and even replace some of the procedures of orthopedic clinicians. In this process, scholars can focus on the biomimetic research and development strategy of nanorobots and refer to the natural motion trajectory of living organisms. Besides, the simplest nanorobots may be constructed with the simplest materials to achieve their biocompatibility and motion controllability. In addition, more sophisticated observation equipment can be combined with imaging to achieve visual operation of nanorobots. Moreover, the mass production of nanorobots may also be achieved in the future. Furthermore, nanorobots can be used as disease treatment prostheses to replace tissue cells in the human body remove affected areas in surgical patients, or carry biological factors to perform treatment for relevant diseases, thus exerting positive impacts on human bone health.

Application of Nanorobots and Related Technologies for Orthopedic Diseases treatment

Orthopedic diseases mainly involve the musculoskeletal system and can be divided into hard tissue-related diseases (such as diseases related to bones and cartilages) and soft tissue-related diseases (such as diseases related to muscles, blood vessels, nerves, ligaments, and tendons). With the advancement of medical technology, there are various treatment methods for orthopedic diseases, ranging from conventional surgical treatment to robot-assisted surgical treatment. Meanwhile, postoperative rehabilitation training can also be assisted by exoskeleton robots and other devices [73] (Fig. 3). Currently, macroscopic robots are the auxiliary robots used in clinical practice, and nanorobots are rarely used. This is also an emerging field for the diagnosis and treatment of orthopedic diseases, which requires further exploration.

Application of nanorobots and related technologies for hard tissue-related orthopedic diseases treatment

The hard tissues in orthopedics are mainly composed of bones and cartilages, with the former being the main component. There are 206 bones in adults to support the body, and these bones are generally connected by joints and ligaments. Common bone diseases include fractures [74] and osteoporosis [75]. Cartilages have lower hardness but stronger toughness compared with bones. However, there is no blood vessel or lymphatic vessel in cartilages. Cartilages can be divided into transparent cartilages, elastic cartilages, and fibrocartilages. The common cartilage diseases in orthopedics are mainly lesions involving transparent cartilages [76] (joint surface) and fibrocartilages [77] (intervertebral disc). Nanorobots can be used in the treatment of orthopedic diseases related to hard tissues, such as bone and cartilage.

Patients with post-traumatic fractures are usually treated with metal plates for open reduction and internal fixation. Some patients with severe injuries and long-term wound opening are prone to infections after surgery, such as the infection after facial fracture implantation of titanium micro steel plates. Ussia et al. designed a light-driven self-propelled tubular black TiO_2/Ag nanorobot, which may or may not move under different light rays and the same wavelength of light, such as ultraviolet light, blue light, and green light. Combined with live fluorescence and digital imaging, the number of bacteria in bacterial biofilms can be detected, thus effectively eliminating infections after titanium microsteel plate implantation [78]. Some researchers also utilize nanorobots to destroy microbial membranes based on the magnetic field in the bone repair process. For example, Mayorga-Martinez et al.

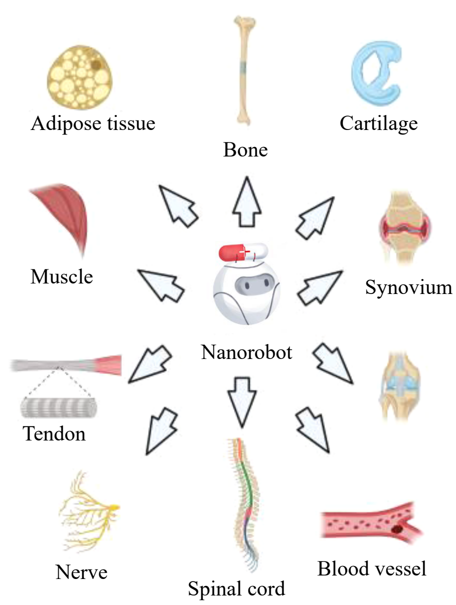

Fig. 3 Application of nanorobots in orthopedics. The robot in the center of the figure represents nanorobot, and the surrounding icons represent the tissues and organs related to orthopedic diseases that nanorobot can be used for diagnosis and treatment. (Note: Created with BioRender.com)

developed a magnetic robot with Eloite nanotubes as the skeleton and Fe_3O_4 nanoparticles as the magnetic material. The surface of this robot was covered with polyethyleneimine to load ampicillin. When multiple robots are combined, they can perform eddy current motion to penetrate and destroy the extracellular matrix of the *Staphylococcus aureus* biofilm on the titanium mesh during bone repair, which is beneficial to bone repair [79]. Rheumatoid arthritis (RA) is also a common immune-related disease in orthopedics. RA is mainly characterized by erosive arthritis, and synovitis is its pathological basis. Xu et al. prepared magnesium-based micro/nanorobots with magnesium microspheres as the core and hyaluronic acid hydrogel and PLGA as the coating. Meanwhile, they adopted the Pickering lotion method to prepare manganese dioxide nanorobots with asymmetric shells. They found that the magnesium-based micro/nanorobots can reduce the joint diameter, arthritis score, and plasma inflammatory factor content in RA rats. Manganese dioxide nanorobots can react with hydrogen dioxide in the inflammatory microenvironment of rats under the precise guidance of ultrasound, inhibit the role of M1 macrophages, and reduce HIF-1α in the synovium, thus alleviating the hypoxia state in synovial tissues. The development of the two types of nanorobots can provide support for the diagnosis, treatment, and drug delivery of inflammatory diseases, such as RA [80]. Cartilage defects also constitute a common orthopedic disease. It is difficult to transport nutrients for cartilage tissues due to a lack of blood vessels within them. A large cartilage defect cannot be repaired spontaneously. Inspiringly, micro/nanorobots provide a new solution to the repair of large cartilage defects. Go et al. constructed a programmable morphological multifunctional biodegradable micro scaffold, which was equipped with a magnetic chitosan skeleton and adjustable pores and sizes according to the target application. Besides, this micro scaffold had strong cell adhesion functions and displayed favorable application prospects in the treatment of liver cancer and *in vivo* regeneration of cartilages [81]. This can be considered an extension of micro/nanorobot technology.

In summary, nanorobots can be used to control bacterial infections in bone tissues after fracture surgery, regulate pathogenic factors of inflammatory diseases in joint tissues, and promote the adhesion of new chondrocytes in cartilage tissues. In the future, nanorobots may be used as bone or cartilage prostheses to perform *in situ* implantation in damaged tissues; nanorobots may also be employed to recruit bone marrow mesenchymal stem cells to differentiate into osteoblasts and chondrocytes; they may also be utilized to accurately identify damaged cells for the repair of intracellular organelles or obtain genetic information. Based on that, the drugs with pain relieving, osteochondral protective, and bone tumor and metastatic cell killing effects may be precisely delivered to single cells. Overall, these scientific efforts may provide new ideas for bone and cartilage repair in the treatment of orthopedic diseases.

Application of nanorobots and related technologies for soft tissue-related orthopedic diseases treatment

The soft tissues in the skeletal motor system also play an important role, such as the control of effectors by the spinal cord and peripheral nerves, the transport of nutrients and metabolites by blood vessels in the skeletal system, the contraction of skeletal muscles leading to body movement, and the strengthening of the stability of the skeletal system by ligaments under loads. Soft tissue lesions can cause paralysis of the skeletal motor system, which necessitates proper treatment [82]. Nanorobots can also play an important role in the treatment of orthopedic diseases related to soft tissues owing to their biological functions.

Shape memory alloys (SMAs) have become a commonly used material in the treatment of diseases related to the skeletal muscle system, and they are also the main materials for preparing nanorobots and artificial muscle fibers. Kim et al. developed an independent twisted structure of nanoscale iron oxide, which was proved to have a huge shape memory effect with recoverable strain. This material can overcome the size limitations of conventional SMAs, providing a new scaffold basis for the design of nanorobots and artificial muscle fibers [83], which is beneficial to the future application of nanorobots in the diagnosis and treatment of orthopedic diseases in muscle systems. The regulation at the DNA level is also of great significance for the study of muscle diseases. Liu et al. reported a construction system for DNA-related nanorobots, which included a photo-driven DNA bipedal walker and a rod-shaped DNA origami platform with a length of 170 nm. The walker and platform can work together to achieve the complex DNA origami technology based on pure mechanical effects, thereby assisting in the development of DNA nanorobots and biomimetic nanomuscle power systems driven by artificial translational molecular motors [84]. Neuromuscular diseases and muscle dynamics-related diseases are relatively common in orthopedic subspecialties, such as trauma orthopedics and hand and foot surgery. Many elderly individuals with osteoporosis may also suffer from muscle-related diseases, such as sarcopenia. Nanorobots and related technologies have also provided new directions for the treatment of such diseases. Yoon et al. reported that femtosecond laser pulses with a focus on the diffraction limit volume can induce an increase in Ca (2+) in irradiated smooth muscle cells without neurotransmitters, which can affect cells and surrounding cells through calcium ion channels, thereby inducing smooth muscle contraction. They maintained that this technology can achieve reversible and reproducible muscle contraction and can be applied to research on muscle diseases and nanorobot control [85]. Vascular injury is also a common orthopedic disease caused by traumas. Some researchers have sent nanorobots to the affected site through blood vessels for relevant treatment. However, the application of these nanorobots is limited by the effective method to move these nanorobots in viscous media such as the blood. Li et al. developed a micro rocket robot with a three-tube structure based on 3D printing and an all-optical driving and imaging system that can achieve high-speed movement in blood vessels under near-infrared light irradiation. The motion trajectory can be tracked by a resolution photoacoustic microscope, which is beneficial to the treatment of vascular diseases [86]. The combination of microneedle technology and nanorobot technology has also provided a method for the treatment of orthopedic diseases. Liu et al. designed a detachable microneedle array, through which extracellular vesicles modified with nitric oxide nanomotors can be delivered to the affected site in Achilles tendon disease. This can inhibit Achilles tendon inflammation, increase the expression of type I collagen, and prevent extracellular matrix degradation [87]. The regulation of the inflammatory microenvironment is also beneficial to disease treatment. Xu et al. designed an *in situ* regulation system for the arthritis microenvironment based on a nanomotion strategy. The synthesized MnO_2 motor can regulate the microenvironment of RA by consuming H_2O_2 to produce O_2, which can detect inflammation and eliminate it. Besides, the nanomotor can inhibit the repolarization of pro-inflammatory macrophages, reduce the secretion of pro-inflammatory factors, and alleviate joint hypoxia, synovial inflammation, bone erosion, and cartilage degeneration [88]. The targeted and precise delivery of drugs is also required in the treatment of spinal cord injury. Bao et al. designed a nanomotor based on poly (2-methacryloyloxyethyl phosphorylcholine) (PMPC)/l-arginine (PMPC/A) and loaded it with an inducible NO synthase inhibitor 1 400 W and nerve growth factor (NGF). With the assistance of choline transporters, the nanomotor can easily pass through the BBB and react with reactive oxygen species in the affected spinal cord to produce nitric oxide, thus promoting drug absorption and penetration in the affected tissue [13]. Diabetes ulcers are a thorny problem for hand and foot surgeons. Zheng et al. developed a DMSNs-Pt-LOX@Nisin (DPLN) nanomotor to act on methicillin-resistant Staphylococcus aureus (MRSA) through cascade propulsion. It was found that this nanomotor promoted the transformation of lactic acid in diabetic foot ulcers into H_2O_2 as a driving dye, which carried antimicrobial peptides with unique binding affinity to MRSA and promoted diabetic ulcer healing by clearing biofilm, thus promoting angiogenesis and reducing inflammation [89].

In summary, nanorobots can be combined with new memory materials to construct soft tissue organs. Besides, the driving and morphology of nanorobots can be improved to achieve visual and controllable movement and precise positioning in soft tissues. In addition, nanorobots can be employed to stimulate the functional status of differentiated cells related to soft tissues to improve and regulate the inflammatory microenvironment. Moreover, they can also be used to cross the body barrier for targeted drug delivery and specific binding to pathogens. These are some attempts made by scholars in the diagnosis and treatment of orthopedic diseases related to soft tissues. In the future, nanorobots may serve as stimulating factors to act on the affected spinal cord or neurons innervated by peripheral nerves to restore the functional state of effectors. Additionally, nanorobots will also be used as materials to induce organoid production in the human body to replace the affected soft tissues, thus achieving normal functions. The physical factors such as ultrasound and ultraviolet radiation in the nanorobot driving system will work together to perform postoperative rehabilitation exercises on the affected soft tissues, which may also become a new research field in the future. Through the transformation of these research results, patients with orthopedic diseases will achieve tissue function recovery more efficiently.

Challenges of Nanorobots Technology in Clinical Application

Firstly, in the process of scholars conducting relevant research, it should be noted that the purpose of nanorobots research is to solve the diagnosis and treatment problems of orthopedic diseases in clinical practice, rather than just conducting research for the purpose of publishing research results. In order to achieve true clinical translation of nanorobots, it is necessary to use methods with wide material sources, low prices, simple preparation, and quantifiable production to construct nanorobots and solve their biological safety and biocompatibility problems. However, current research often designs complex and difficult to prepare nanorobots for richer functions, which results in their lack of clinical translation benefits and inability to be truly applied in clinical practice. There are also not many related patents, which is currently the bottleneck for nanorobots to be transformed into achievements in the field of orthopedics.

Moreover, with the application of nanorobots, a series of standardized operation process problems will also arise. Scholars not only need to pay attention to the research on the preparation and functions of nanorobots, but also focus on the standardized production process, quality control system, application schemes in different orthopedic diseases, and what qualifications of hospitals or doctors can use nanorobots. These studies will also be accompanied by the development of nanorobots. Only by solving such problems can we help the transformed nanorobots better treat orthopedic diseases.

Finally, the emergence of a new thing will also bring about ethical problems, and the field of nanorobots is no exception. Regulatory authorities need to introduce policies to explain ethical problems, such as to what extent nanorobots can replace orthopedic doctors' diagnosis and treatment, how nanorobots should coexist with orthopedic doctors, what types of orthopedic diseases should use nanorobots, how to avoid excessive application of nanorobots in the orthopedic field, and whether the medical security system should include nanorobots therapy in the free catalog. These are also areas that need to be studied.

Conclusions and Prospects

The incidence of orthopedic diseases gradually increases with the aging of the population in the context of technological advancement. It is necessary to further improve the efficacy of conventional treatment methods for patients with orthopedic diseases. The application of nanorobots in biomedicine is an interdisciplinary field developed with the advancement of nanotechnology. These nanorobots can operate at the nanometer level owing to their nanoscale size. Hence, they can play an important role in drug delivery as well as the diagnosis and treatment of orthopedic diseases related to hard tissues (such as bones and cartilages) and soft tissues (such as muscles, nerves, blood vessels, and ligaments) in the bone movement system. In the future, scientific attempts at the treatment of orthopedic diseases based on nanorobots may be made by strengthening the targeted differentiation of cells induced by nanorobots, repairing damaged cells from a genetic perspective, developing precise targeted delivery systems for therapeutic drugs, generating stimulation signals to improve the state of damaged cells, forming damaged tissue substitutes, and coordinating driving factors for physical therapy (Fig. 4). It is expected that nanorobots will replace some conventional diagnosis and treatment models in orthopedics with the advancement of technology, thus benefiting orthopedic patients through the transformation of achievements.

CRediT Author Statement

Q. Yang and **F. Yu** designed the review. **Q. Yang** and **F. Yu** collected the references. **F. Yu** made the figures. **Q. Yang** wrote the original manuscript. **F. Yu** revised the manuscript. **Q. Yang** and **F. Yu** supported the fundings. All authors contributed to the article and approved the submitted version.

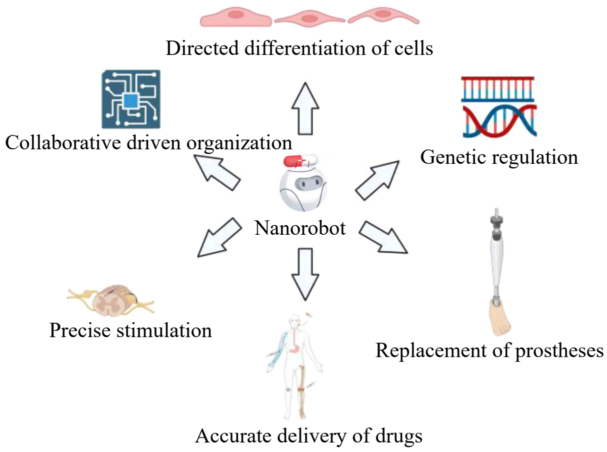

Fig. 4 The future development directions of nanorobots for the orthopedic diseases treatment. The robot in the center of the figure represents nanorobot, and the surrounding icons represent the research content that nanorobot can conduct in the field of orthopedics. (Note: Created with BioRender.com)

Acknowledgements

This research was supported by grants from National Natural Science Foundation of China (No. 82102568; No. 82102076), Shenzhen Key Medical Discipline Construction Fund (No. SZXK023), Shenzhen "San-Ming" Project of Medicine (No.SZSM202211038), Shenzhen Science and Technology Program (No. ZDSYS20220606100602005), Guangdong Basic and Applied Basic Research Foundation (No.2022A1515220111) and The Scientific Research Foundation of PEKING UNIVERSITY SHENZHEN HOSPITAL (No. KYQD2021099).

Conflict of Interests

There is no conflict of interests between the authors.

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