Review

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Viewpoints about the Application of Nanoprobe Technology in Orthopedic Diseases Based on Its Functions in Disease Diagnosis and Treatment

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Abstract

Orthopedic diseases are common clinical disorders that impose a heavy burden on patients and their families, and they have presented considerable challenges to clinicians. Nanoprobes have emerged as a new microbial sensor technology with the advancement in nanotechnology. They can be employed to detect individual living cells, thus playing an important role in the diagnosis and treatment of diseases. Nanoprobes can target specific cells, subcellular organelles, exosomes, extracellular matrix, disease microenvironment, and metabolic products such as specific diagnostic substances and inflammatory factors produced in the progression of orthopedic diseases. Through the combination with photothermal, electromagnetic, ultrasound, and other excitation methods, they can achieve multidimensional targeted treatment for such orthopedic diseases as osteoarthritis, rheumatoid arthritis, malignant bone tumors, and bone fractures and defects by carrying drugs, proteins, peptides, and cytokines. Given these facts, the role of nanoprobe technology in the diagnosis and treatment of common orthopedic diseases is explored in this study.

Keywords: nanoprobe; diagnosis and treatment; osteoarthritis; rheumatoid arthritis; bone tumor; bone fracture and defect; imaging

Introduction

Orthopedic diseases mainly affect the skeletal and motor systems composed of bones, bone connections, and skeletal muscles, which can provide support, protection, and movement functions for the human body. After the occurrence of disorders in the motor system, there would be disruptions to the body's ability to support the weight, protect internal organs, maintain postures, and maintain basic forms, which may result in a series of symptoms such as pain and deformities. In severe cases, these disorders can lead to disability, imposing a heavy burden on patients and their families. In the context of technology development and medical level improvement, trauma and population aging are intensifying worldwide, resulting in an increasing incidence of orthopedic diseases [1–4]. Improving the diagnosis and treatment level of orthopedic clinicians will bring benefits to such patients and reduce the burden of national

medical security.

In recent years, with the advancement of technology, orthopedics has made significant progress in both diagnosis and treatment. Many new technologies continue to emerge to assist in the diagnosis and treatment of orthopedic diseases, including nanoprobe technology [5], an emerging technology developed based on nanotechnology. Nanomaterials have at least one dimension in the nanometer range, exhibiting excellent properties compared with conventional materials [6]. Based on nanomaterials, new biosensor nanoprobes have been fabricated with favorable performance in the diagnosis and treatment of diseases owing to their advantages such as the small size, high penetration, long half-life, and ability to carry substances [7, 8]. In this study, the advantages of nanoprobe technology in the medical field and its applications in osteoarthritis (OA) [9], rheumatoid arthritis (RA) [10], bone tumors [11], and bone fractures and defects [12] are reviewed systematically. These findings may provide evidence for subsequent research on the treatment of orthopedic diseases.

The nanoprobe is a new microbial sensor capable of detecting individual living cells. The probe of the sensor is designed with a small size at the nanometer level (1–100 nm), and they can achieve non-invasive, real-time, trace, accurate, and sensitive substance monitoring in living organisms. As a new field combining nanotechnology and biosensor technology, nanoprobes have wide applications in medicine [13], environmental protection [14], biological evolution [15], food production [16], mineral discovery [17], energy exploration [18], industrial raw material preparation [19], and plant research [20]. In the medical field, nanoprobes can be used for the diagnosis and treatment of diseases. For example, their usage as contrast agents for targeted imaging of disease sites can achieve an accurate diagnosis of diseases [21]. Besides, nanoprobes can also be used as carriers to achieve favorable outcomes in various therapies, such as radiotherapy, chemotherapy, photothermal therapy, photodynamic therapy, chemodynamic therapy, ferroptosis-related therapy, and immunotherapy [22]. In the environmental protection field, nanoprobes can be used to accurately detect substances that have an impact on the environment. For example, the upconversion luminescence sensing strategy based on nanoprobes can accurately monitor H_2O_2 in lake water [23]. In terms of biological evolution, nanoprobes can simulate catalytic enzymes involved in various reactions within living organisms, thus providing new research strategies for the co-evolution of the lithosphere and biosphere on the Earth [24]. In beverage production, dual-mode fluorescent and colorimetric nanoprobes can be used to evaluate the harmful indigo carmine dye in juice and soft drinks, thereby ensuring food safety [15]. In oil and gas exploration, metal nanoprobes can accurately characterize the pores of insulating rocks and improve the imaging contrast of the underlying morphology [18]. In chemical raw material preparation, fluorescent nanoprobes can be combined with layer fault modeling and other new technologies to achieve the preparation of some powdery raw materials such as $AgFeO_2$. On that basis, the structure of raw materials can be characterized accurately, thus ensuring the production of raw materials [19]. In crop research, nanoprobes can be used for three-dimensional (3D) labeling and precise imaging of the cellular structure of such plants as potatoes, thus facilitating the study of non-signal target cells in plants [25]. Overall, nanoprobe technology can be applied to various aspects of our lives and may provide powerful impetus for changing life patterns.

Application of Nanoprobe Technology in the Biomedical Field

The exploration of the microscopic world has always been a hot topic in scientific research, and a more thorough insight into the microscopic field contributes to medical development. As an indispensable technology in the field of microscopic medicine, nanoprobe technology can visualize various diseases for clinicians. The application of nanoprobes can enhance the spatial resolution of imaging, thus achieving higher resolution quality, sensitivity, and accuracy [26]. The integration of nanoprobes with photothermal [27], magnetodynamic [28], and ultrasound [29] excitation conditions can synergistically target the local area of the disease with the carried substances. Moreover, they can reduce the side effects on healthy tissues and accelerate the diagnosis and treatment process in the multimodal treatment of diseases (Fig. 1).

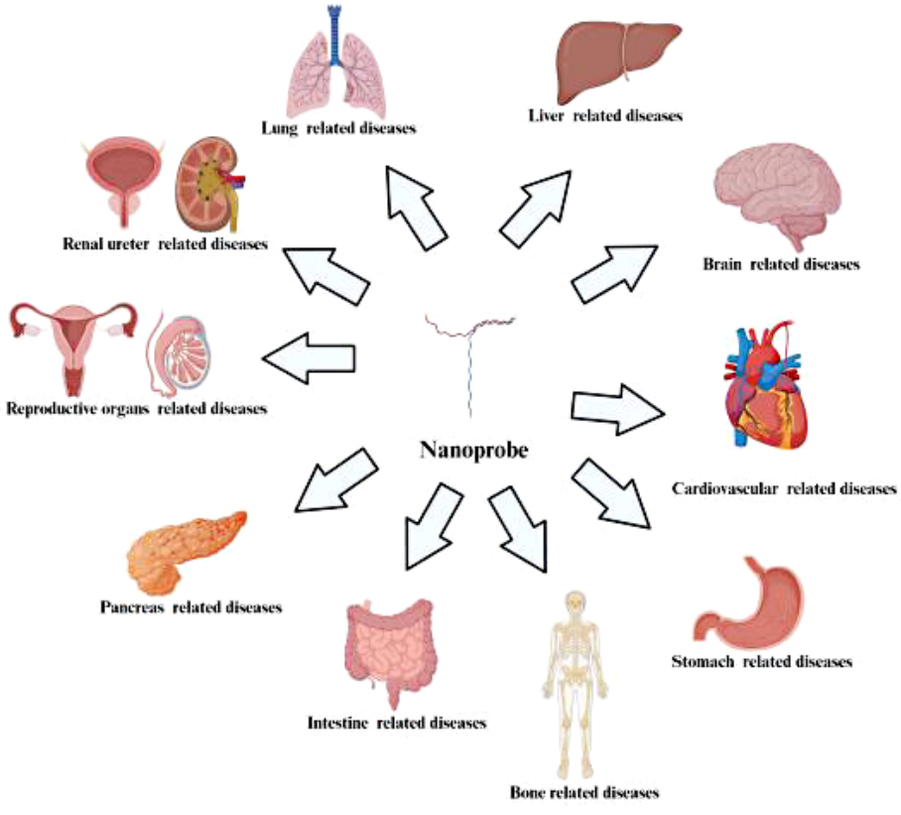

Fig. 1 Application of nanoprobe technology in biomedical research. (Note: Created with BioRender.com)

Application of nanoprobe technology for disease diagnosis

The accurate diagnosis of diseases is considered a prerequisite for effective treatment, and it is often difficult to make a clear diagnosis for some diseases in the early stage. On the one hand, the early symptoms of some diseases may not be specific, making it impossible to receive targeted treatment. On the other hand, some diseases may not have special symptoms in the early stage and hence fail to attract the attention of doctors and patients, resulting in delayed treatment and ultimately poor treatment outcomes. Some diseases are not even diagnosed until advanced stages based on specific symptoms. However, due to the existence of subtypes or heterogeneity among different patients, the diagnosis of diseases may be inaccurate, which affects the effectiveness of treatment. With the assistance of nanoprobes, an early or accurate diagnosis of diseases may be achieved [30, 31].

Macrophages play an important role in the immune process of various diseases, and imaging macrophages through nanoprobes is beneficial to the diagnosis and treatment evaluation of diseases. Liu et al. developed a molecular imaging nanoprobe based on ultra-small superparamagnetic iron oxide nanoparticles. The probe designed based on nuclear magnetic resonance can target the intracellular NO reaction in the repolarization process of M2 macrophages to M1 macrophages, leading to probe electrical attraction and colloidal aggregation. Besides, imaging the repolarization process of macrophages can also provide a non-invasive diagnosis and treatment method for such immune-related diseases as tumors [32]. Extracellular vesicles are natural nanoparticles secreted by cells that play an important role in regulating diseases. Identifying the specific proteins carried by extracellular vesicles can assist in the diagnosis and treatment of diseases. Gu et al. proposed a fluorescence resonance energy transfer nanoprobe labeling strategy consisting of gold nanoclusters (AuNCs) and polydopamine nanospheres (PDANS) for specific detection of amyloid beta (1-42) (Aβ(1-42)) and CD63 on extracellular vesicles, thereby achieving the early and advanced diagnosis of Alzheimer's disease [33]. Although the early diagnosis of diseases often results in favorable treatment outcomes, it is often difficult to detect physiopathological changes in an early stage through existing detection methods. Lu et al. introduced a novel nanoprobe PCN@FL and realized

two-photon microscope imaging based on fluorescent nanoprobes for hypochlorite changes and phosphorylation level alternations in early atherosclerosis. Based on this nanoprobe, early atherosclerosis was evaluated in a mouse model [34]. Nanoprobes derived from biological components also have powerful diagnostic functions. Li et al. constructed nanoprobes using synthetic biological nano-fragments (SynBioNF) generated by the fragmentation of genetically programmed yeast cells. They achieved targeted quantitative detection of virus particles in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with a sensitivity similar to that of the gold standard reverse transcription-quantitative polymerase chain reaction [35]. Ischemia-reperfusion injury (IRI) is a research hotspot in the field of heart diseases. Luo et al. prepared a near-infrared (NIR) light-activated dual responsive nanoprobe UCNP@mSiO$_2$ @SP-NP-NAP, which can be employed to monitor the levels of hydrogen sulfide (H_2Sn) and sulfur dioxide (SO_2) during iron death in myocardial cells. Besides, they applied this nanoprobe to the diagnosis and targeted treatment of IRI in heart diseases [36]. Some special substance types also constitute the basis for the preparation of nanoprobes. Exploring these substance types can also promote the application of nanoprobes in the field of disease imaging. Zeng et al. constructed a self-assembled amphiphilic polymer micelle by using copper-free bacterial strains to promote the azide cycloaddition click reaction between a dibenzocyclooctyne-modified antisense oligonucleotide and an azide-containing aliphatic polymer polylactic acid. Two endogenous enzyme-activated spherical nucleic acid (Ep SNA) nanoprobes with no purine/pyrimidine (AP) sites containing tailing DNA hairpins were connected to the micelle, and tailing DNA hairpins, antisense oligonucleotides, and doxorubicin can be released after the cleavage by specific enzymes in tumor cells, thus achieving specific mRNA expression, imaging, and gene and drug therapy for tumors [37]. Li et al. designed an integrated nano Cas12a sensor by combining genetic engineering tools with mitochondrial DNA. This sensor can be used to transport Cas12a/crRNA, fluorescence quencher reporter, and Mg^{2+} to mitochondria, thereby accurately reporting single nucleotide variations in cells. The nanoprobe can accurately identify tumors and their metastases at the genetic level [38]. Apart from thrombosis and tumor-related diseases, nanoprobes can play an important role in many other diseases, like neurodegenerative diseases of the brain such as Alzheimer's disease and Parkinson's disease. Bodily et al. developed a portable, wireless readout-based graphene field-effect transistor (GFET) biosensor platform, which enabled the precise detection of small molecule proteins amyloid beta (Aβ), Tau (τ), and alpha synuclein (αS). This method based on nanoprobe technology for accurate detection of small molecule substances can achieve real-time monitoring of neurodegenerative diseases of the brain without geographical limitations [39].

The materials used to construct nanoprobes have special properties that contribute to energy conversion. In addition, the physical way of exciting the probe function and the targeting effect of the carried substance can also conduce to the precise imaging of various diseases or the accurate quantification of trace diagnostic substances in multiple dimensions. Therefore, nanoprobes can achieve an early and accurate diagnosis of diseases, and hence they are also essential for orthopedic diseases. This can control the progression of orthopedic diseases in time, eliminate pathogenic factors, and ultimately achieve favorable outcomes.

Application of nanoprobe technology for disease treatment

After the accurate diagnosis of a disease, the therapeutic effect also depends on whether the drug can be accurately delivered to the affected site, whether the drug concentration reaching the affected site can be effective, whether the drug can be released on time and as needed during the special stage of pathogen action, and whether the drug can penetrate the protective barrier of the body and only act on the affected site or even disease cells while excluding normal tissues. With the transformation of nanoprobe products in clinical applications, the treatment conditions for the above-mentioned diseases are no longer complex [40, 41].

Pulmonary embolism and cerebral vascular embolism are life-threatening vascular diseases, and imaging them with nanoprobes contributes to the timely clearance of blood clots and life-saving. Song et al. constructed a nanoplatform using a thermosensitive nitric oxide donor-doped homopolymer with absorption and molecular motion functions in the long wave second NIR-II region. This

nanoplatform was loaded with a functionalized fibrin-specific ligand that can target thrombi. Under the action of photoacoustic nanoprobes, thrombi were accurately and specifically displayed, and thrombus clearance was achieved under a local photothermal effect and the controlled release of NO, thus restoring the blood flow to unobstructed blood vessels [42]. Wang et al. designed a hollow mesoporous silica nanoprobe RGD/ICG/ PFP@HMSN by combining light (near-infrared light) and sound (ultrasound), and this nanoprobe was composed of arginine glycine aspartic acid (RGD) peptide, perfluoropentane (PFP), and indocyanine green. In addition, this probe can target and penetrate blood clots, further achieving the accurate diagnosis of arterial thrombosis and implementing photoacoustic synergistic therapy [43]. Pancreatic cancer progresses rapidly and may become life-threatening in case of poor treatment. Pu et al. prepared a therapeutic nanoprobe for pancreatic cancer based on polyetherimide superparamagnetic iron oxide nanoparticles (PEI-SPION) and siRNA nanoprobe. This therapeutic nanoprobe realized gene silencing of tumor antigen mucin 4 in pancreatic cancer cells and achieved genetic treatment of pancreatic cancer [44]. Of course, nanoprobes can also be used for detecting the efficacy of tumor therapies. Ge et al. developed a novel fluorescent nanoprobe COF-H1/H2 peptide by using low abundance miRNA-221 amplification imaging and caspase-3 imaging through catalytic hairpin assembly loop amplification in living cells. With the aid of this nanoprobe, the efficacy of miRNA-based treatment strategies in the treatment of tumors can be evaluated [45]. Nanoprobes that simultaneously maintain the biological effects of imaging and therapeutic components are a hot topic in the field of probe development. Wang et al. prepared gadolinium-doped $CuWO_4$ (CWG) nanoprobes, which exhibited a strong near-infrared absorption ability and enhanced magnetic resonance imaging (MRI) and photoacoustic imaging (PAI) capabilities due to the presence of gadolinium element. In addition, the presence of copper elements can promote the production of OH and achieve chemical kinetic therapy, thus realizing synergistic treatment under multi-mode guided imaging [46]. Ma et al. developed a near-infrared malondialdehyde (MDA) response molecule acid unlocking ratio photoacoustic nanoprobe with dual functions of PAI and urine analysis for the specific substance MDA in the plaque. It can be employed to specifically detect the difference in the MDA in the urine among patients with atherosclerosis. Based on a combination of the two functions, it can be utilized to track the progression of atherosclerosis and relevant treatment and monitor pneumonia infection by detecting the level of oxidative stress in the body [47]. Apoptosis is an important process with complex mechanisms in disease treatment. Liu et al. modified double-stranded DNA (dsDNA) and cysteine protease 3-specific cleavable peptide (pep) onto the surface of dual responsive integrated Au nanoparticles to prepare a composite fluorescent probe for *in situ* real-time monitoring of the apoptosis process. After entering the cell, the probe specifically recognized and silenced miRNA 21, and cysteine protease 3 was cleaved, both of which can be imaged, thus achieving apoptosis of HeLa and A549 cells [48]. The application of *in situ* catalysis regulated by nanoenzymes in the tumor cell microenvironment and tumor markers is helpful for the treatment of tumors. Wang et al. prepared chiral photoacoustic (PA) nanoprobes D/L-Ce@MoO_3 based on H_2O_2-catalyzed tumor microenvironment activation reaction using D-cysteine or L-cysteine, molybdate, and cerium nitrate by a one-step method. This nanoprobe can be applied to the accurate, non-invasive, and *in situ* imaging of acute lung injury in the progression of tumors. Meanwhile, Ce ions in the probe can catalyze the damage of H_2O_2 in the liver to produce OH, which further killed tumor cells and converted chiral probes into non-chiral polyoxometalates for PAI [49]. Dallari et al. reported a multi-layer nanoprobe Au@RRs @AuNP, which consisted of a bioorthogonal Raman reporter embedded in two layers of gold nanoparticles. This probe can achieve precise detection of Aβ(1-42) in human cerebrospinal fluid, enabling the early diagnosis and treatment of neurodegenerative diseases, such as Alzheimer's disease [50].

The substances carried by nanoprobes are the main components for the treatment of diseases. Besides, the materials used to construct nanoprobes are often biocompatible, and they can also exert therapeutic effects. The excitation methods of nanoprobes, such as photothermal, electromagnetic, and acoustic waves, are also commonly used auxiliary treatment methods in clinical practice. Therefore, nanoprobes can also fulfill therapeutic effects in multiple dimensions. Benefitting from their accurate targeting, penetration, and controlled release properties, these nanoprobes can enhance the therapeutic effect, which

is also conducive to the treatment of orthopedic diseases.

Application of Nanoprobe Technology in Orthopedics

Different from other systems, the skeletal motor system is an important system that functions based on both hard and soft tissues, such as harder bones and cartilage, as well as softer muscles, blood vessels, and nerves. Most orthopedic diseases may affect both hard and soft tissues, which complicates relevant treatment (Fig. 2). For example, the cartilage, bone, meniscus, synovium, and muscle are all affected in arthritis; the bone, cartilage, blood vessels, and muscle can all be affected in bone tumors. Consequently, there are effective treatment measures for other systemic diseases but not for orthopedic diseases. Hence, there is an urgent demand for a change in treatment methods to improve the efficacy in the treatment of orthopedic diseases. Nanoprobe technology provides new strategies for the clinical diagnosis and treatment of orthopedic diseases owing to its good penetration effect, targeting effect, and material carrying capacity [51, 52] (Fig. 3).

Application of nanoprobe technology in osteoarthritis

Osteoarthritis (OA) is a chronic bone and joint disease characterized by degenerative changes in articular cartilage and secondary bone hyperplasia, and this disease often affects joints with a higher weight-bearing capacity and mobility. OA is more common in the population aged over 50 years, with a slightly higher incidence in females. About 90% of elderly people over 80 years old have imaging manifestations of OA [53]. The treatment methods for OA include avoiding weight-bearing, drug therapy, and surgical treatment. With the improvement of medical technology, new technologies and methods have been developed for the diagnosis and treatment of OA [54], and nanoprobe technology has been highlighted among them. With the development of this technology, the early and accurate diagnosis and targeted treatment of OA have become more convenient.

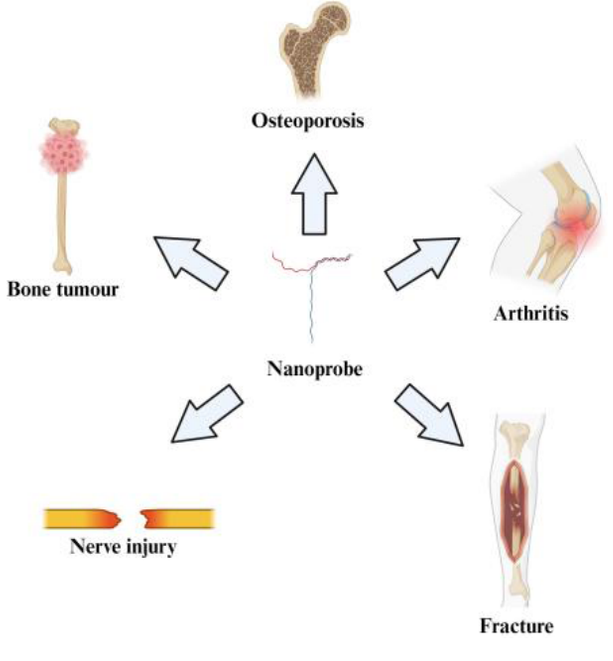

Fig. 2 Application of nanoprobe technology for orthopedic diseases treatment. (Note: Created with BioRender.com)

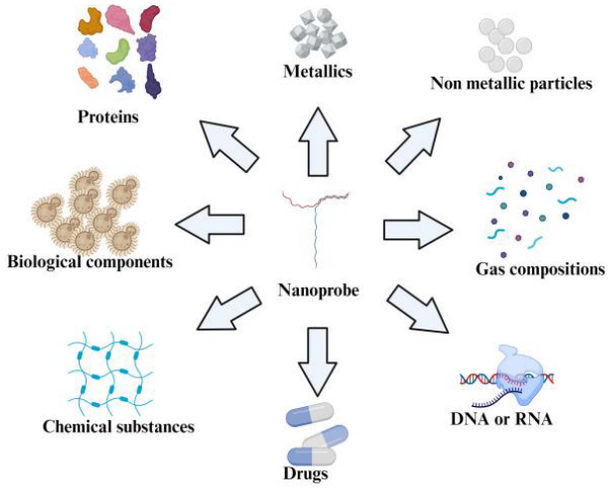

Fig. 3 Substances that can be carried by nanoprobes. (Note: Created with BioRender.com)

Due to the lack of accurate and timely detection methods, the treatment effectiveness of OA is often affected. The application of nanoprobe technology can improve the diagnosis and treatment of OA. Xiao et al. used PAI to explore the role of nanoprobes in the diagnosis of OA. They synthesized nanoprobes by electrostatic attraction between polylysine and melanin (PLL-MNPs) and incubated cartilage explants with different concentrations of anionic glycosaminoglycans (GAGs) using the nanoprobes. The nanoprobes were also used to detect instability-induced OA in mouse models. It was found that the PLL-MNP nanoprobes could accurately detect the concentration of GAGs. In addition, the enhanced PAI could further demonstrate the progression of OA by detecting changes in the GAG content, thus

validating favorable clinical application potential [55]. Nanoprobes can achieve precise imaging for the guided treatment of OA. Shen et al. used PEG micelles modified by reactive oxygen species (ROS)-sensitive thioketide linkers (TK) and cartilage-targeting peptides (TKCP) to fabricate nanoprobes that can locate cartilage and target ROS. The nanoprobes were encapsulated with dexamethasone (DEX) to form nanoparticles, which achieved precise imaging and disease diagnosis through dose-dependent fluorescence expression of ROS. Additionally, the nanoprobes were also used to control the release of DEX at the cartilage injury site for the treatment of OA [56]. Chen et al. synthesized a hyaluronic acid gold nanoparticle optical probe (HA-GNP) by a one-step method. The probe exhibited good stability and biocompatibility, and it was superior to conventional probes constructed with sodium citrate gold nanoparticles. Further, this probe can also exert inhibitory effects on joint chondrocytes, providing a new approach to the treatment of OA [57]. Shen et al. also prepared a nanoprobe, Au@PDA-WLNP, with a PAI function. The nanoprobe can be used to monitor early degeneration of articular cartilage. By targeting type II collagen, the nanoprobe was more easily enriched in cartilage tissues, which caused a local plasmon resonance coupling effect and enhanced the photothermal conversion ability, thus delaying the progression of bone and joint diseases by eliminating ROS [58]. Some researchers have also developed specific nanoprobes to resolve pain symptoms in the procession of OA. Au et al. constructed a therapeutic nanoprobe based on MoS_2-AuNR coated with molybdenum disulfide nanosheets, which can target pain-related nerve growth factor and exhibit favorable photoacoustic and photothermal properties. The nanoprobe can actively act on the knee joint pain site of OA mice, and the imaging-guided photothermal therapy can also alleviate OA-related pain and improve gait, making it an active and specific treatment method [54]. Nanoprobe technology can also be combined with targeted drug delivery to enhance the time and accuracy of drug action. Xiong et al. established a pH-responsive metal-organic framework (MOF) system modified with hyaluronic acid (HA) and loaded with anti-inflammatory catechins (PCA). The MOF@HA @ PCA probe system was regulated by the acidic environment in OA joint fluid and can release PCA, thus down-regulating the expression of synovitis markers while up-regulating the expression of cartilage-specific markers. Hence, nanoprobes are expected to contribute to the precise diagnosis and treatment of OA in the future [59]. Li et al. developed a novel core satellite gold nanostructure for chronic inflammation and tumors caused by oxidants. Under the induction of H_2O_2, PAI, and surface-enhanced Raman scattering imaging were combined to accurately distinguish inflammatory tissues from normal ones. The silica shell on the probe can also target drug delivery to achieve precise treatment of inflammation, enabling real-time and accurate detection of H_2O_2 produced in inflammatory diseases such as OA or tumors [60]. On that basis, they also grafted a thermosensitive polymer with NIR-II fluorescence dye onto the surface of a nanoporous core satellite gold nanostructure to construct a temperature-responsive nanoprobe. The disease was monitored by the temperature difference between the affected site and normal tissues, and the nanoprobe served as an evaluation method for the early diagnosis and treatment of arthritis [61]. Zerrillo et al. used a novel fluorinated polymer nanoparticle as a carrier for multimodal nanoprobes and constructed a novel fluorinated polylactic acid co-glycolic acid and polyethylene glycol (PEG) nanoparticle. The probe particles were non-cytotoxic and could deliver contrast agents into OA joints. The visualization and tracking of OA treatment were achieved through MRI and optical imaging [62].

In summary, nanoprobes can target chondrocytes and extracellular matrices to accurately image and assist in the diagnosis and treatment of OA. Besides, they can also respond to changes in metabolic products such as inflammatory factors produced during the procession of OA, as well as changes in acidity and alkalinity in the inflammatory microenvironment, thus reducing or eliminating harmful factors. In addition, they can also carry specific drugs or cytokines to directly repair damaged joint tissues or accurately locate the affected site, thereby promoting the treatment of OA (Fig. 4). Based on the research on the application of nanoprobe technology in the diagnosis and treatment of OA, the therapeutic effect has been improved. In the future development process, more targeted nanoprobes can be developed for different risk factors of OA, such as aging, obesity, trauma, and genetics. The diagnostic and therapeutic factors carried by nanoprobes can be

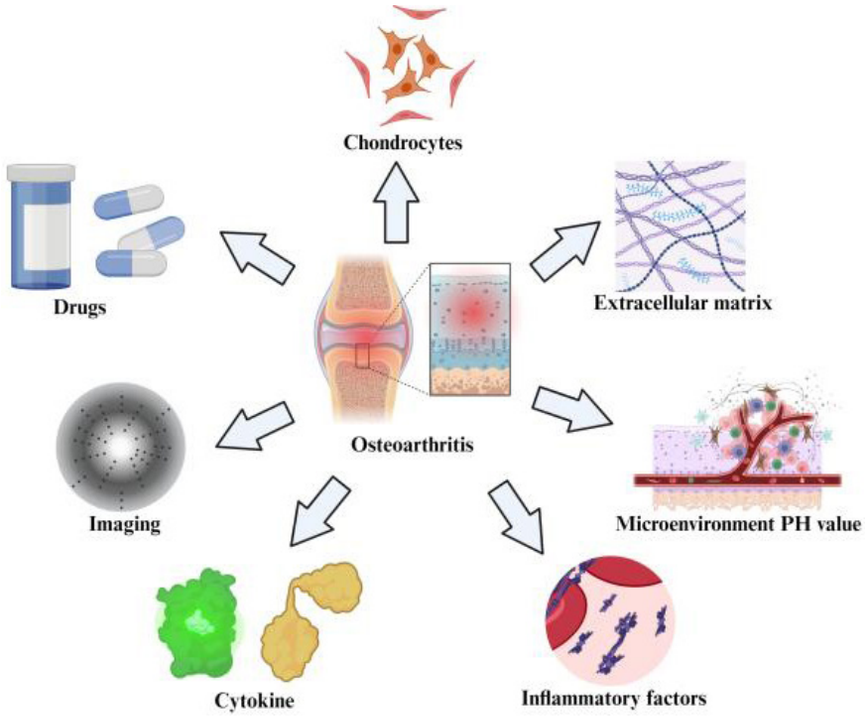

Fig. 4 The diagnostic and therapeutic effects of nanoprobe technology on osteoarthritis. (Note: Created with BioRender.com)

accurately applied to the treatment of patients with OA caused by different risk factors, thus providing personalized therapies for these patients.

Application of nanoprobe technology in rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disease caused by connective tissue disorders, with synovitis as the pathological basis and erosive arthritis as its characteristic. This disease is more common in the population aged more than 15 years, especially those between 20 and 45 years old, with a high incidence reported in females. RA is mainly manifested as multiple symmetrical joint pain and swelling [53]. The treatment methods for RA include muscle exercise, drug therapy, and surgical treatment. The treatment methods for RA also need to be improved, which may be achieved with the aid of nanoprobes [63]. Nanoprobes can not only provide orthopedic clinicians with novel ideas but also offer new treatment models for clinicians in the department of rheumatology and immunology.

Different from OA, RA first appears in the synovial tissue of joints and is an autoimmune disease with high disability and recurrence rates, which complicates relevant treatment. Wang et al. constructed a dually triggered therapeutic nanoprobe, mZMI@HA, based on NIR persistent luminescent nanoparticles (NIR PLNPs). This nanoprobe can exert pioneering diagnostic and therapeutic effects on RA, exhibiting better imaging signal intensity. Besides, it can control drugs through photothermal effects and pH responses, thus decelerating the progression of collagen-induced RA and facilitating the precise treatment of RA [64]. The early diagnosis of RA contributes to decelerating disease progression and improving therapeutic effects. Zhang et al. constructed an organic nanoprobe ONP-CySe using near-infrared fluorescent selenomethionine cage-shaped cyanine dye as the sensing component and amphiphilic triblock copolymer triphenylphosphine derivative as the mitochondrial targeting component. This organic nanoprobe presented favorable targeting and biocompatibility towards mitochondria in macrophages, and it can be employed to monitor the therapeutic effects of drugs such as selenocysteine and methotrexate in RA mice, thus conducing to the early diagnosis of RA and the screening of therapeutic drugs [65]. ROS also plays an important role in the pathogenesis of RA, which is similar to OA. Chen et al. developed a ratio nanoprobe using upconversion nanoparticles (UCNPs) coupled with chromophore-labeled HA. This nanoprobe could affect light energy transfer by binding or separating HA on the probe. Besides, it exhibited favorable water solubility, biocompatibility, and ROS targeting

properties, which contributed to the early diagnosis of RA and higher efficacy of methotrexate in the treatment of RA [66]. Hwang et al. synthesized two anisotropic bimetallic core satellite polyaniline nanohybrids using small or polymer ligand-coated gold nanospheres or gold nanorods as seeds. The nanoprobes exhibited good optical properties and could be employed to quantitatively analyze cyclic citrullinated peptide autoantibodies for the early diagnosis of RA [67]. Subsequently, they prepared anisotropic bimetallic polymer nanoparticles (ABPNs) through a combination of oxidative polymerization and additional growth of metal nanoparticles on gold seeds under the directed aggregation mediated by non-covalent interactions. Under the action of a magnetic field, the probe exhibited good optical performance and could bind to antibodies, ultimately achieving quantification of such RA-related diagnostic factors as cyclic citrullinated peptide and rheumatoid factors, thus facilitating the early diagnosis of RA [68]. Some scholars have also developed other probes targeting autoantibodies in RA lesions. For example, Veigas et al. developed a colorimetric immunosensor based on cross-linked gold nanoprobes, which could achieve wide aggregation in the presence of pentameric IgM rheumatoid factors, while producing visible color changes from red to purple. This detection method had low costs, which promoted its large-scale application in the diagnosis of rheumatoid factor antibodies [69]. Human immunoglobulin G (HIgG) also plays an important role in the diagnosis of RA. Wen et al. prepared a method with luminescence effects, multi-phonon resonance Raman scattering effects, and infrared absorption effects. Based on this method, they developed a nanorod CdS@SiO_2 for the precise detection of HIgG in different scenarios, thus providing new ideas for the detection of diseases such as RA [70]. Lee et al. fabricated a multifunctional gold nanoprobe for detecting ROS and hyaluronidase by immobilizing the end of HA labeled with NIR fluorescence dye on the surface of gold nanoparticles (AuNP). When HA was cleaved by the latter two, different fluorescence signals were displayed, allowing for the precise imaging of RA inflammation and tumor sites, thus serving as an optical imaging agent to assist in disease diagnosis [71]. Nanoprobes can also be used to detect joint damage caused by various inflammatory factors secreted in the progression of RA. Lee et al. used matrix metalloproteinase 3-specific nanoprobes to monitor the joint inflammation and bone damage after the preparation of a nanocomposite of tumor necrosis factor-α targeting poly siRNA and thiolated ethylene glycol chitosan polymers [72]. Although the nanoprobes were only used as a disease progression monitoring method in this study, they also demonstrated the important role of this technology in the diagnosis and treatment of RA.

Based on the above facts, in the diagnosis and treatment of RA, nanoprobes can be employed to detect disease antibodies at lower levels with high sensitivity and accuracy, thus realizing the accurate and rapid early diagnosis after imaging disease-specific components. Besides, nanoprobes can also target fibroblasts, macrophages, extracellular matrix such as collagen, and even subcellular organelles in synovial tissues, thereby synergistically resolving autoimmune inflammation with loaded drugs or probes under stimulation conditions. Additionally, novel proteins and peptides can also be used to resolve inflammatory factors or environments, thus alleviating relevant diseases (Fig. 5). However, it remains unclear about the etiology of RA, and autoimmune and genetic factors are currently two causes with higher attention. The small size of nanoprobes and their ability to integrate multiple detection factors can be used to explore the etiology of RA, educate susceptible populations, and reduce disease occurrence, which may better benefit the public.

Application of nanoprobe technology in bone tumors

Bone tumors can be categorized into benign and malignant types. Benign bone tumors usually have asymptomatic protrusions on the bone and cartilage, and they can be left untreated in the absence of symptoms. Nevertheless, it is necessary to conduct a close observation, and surgical resection is required after the appearance of symptoms. For malignant bone tumors such as osteosarcoma and Ewing's sarcoma, chemotherapy combined with surgery (limb salvage surgery or amputation surgery) is required [53]. The development of nanoprobe technology can provide a more comprehensive and extensive treatment plan for precise imaging and adjuvant therapy of bone tumors [73], which is similar to other systemic tumors. Additionally, nanoprobe technology can be used in various aspects of preoperative,

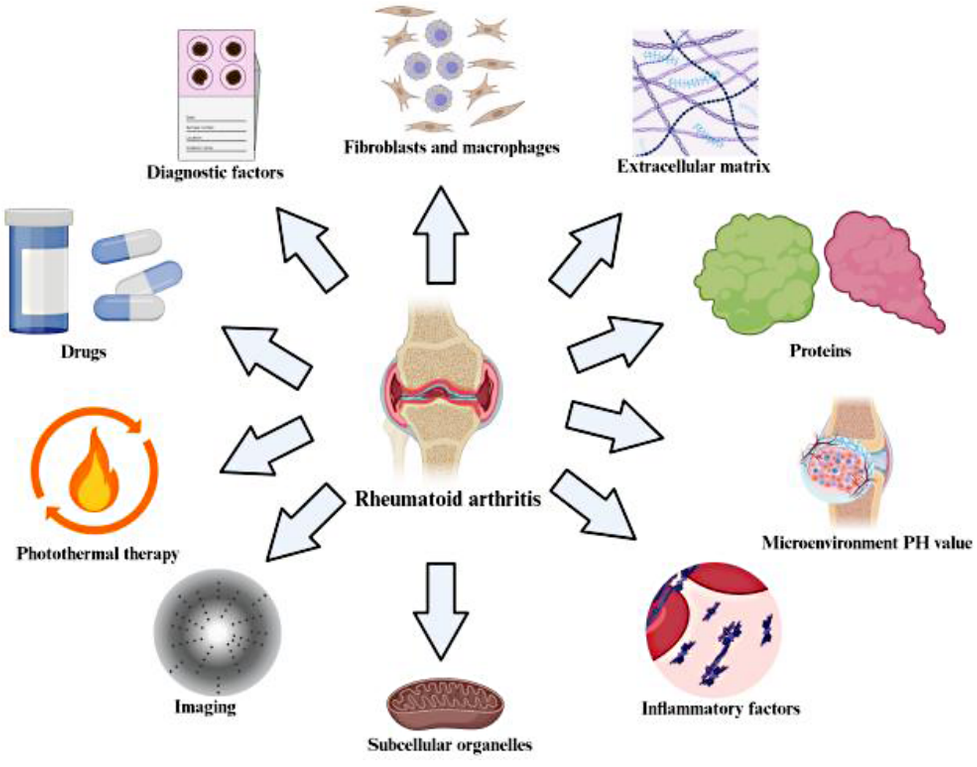

Fig. 5 The diagnostic and therapeutic effects of nanoprobe technology on rheumatoid arthritis. (Note: Created with BioRender.com)

intraoperative, and postoperative diagnosis and treatment of bone tumors.

Nanoprobes play an important role in the diagnosis and treatment of malignant bone tumors. Xiong et al. constructed a gold nanotriangle loaded with CD33, hyaluronic acid, and near-infrared photosensitizer on its surface. CD33 and hyaluronic acid achieved dual targeting of tumors, and the photosensitizer enhanced the photothermal and photodynamic effects of the probe, which could be protected by hyaluronic acid from decomposition. Combined with relevant therapeutic drugs, the probe can exert anti-tumor effects and significantly inhibit tumor growth in osteosarcoma mice [74]. Based on the screening of two novel tumor-specific oligopeptides PT6 and PT7, Ma et al. coupled them with PEGylated gold nanorods. This probe can specifically target the osteosarcoma cell line UMR-106 or the formed graft both *in vitro* and *in vivo* and improve the contrast of osteosarcoma imaging *in vivo*, thereby facilitating the diagnosis of specific osteosarcoma diseases [75]. Fu et al. developed a nanoprobe-based nanodrug by integrating ferrate and doxorubicin into a hollow mesoporous silica nanoplatform, which was then combined with a solid-liquid phase change material of n-eicosane to construct the drug. The nanodrug can be activated by ultrasound induction to target the release of the two loaded drugs. This further depleted glutathione in tumors, down-regulated glutathione peroxidase 4, hypoxia-inducible factor 1 alpha, and multidrug resistance gene/transporter P-glycoprotein, and inhibited hypoxic osteosarcoma through iron-dependent cell death [76]. Tumors can cause the erosion of bone tissues, thus leading to pathological fractures. Lou et al. developed a novel small molecule near-infrared IIb dye, IT-TQF, which exhibited high fluorescence intensity in the near-infrared window. The dye was encapsulated in DSPE-PEG2000 to construct an IT-TQF NP nanoprobe. Based on this nanoprobe, the high resolution and tissue penetration of imaging can be achieved, which can accurately display *in situ*, subcutaneous, and metastatic tumors as well as bone tissue erosion caused by tumors, thus providing effective guidance for surgical resection of *in situ* tumors [77]. In the detection of bone tumors, it is necessary to achieve favorable imaging effects on bone mineral substances. Lim et al. chemically coupled a specific targeting ligand alendronate with a Cy5.5-labeled dibenzocyclooctyne to the two ends of azide polyethylene glycol N-hydroxysuccinimide (NHS) ester to construct a novel nanoprobe. This nanoprobe can be self-assembled and exhibited a high

affinity for calcium phosphate with a high bone mineral content, which contributed to imaging calcium ions in normal bone tissue cells *in vitro* and *in vivo* [78]. This may also facilitate the accurate diagnosis of bone tumors. The postoperative prognosis evaluation of bone tumors is conducive to the formulation of treatment plans, and nanoprobes can assist in assessing the prognosis of bone tumors. Yang et al. developed an APPAD (AuNBPs @ PDA-pe-AS1411-Dox) nanoprobe composed of core-shell nanoparticles and a gold nanocone core coated with dopamine, which can carry peptide chains, AS1411, and doxorubicin. Besides, this nanoprobe can release doxorubicin in an acidic tumor microenvironment, initiate cell apoptosis, and fulfill precise fluorescence imaging functions. Hence, this nanoprobe can be used to evaluate the efficacy and prognosis of patients with advanced osteosarcoma after surgery [79]. The imaging of lymph nodes also plays an important role in the diagnosis of osteosarcoma. Xu et al. proposed a 99mTc-labeled biomineralization nanoprobe, which can be targeted and localized in osteosarcoma based on MRI, radionuclide bone imaging, and near-infrared imaging. Additionally, this nanoprobe can image the lymphatic system network of mice in real time, providing high-definition imaging of lymphatic drainage *in situ* in osteosarcoma mice [80]. Metastatic bone tumors usually occur in the late stages of various malignant tumors. When metastatic tumors appear in the bone, most patients can only be treated by conservative methods with poor outcomes. An accurate diagnosis can help clinicians make the most accurate diagnosis and treatment plan for patients. Wang et al. developed a multifunctional nanoprobe (the Au/Gd nanodot) for malignant tumors in the urinary system that frequently undergo bone metastasis, which can assist in the multimodal imaging and accurate diagnosis of bone metastatic tumors. Multimodal imaging can complement each other and accurately detect and guide the treatment of metastatic prostate malignancies, providing a new insight into the diagnosis and treatment of bone metastatic tumors [81]. Exosomes also play an important role in the diagnosis of tumor diseases. Qin et al. designed a dual-cycling nanoprobe (DCNP) that can directly quantify programmed cell death ligand 1 (PD-L1) (ExoPD-L1, ExomiR-21) and miRNA-21 (ExomiR-21, ExomiR-2) on the surface of cancer exosomes in their lysate, enabling precise quantification of tumor markers on the surface or *in vivo* [82]. This diagnostic approach also facilitates the use of exosomes related surface markers for the diagnosis and treatment of bone tumors.

Different from other orthopedic diseases, malignant bone tumors may endanger patient life in a short period, highlighting the urgent demand for enhanced treatment. Nanoprobe technology can display the boundary between bone tumors and normal tissues based on specific markers (cell related, subcellular organelle related, or exosome related) of bone tumors, thus assisting in the development of more feasible preoperative, intraoperative, and postoperative treatment plans. With the aid of this technology, orthopedic clinicians can completely remove tumor tissues, thus preventing a poor prognosis. Regarding the survival mechanism of tumor cells, nanoprobe technology can also more accurately target drugs, new substances that promote tumor cell death, or physical therapy methods on tumor cells or their living environment, further causing apoptosis, shrinking tumors, and providing convenience for surgical procedures (Fig. 6). Due to the existence of various malignant bone tumors and the younger onset age compared with other orthopedic diseases, some bone tumors are even more common in adolescents and children. Therefore, it is necessary to emphasize the protection of healthy tissues and limb contours in the treatment process. Besides, the application of nanoprobes can reduce the side effects in the treatment of bone tumors. Additionally, nanoprobes can be employed to explore the reasons why malignant bone tumors are more common in adolescents.

Application of nanoprobe technology in bone fractures and bone defects

Bone fractures refer to the interruption of bone integrity and continuity, which can be caused by direct violence, indirect violence, or accumulated fatigue [53]. With the development of transportation, the incidence of bone fractures caused by trauma has been increasing year by year, and the number of patients with brittle bone fractures caused by elderly diseases such as osteoporosis or pathological bone fractures caused by bone tumors has also been increasing. Nanoprobes can be applied to the treatment of bone fractures and bone defects. They can activate multiple endogenous or exogenous repair mechanisms to promote bone repair, thus accelerating bone fracture and bone defect healing [83].

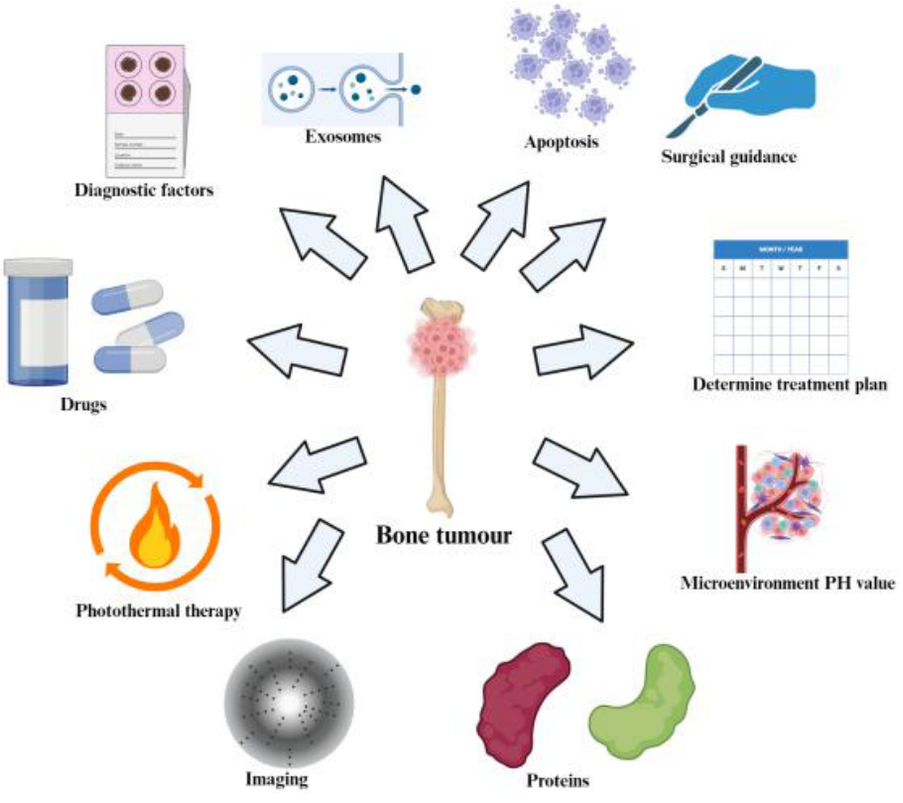

Fig. 6 The diagnostic and therapeutic effects of nanoprobe technology on bone tumors. (Note: Created with BioRender.com)

The osteogenic differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) can be applied to bone defect repair in fractures. Sun et al. used 5-bromo-4-chloro-3-indolyl phosphate (BCIP) to modify the surface of gold nanoparticles (AuNPs) and prepared an intelligent nanoprobe Au@BCIP. When co-cultured with BMSCs in different states, this probe can be used to detect the activity of alkaline phosphatase and track it *in vivo*. With the assistance of this probe, they identified the role of the p38 mitogen-activated protein kinase signaling pathway in the osteogenic differentiation of BMSCs [84]. Wan et al. also performed surface modifications on gold nanoparticles using silica and DNA Transmittin 3000 (TS) for preparing the AuNPs@SiO_2-TS nanoprobe, which can operate within 14 days after the co-incubation with BMSCs. There was no cytotoxicity to BMSCs and no effect on their multi-directional differentiation potential. Dual-energy computed tomography (DECT) can present the migration of BMSCs to the cortical bone during bone repair, which can be used to observe bone regeneration [85]. Ma et al. designed a NIR light-triggered NO release nanoplatform based on upconversion nanoparticles (UCNPs), achieving photo-controlled NO release. The platform exhibited excellent nanoprobe performance and can control and enhance the osteogenic differentiation of mesenchymal stem cells (MSCs), thus facilitating the treatment of bone loss related diseases such as osteoporosis [86]. Yang et al. explored through nanoprobe technology that m6A methyltransferase METTL14 released from extracellular vesicles can increase the m6A methylation level of nuclear gactor of activated T-cells, cytoplasmic 1 (NFATc1), inhibit osteoclasts, and help postmenopausal women preserve bone mass, providing a new bioactive substance as a treatment for related diseases [87]. In the imaging of fracture repair with titanium alloy in clinical practice, metal artifacts may affect the judgment of diagnosis and treatment results. Lin et al. designed a highly specific osteogenic biomarker and nano gold Pamidronate nanoprobe. They confirmed that it can accurately identify image loss caused by metal artifacts in the imaging process after the implantation of titanium alloy screws in the anterior hard palate of rats. Besides, this nanoprobe can be employed to monitor the maturity of the surrounding bone of the implant [88], which contributed to the diagnosis and treatment of orthopedic diseases and the research on the transformation of orthopedic implant products. At present, imaging technology for bone diseases in clinical practice has a radiation impact on the human body, and developing imaging

techniques without hazards to the human body is also a hotspot in the development of nanoprobe technology. Zhang et al. constructed carboxyl-rich nanoprobes using PbS/CdS core-shell quantum dots with NIR IIb (1 500–1 700nm) emission as a substrate. The probes exhibited favorable imaging and penetration effects on bone tissues. Additionally, they can bind to macrophages and exert targeted effects on the bone marrow, which is helpful for imaging-guided fracture diagnosis [89]. Chen et al. designed a surface-modified upconversion nanoparticle probe material with multimodal imaging capabilities of fluorescence, MRI, and DECT. This probe can achieve the efficient and long-term labeling of stem cells and clear imaging of stem cell aggregation during bone defect repair presenting cell compatibility and no cytotoxicity. This probe can be considered as a non-invasive and real-time cell tracking imaging method for implementing visualized cell therapies in the treatment of bone regeneration related diseases [83]. Li et al. established a synergistic nano-library based on biologically active core-shell CaF_2 upconversion nanostructures. It can be regarded as a nanoprobe that can regulate metabolism through integrated mineral particles, and it also plays a role in accelerating and monitoring biomineralization. Besides, this probe can be employed to specifically recognize osteoblasts and synergistically promote bone defect regeneration [90]. Salgado et al. developed a novel functionalized hybrid semiconductor bioconjugate made from fluorescent quantum dots, which was coated with chitosan and chemically modified with O-phospho-L-serine. The nanoprobes can target and get aggregated on BMSCs to promote osteogenic differentiation of these cells and increase extracellular matrix mineralization. Therefore, they can play an important role in cell imaging and targeted intracellular material transport [91].

Given the above findings, nanoprobe technology also has favorable diagnostic and therapeutic effects on bone fractures and bone defects. The drugs or substances carried by nanoprobes can directly act on cells or activate BMSCs in the body to differentiate into osteoblasts through extracellular matrix activation. Besides, nanoprobes can also promote the integration of artificial materials with the defect site, thereby conducing to the healing of bone fractures and bone defects. Additionally, nanoprobes can also avoid the influence of internal plants on treatment decisions during the diagnosis and treatment process, which contributes to the recovery of limb functions (Fig. 7). The human skeleton can be categorized into different types, including long bones, short bones, flat bones, and irregular bones. Different bones are located in different positions and have different degrees of importance in the human body. Bone fractures and bone defects may occur in various bones, and screws and steel plates are currently the

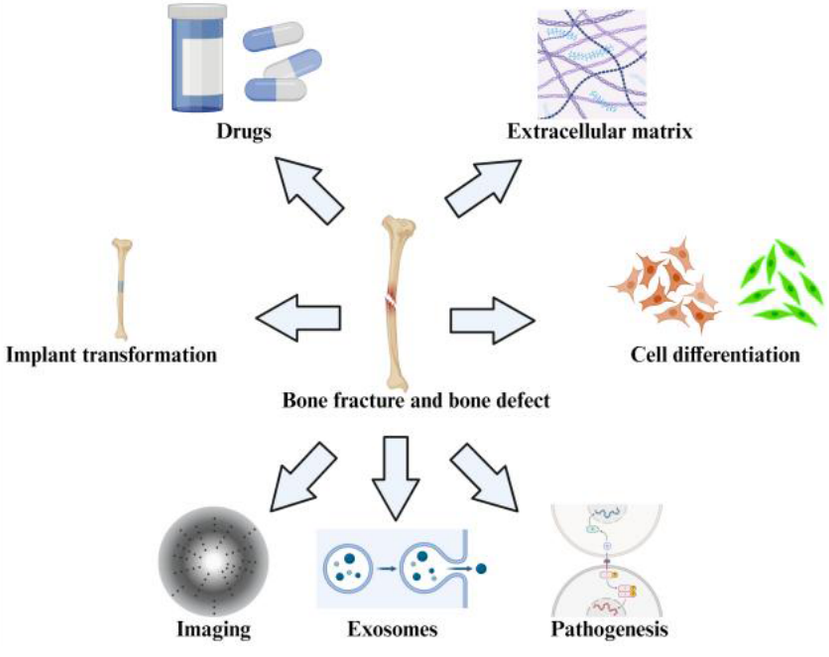

Fig. 7 The diagnostic and therapeutic effects of nanoprobe technology on bone fractures and defects. (Note: Created with BioRender.com)

main methods for the treatment of these diseases. With the development of nanoprobe technology, internal plant materials with favorable targeted memory, degradability, and biocompatibility may be endowed with the diagnostic and therapeutic effects of probes, thus contributing to the complete restoration of damaged limbs and less disability.

Conclusions and Prospect

As a novel microbial sensor capable of detecting individual living cells, the nanoprobe enables non-invasive, real-time, trace, accurate, and sensitive substance monitoring within living organisms. Nanoprobes are widely used in the medical field and assist in the diagnosis and treatment of diseases. By targeting specific cells, subcellular organelles, exosomes, extracellular matrix, disease microenvironment, and metabolic products such as diagnostic substances and inflammatory factors produced in the disease process, and by carrying drugs, novel proteins or peptides, and cytokines, combined with their photothermal, electromagnetic, ultrasound, and other excitation methods, nanoprobes can target orthopedic diseases in multiple dimensions, thus achieving an early and accurate diagnosis based on precise imaging of orthopedic diseases. Moreover, nanoprobes contribute to the precise treatment of orthopedic diseases at different stages, thereby benefiting patients with these diseases.

In future research, nanoprobe technology may be employed to investigate different risk factors and causes of orthopedic diseases and explore the pathogenesis of orthopedic diseases, thus contributing to the transformation of plant-based products in orthopedic diseases. Based on nanoprobes technology, we can achieve large-scale and precise screening of orthopedic diseases through commercial test kits. Then, based on the results of the screening, we can customize personalized and precise screening test kits for early and accurate diagnosis of patients' subtypes of orthopedic diseases, and treat patients based on the precise test kits. This is not only a feasible research direction in the field of nanoprobes, but also a feasible content for the transformation of its achievements.

Of course, we should also be aware that the current application of nanoprobes technology in orthopedics is still limited to basic research. The design of nanoprobes is relatively complex and the cost is relatively high, which makes it impossible to carry out large-scale commercial production. At the same time, there is a lack of standard operating procedures for the design and use of nanoprobes, which means that there is still a long way to go before they can be truly used in clinical practice. Finally, not all patients with orthopedic diseases are suitable for using nanoprobes for auxiliary diagnosis and treatment, and regulatory authorities should also introduce corresponding policies to avoid excessive medical applications in this field. But we believe that with the advancement and development of technology, these will not become obstacles to the clinical application of nanoprobes technology. Based on that, nanoprobe technology will truly move from the research field to clinical practice, thus reducing pain and limb deformities in orthopedic patients and enhancing human health.

CRediT Author Statement

Q. Yang and **F. Yu** designed the review. **Q. Yang** and **F. Yu** collected the references. **F. Yu** drew the figures. **Q. Yang** wrote the original manuscript. **F. Yu** revised the manuscript. **Q. Yang** and **F. Yu** provided the funding. Both authors contributed to the article and approved the submitted version. Graphical abstract was created with BioRender.com.

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Conflict of Interests

There is no conflict of interests.

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