

Biomaterials for orthopedic diagnostics and theranostics

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Abstract

Despite widespread use of conventional diagnostic methods in orthopedic applications, limitations still exist in detection and diagnosing many pathologies, especially at early stages when intervention is most critical. The use of biomaterials to develop diagnostics and theranostics, including nanoparticles and scaffolds for systemic or local applications, has significant promise to address these shortcomings and enable successful clinical translation. These developments in both modular and holistic design of diagnostic and theranostic biomaterials may improve patient treatments for myriad orthopedic applications ranging from cancer to fractures to infection.

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Clinical rationale for orthopedic diagnostics and theranostics

Orthopedic diseases and disorders are the second leading cause of disability worldwide and result in \$880

billion in direct health care costs annually in the United States alone [1,2]. As life expectancy increases, bone-related diseases and disorders have become increasingly prevalent. This trend will have significant impact on conditions such as osteoporosis, which affects >50% of Americans over the age of 50 [3].

Early and efficient detection and treatment of bone-related diseases and disorders is important to limit morbidity and mortality. For example, metastasis of aggressive cancers, such as osteosarcoma, severely impacts survival rates. Indeed, osteosarcoma survival rates fall from 70–80% to only 20–30% after metastatic progression [4]. Early diagnosis is critical for improving overall prognosis, as time to osteosarcoma diagnosis is correlated with greater rates of metastases and poorer prognosis [5,6]. Furthermore, early initiation of treatment can delay onset, number, and size of pulmonary metastases, increase the success of tumor resections, and improve overall survival [4,7,8]. In addition, bone metastases from lung, prostate, and breast cancer require early detection to circumvent severe complications such as pathologic fractures or spinal cord compression [9].

Orthopedic imaging plays a key role in current clinical diagnoses and disease progression and/or treatment monitoring. Modalities such as radiographs (x-rays), computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, and nuclear imaging studies, including scintigraphy, single-photon emission CT, and positron emission tomography (PET), are the mainstays of bone-related diagnostics. These techniques have distinct advantages and limitations, as detailed in Table 1. Specific to orthopedics, features of bone-related disorders can be nonspecific and overlap with other etiologies, making accurate diagnosis a challenge [10]. Surgical management of bone-related pathologies can lead to anatomic changes and often requires hardware, which can also significantly interfere with image quality, limiting treatment monitoring [10]. Moreover, traditional diagnostic techniques may have a limited ability to detect bone disorders at early stages when intervention is most efficacious at preventing or mitigating progression [10,11].

Given the limitations of traditional bone diagnostic techniques, there is a clear clinical rationale for

Table 1

Advantages and disadvantages of clinical diagnostic modalities^a.

Modality	Advantages	Disadvantages
Radiography (x-ray)	High spatial resolution	Uses ionizing radiation (minor exposure)
CT	Portability Less expensive Volumetric data with multiplanar/3D reconstructions High spatial resolution	Superimposition of structures Lower sensitivity Uses ionizing radiation (moderate exposure) Lower soft tissue contrast than MRI Less useful for soft tissue evaluation
MRI	Gives detailed information about complex fractures Few contraindications when compared with MRI High soft tissue contrast/spatial resolution Does not use ionizing radiation Better for evaluation of occult fractures, infections, articular cartilage, ligaments, and soft tissues	Expensive Longer scanning time Image artifact from metal surgical implants Contraindications (pacemakers, claustrophobia, etc.) Operator dependent
Ultrasound	Does not use ionizing radiation Less expensive Portability Ability for dynamic examinations and procedural guidance	Limited evaluation of deep structures Lower resolution
Nuclear medicine (scintigraphy, SPECT, PET)	Accessibility to functional information High sensitivity	Uses ionizing radiation/radiotracers Low specificity Low spatial resolution/anatomic localization Expensive

SPECT = single-photon emission computed tomography; 3D = three-dimensional.

^a Adapted from the study by Kumar and Hayashi (2016) [12].

developing advanced diagnostics and theranostics. In particular, biomaterials designed to target or localize to bone or disease sites are promising to increase the precision and prognostic value for cancer, osteoporosis, nonunions, fracture, and/or infection (Figure 1a). Furthermore, orthopedic theranostics may have even greater clinical impact. Multimodal imaging can be used to overcome the limitations of individual modalities, mediated through the use of multifunctional biomaterials. Herein, we discuss current advances and the promise in future biomaterial designs that can be used systemically or locally for diagnosing and treating bone-related disorders (Figure 1b and c).

Biomaterial design considerations for bone diagnostics and theranostics

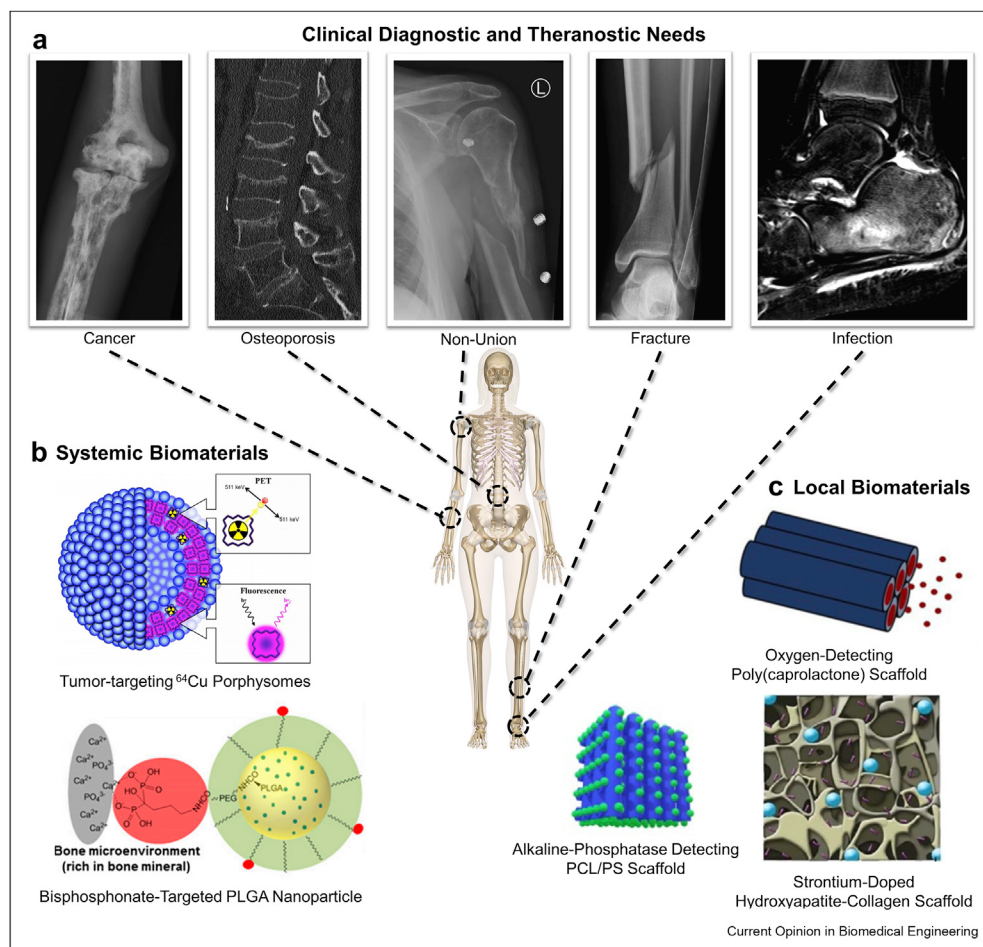
Biomaterials, including polymers [19,20], liposomes [13,21], gold nanoparticles (NPs) [22–24], mesoporous silica (MeSi) NPs [25–27], quantum dots (QDs) [28,29], upconversion (UC) NPs [30–32], hydroxyapatite [33–39], and superparamagnetic or ferromagnetic elements, nickel, iron (superparamagnetic iron oxide NPs [SPIONs]), cobalt, and their combinations [40–42], are commonly used for diagnostics and theranostics (Figure 2, Table 2). Indeed, a recent review discusses diagnostic and theranostic biomaterials development for bone tumors [43]. However, far fewer approaches exist for other orthopedic applications,

which have unique diagnostic and theranostic requirements including deep and dense tissue penetration, robust, tissue-specific accumulation, and confounding factors from implants or other treatments. Diagnosing osteoporosis, for example, requires methods that can accurately measure skeletal biomechanics and physiochemical properties (i.e. size, shape, structural properties), whereas diagnosing osteomyelitis may be challenged by orthopedic hardware and anatomical location [44]. These requirements are met through multifunctional biomaterials designed for both systemic administration and local applications that primarily enhance and extend the capabilities of existing orthopedic diagnostic techniques.

Systemically administered biomaterials for bone diagnostics and theranostics

Systemically administered biomaterials, often in the form of NPs, are commonly used for bone diagnostics/theranostics (Figure 2 and Table 2). NPs have many advantages as diagnostic/theranostic biomaterial platforms. NPs can be systemically administered noninvasively and, if properly designed, can achieve tissue specificity through targeting moieties [43]. NPs are inherently multifunctional and/or modular to enable tissue targeting/selectivity, enable sustained and/or responsive drug release, and protect sensitive drug payloads from degradation [45]. NP chemistry can also be altered to modulate circulation half-life, for example,

Figure 1



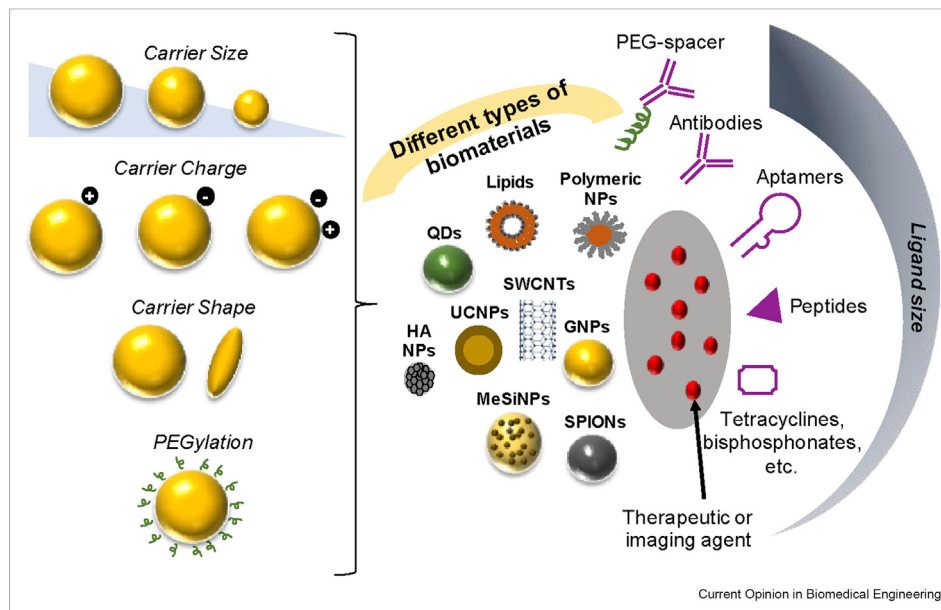
Overview of clinical needs for diagnostic/theranostic biomaterials and example biomaterials. (a) Representative clinical images of orthopedic applications. (b, c) Systemic and local biomaterials highlighted in this review. PLGA: Poly(lactic acid-co-glycolic acid); PCL/PS: Poly(caprolactone)/calcium silicate. Porphysome is reproduced from Ref. [13] (<https://pubs.acs.org/doi/10.1021/nn400669r>) with permission. Further permission related to the material excerpted should be directed to the ACS. BP-targeted PLGA nanoparticles reprinted with permission from Ref. [14]: Low SA, Galliford CV, Yang J, Low PS, Kopeček J. Biodistribution of Fracture-Targeted GSK3 β Inhibitor-Loaded Micelles for Improved Fracture Healing. *Biomacromolecules*. 2015; 16(10):3145–53. <https://doi.org/10.1021/acs.biomac.5b00777>. Copyright 2015 American Chemical Society. Alkaline phosphatase detecting PCL/PS scaffold originally published in Ref. [15] reprinted from *Chemical Engineering Journal*, 408, Yang C, Gao X, Younis MR, Blum NT, Lei S, Zhang D, Luo Y, Huang P, J Lin J, Non-invasive monitoring of *in vivo* bone regeneration based on alkaline phosphatase-responsive scaffolds, 127959, Copyright 2021, with permission from Elsevier under CC BY-NC 4.0 license #5036610232181. Oxygen-detecting poly(caprolactone) scaffolds reprinted with permission from Ref. [16]: Schilling K, El Khatib M, Plunkett S, Xue J, Xia Y, Vinogradov SA, Brown E, Zhang X. Electrospun Fiber Mesh for High-Resolution Measurements of Oxygen Tension in Cranial Bone Defect Repair. *ACS Appl Mater Interfaces*. 2019 Sep 18; 11(37):33548–33558. Copyright 2019 American Chemical Society. The strontium-doped hydroxyapatite-collagen scaffold image [17] is reproduced under CC BY-NC 4.0 license #5036610940442. The center skeleton is from Ref. [18]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

through poly(ethylene glycol) functionalization [46,47]. NPs can be developed to deliver synergistic, multidrug combinations of myriad drug cargos (hydrophobic or hydrophilic drugs/contrast agents, nucleic acids, etc.) and/or to localize heating or radiation in conjunction with multimodal imaging [45]. In addition, some NP compositions, namely metallic NPs, are inherently magnetic or fluorescent for imaging [48].

Facile incorporation of bone targeting, either through passive or active mechanisms, is another advantage of

NPs that can lead to the specificity and contrast necessitated by bone diagnostics/theranostics (Figure 2). Indeed, systemically delivered small molecule biodistribution to bone is poor with <1% of doses successfully reaching bone [14]. Tissue specificity arises from the incorporation of targeting moieties and/or enhanced permeability and retention due to tumor growth, infection, or injury [58–61]. For example, first-generation small-molecule radiotracer-based bone diagnostics were developed based on molecules with bone affinity including ^{18}F -NaF,

Figure 2



Schematic representation of systemically delivered NP-based biomaterials used in diagnostics and theranostics for orthopedic applications detailing the features that can be modulated. SWCNT, single-walled carbon nanotube; GNPs, gold nanoparticles; HA NPs, hydroxyapatite nanoparticles. The figure was adapted from Ref. [49]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

^{99m}Tc -methylene diphosphonate, and ^{99m}Tc -hydroxymethylene diphosphonate [62–66]. Similarly, facile incorporation of bone-targeting moieties is a key asset of NPs to provide specificity and contrast necessitated by bone diagnostics/theranostics (Figure 2). For bone targeting of NPs, bisphosphonates (BPs) are commonly used. BPs bind generally to bone tissue because of high affinity for hydroxyapatite [67,68] and also to regions of high metabolic activity, such as primary bone tumors (e.g. osteosarcoma) or metastases [69,70]. Other targeting groups have been explored, including phytic acid, aspartic acid, and glutamic acid and (co)polypeptides thereof, tetracycline, as well as aptamers and other peptides with affinity to the bone matrix or relevant cells, as recently reviewed [43].

Macroscale biomaterials for diagnostics and theranostics

Macroscale or bulk biomaterials placed locally within or juxtaposed to bone during surgical procedures also have significant value for diagnostics and theranostics. These include tissue engineering scaffolds, bone fixation devices or implants, and drug reservoirs using a variety of biomaterials including poly(caprolactone), poly(lactic acid), poly(glycolic acid), poly(methyl methacrylate), poly(butyl terephthalate), poly(carbonate), hydroxyapatite, calcium phosphate, and calcium silicate [71,72]. The general design principles and biomaterials used for implantable orthopedic devices can be found in recent

reviews [71–73]. Common orthopedic biomaterials are adapted for diagnostic/theranostic applications through the incorporation of contrast agents and responsive moieties, which enable sensing of the local microenvironment, therapeutic efficacy, or the rate and/or extent of degradation of scaffolds. Because of localized placement, macroscale biomaterials are subject to different delivery constraints (e.g. surgical placement or injectable localization) compared with systemically administered biomaterials, which can lessen the risk for off-target effects and background and may also enable use of more varied materials with greater quantities/varieties of detection moieties. Diagnostic capabilities are often used in biomaterial development in the preclinical stage to better understand *in vivo* interactions and provide examples of potential future clinical applications.

Biomaterial diagnostics and theranostics in development for orthopedic applications

The goal of biomaterial-based diagnostics is to improve existing detection modalities, either through enhanced contrast or increased pathological specificity. The examples of diagnostic and theranostic biomaterials developed for both systemic and local orthopedic applications since the year 2010 are highlighted in Table 3, with selected examples discussed in subsequent sections.

Systemic diagnostics and theranostics

Several examples of orthopedic diagnostics and theranostics in development involve systemic delivery of

Table 2

Advantages and limitations of existing and emerging biomaterial diagnostics and theranostics [50–57].

Biomaterial	Advantages	Limitations
Polymers	Flexible compositions, functionalities, and morphologies to enable drug/tracer incorporation and targeting	Translation can be challenging because of difficult scale-up of manufacturing processes
Lipid-based NPs	Tunable size, shape, responsive behavior, and degradation	No inherent diagnostic capabilities
Gold NPs	Flexible compositions, functionalities, and morphologies to enable drug/tracer incorporation and targeting	No inherent diagnostic capabilities
	Favorable photostability	Limited surface area for drug/tracer, targeting group, and stabilizing molecule incorporation
	Tunable absorptive/scattering properties based on size and shape	Rapid clearance
	Excellent biocompatibility	Toxicity
	Simple conjugation of targeting groups and/or therapeutic molecules via thiol chemistry	
Superparamagnetic iron oxide (SPIONs)	Biocompatible	Dose accumulation over time leading to toxicity
	Exhibit <i>in vivo</i> biodistribution tunability based on size and surface modifications	
	Detectable via MRI, which enables robust resolution	
	Induction of localized heating in magnetic fields	
Upconversion nanoparticles (UCNPs)	Absorb multiple photons to produce high energy anti-Stokes luminescence	Non-negligible heating of exposed tissues
	Minimal tissue autofluorescence	Low brightness relative to other NIR fluorescent probes
	Enable multiplexed imaging	
	Highly resistant to photobleaching	
Quantum dots	High quantum yield	Sometimes formed from cytotoxic elements
	Photostable	Rapid clearance
	High signal-to-noise ratio	
	Simultaneous excitation of multiple wavelengths	
Hydroxyapatite	Biocompatible	Rapid clearance
	Bioactive	
	Osteoconductive	
	Excellent drug loading capabilities	
Mesoporous silica NPs	High cargo loading capacity, controllable release	Cargo leakage
	Excellent chemical, thermal, and mechanical stability	Biocompatibility and toxicity
	Tunable size and porosity	
	Flexible morphology	

biomaterials (Table 3). Systemic diagnostics and theranostics are defined here as technologies injected intravenously, subcutaneously, intradermally, or otherwise. One critical hurdle for systemically delivered diagnostics/theranostics is tissue specificity to ensure high levels of contrast between dysfunctional and healthy tissues for reliable disease detection. Despite this hurdle, a variety of designs developed in the absence of targeting ligands have resulted in dramatic improvements in traditional diagnostics. For example, porphyrin–lipid conjugates combined with small fractions of radioactive ^{64}Cu were used to detect metastatic bone lesions via porphyrin fluorescence and ^{64}Cu PET/CT imaging in a prostate cancer animal model [13]. While traditional PET scans are limited to detection of metastatic lesions larger than 1 cm, the porphosome technology improved sensitivity to 2 mm. Porphosomes also accumulated at naïve bone, suggesting inherent bone diagnostic capabilities, which may be useful for adapting the technology to other orthopedic applications (e.g. bone mineral density, stress fracture detection). In another example, PEGylated mesoporous silica

NPs (MeSiNPs) were used to deliver ammonia borate, which releases H_2 in acidic environments, resulting in negative CT contrast [33]. While a clinical contrast agent was unable to differentiate between osteosarcoma and healthy bone in a rat model, intravenous injection of MeSiNPs enabled 20x greater contrast on CT [33], therefore highlighting the utility of MeSiNP–ammonia borate diagnostics.

To achieve the goal of high tissue specificity and diagnostic or theranostic sensitivity, bone-targeted biomaterials have been developed based on existing clinical diagnostic modalities. For example, gold NPs functionalized with bone-targeting glutamic acid were developed to noninvasively detect bone microdamage, which is indicative of impending bone fracture but is undetectable using x-ray [81]. *Ex vivo* x-ray revealed preferential binding to damaged bone owing to glutamic acid binding to exposed calcium versus healthy bone tissue, suggesting the utility of gold NPs for bone diagnostics and theranostics. For cell targeting, HER2 antibody–conjugated PEGylated polymersomes loaded

Table 3

Examples of biomaterials explored for bone diagnostics and theranostics from 2010 to 2021.

Biomaterial type	Diagnostic/therapeutic entity	Surface modification	Rationale for use in bone applications	Clinical application	Diagnostic modality*	Ref
SPIONs	Iron oxide NPs	1,5-dihydroxy-1,5,5-tris-phosphono-pentyl-phosphonic acid (di-HMBPs)	High affinity for calcium ions/hydroxyapatite	Osteoporosis	MRI	[74]
	Iron oxide NPs	Alendronate	High affinity for hydroxyapatite	Bone metabolic activity	MRI	[75]
	^{99m} Tc-labeled iron oxide NPs	Alendronate	High affinity for hydroxyapatite	Bone	SPECT/PET-MRI	[76,77]
	^{99m} Tc-labeled PEGylated iron oxide NPs	Bisphosphonate	High affinity for hydroxyapatite	Bone	SPECT/PET-MRI	[77]
	Iron-doped hydroxyapatite NPs	N/A	Bone substitute	Bone	SPECT/PET MRI	[34]
Carbon nanotubes	^{99m} Tc-labeled carbon nanotubes	Alendronate	High affinity for hydroxyapatite	Active bone metabolism	Photoacoustic imaging	[78]
Quantum dots	Quantum dots	Various antibodies	Binding to unique cell populations in bone marrow	Targeted cell imaging	NIR	[79]
Gold NPs	Ag ₂ S QD/doxorubicin	Alendronate	High affinity for hydroxyapatite	Bone tumors	NIR	[80]
	Gold NPs	Glutamic acid	Targets microcracks by chelating with calcium ions	Damaged bone	CT	[81]
	Gelatin methacrylate–gold NP scaffold	N/A	N/A	Bone defects	CT	[20]
Mesoporous silica NPs	Gold nanorods	Tumor-specific oligopeptides	Binding to osteosarcoma cells	Osteosarcoma	Photoacoustic imaging	[82]
	Mesoporous silica NPs loaded with ammonia borate	N/A	Nanocomposites respond to the acidic environment of the tumor to release H ₂ , improving contrast between osteosarcoma and surrounding healthy bone	Osteosarcoma	CT	[33]
	Mesoporous silica NPs/gold nanorods	Zoledronic acid	High affinity for hydroxyapatite	Bone metastasis	Photoacoustic imaging	[83]
	Mesoporous silica NPs containing an iron oxide core immobilized with BMP-2, coated with calcium phosphate/bone morphogenic protein 2 (BMP-2)	N/A	Resemblance to native bone	Critical sized defects	MRI	[84]
	Mesoporous silica-coated bismuth sulfide NPs/ doxorubicin	RGD-peptide	Target tumor vasculature and tumor cells	Osteosarcoma	NIR/CT	[85]
Hydroxyapatite	Silicate-substituted HAp doped with Eu(III) and Bi(III) ⁺	N/A	Bone substitute	Bone	Luminescence	[39]
	Calcium-deficient hydroxyapatite scaffold labeled with the NIR probe	N/A	Bone substitute	Monitor bone healing	NIR	[86]
	Hydroxyapatite NPs/ Ho-166	N/A	High affinity to bone	Bone cancer	Unclear	[38]
	Folic acid–modified hydroxyapatite NPs/ ⁶⁴ Cu	Medronic acid (MDP)	High affinity to areas of active bone metabolism	Osteosarcomas and other bone disorders	PET	[36]
	Nano-hydroxyapatite rods/molybdenum oxide	N/A	Bone substitute	Bone infection	Fluorescence	[37]

Table 3 (continued)

Biomaterial type	Diagnostic/therapeutic entity	Surface modification	Rationale for use in bone applications	Clinical application	Diagnostic modality*	Ref
Upconversion nanoparticles (UCNPs)	Eu(III)/Gd(III)-doped hydroxyapatite nanorods/ibuprofen (as a model drug)	N/A	Bone substitute	Bone	Luminescence/MRI/CT	[35]
	Iron-doped hydroxyapatite alginate–gelatin scaffold/ <i>bone morphogenic protein 2 (BMP-2)</i>	N/A	Osteoconductive properties of scaffold	Localized monitoring of cell infiltration in bone	MRI	[87]
	¹⁸ F labeled NaGdF ₄ : Yb, Er UCNPs	Etidronic acid, alendronic acid, and nitrile (trimethylphosphonic acid)	High affinity for hydroxyapatite	Bone	MRI/PET	[88]
	Yb(III)/Ho(III)-doped UCNPs	N/A	The apatite component of the UCNP mimics bone	Bone regeneration	Luminescence	[30]
	NaYF ₄ :Yb ³⁺ , Er ³⁺ UCNPs	Positive and negative charged polymers	Use for tracking without affecting intrinsic cell properties	Cell localization	Fluorescence	[31]
	NaYbF ₄ :Gd ³⁺ /Er ³⁺ UCNPs	Iminodiacetate	Chelates exposed calcium ions	Damaged bone	Gemstone spectral CT	[89]
	Mesoporous silica-coated upconversion NPs doped with gadolinium (III)/plumbagin and poly (acrylic acid)	Zoledronic acid	High affinity for hydroxyapatite	Bone metastasis	MRI/luminescence	[32]
	Electrospun polycaprolactone fibers encapsulating PtP–C343 (phosphorescent probe)	N/A	Electrospun fibers mimic ECM	Bone healing	Two-photon phosphorescence lifetime microscopy	[16]
	PLGA-PEG loaded with iron oxide NPs and NIR dye	Alendronate	High affinity for hydroxyapatite	Bone	MRI/NIR	[90]
	Fluorescein isothiocyanate–labeled PEGylated poly (γ-benzyl-L-glutamate) (PBLG)	Alendronate	High affinity for hydroxyapatite	Bone	Fluorescence	[91]
Polymer-based systems	Poly(trimethylene carbonate)-b-poly(glutamic acid) loaded with SPIONS	HER2 antibody	Tumor targeting	Bone metastasis	MRI	[19]
	^{99m} Tc-labeled polymers	N/A	Binds to bone surface	Bone metastasis	PET	[92]
	SPION coated with chitosan-PEG copolymer	HER2 antibody	Binds to metastatic tumor cells	Bone metastasis	MRI	[19]
	Polycaprolactone/calcium silicate, (PCL/CS) scaffold labeled with hemicyanine dye	N/A	Alkaline phosphatase (ALP)–responsive dye	Monitor bone healing	NIR/photoacoustic imaging	[15]
	Poly(lactide-co-glycolide) (PLGA) loaded with SPIONS/paclitaxel	Alendronate	High affinity for hydroxyapatite	Bone tumors	MRI	[93]
	HAp composite PLGA scaffold/SPIONS	N/A	Bone substitute	Bone defect	X-ray	[94]
		Alendronate	High affinity for hydroxyapatite		MRI	[95]
						(continued on next page)

Table 3 (continued)

Biomaterial type	Diagnostic/therapeutic entity	Surface modification	Rationale for use in bone applications	Clinical application	Diagnostic modality*	Ref
Lipid-based systems	Iron-doped polydopamine NPs/7-ethyl-10-hydroxycamptothecin (SN38)			Bone tumor/osteolysis		
	Chitosan-grafted PEG HER2/neu copolymer with SPION core		Tumor targeting	Bone metastasis	MRI	[96]
	⁶⁴ Cu-porphysomes	N/A	Binds to metastatic tumor cells	Bone metastasis	PET/fluorescence	[13]
	PEGylated liposomes loaded with CdSe QDs and iron oxide NPs	cRGDyk peptide	Binds to metastatic tumor cells	Bone metastasis	MRI/fluorescence	[21]

Additional examples pertaining to bone tumor diagnostics and theranostics can be found in the following review [43].

HAp = hydroxyapatite; ECM = extracellular matrix; HER2 = human epidermal growth factor receptor 2; PLGA = poly(lactic acid-co-glycolic acid); PEG = poly(ethylene glycol); Yb = ytterbium; Ho = holmium; Gd = gadolinium; Er = erbium; Eu = europium; Bi = bismuth; RGD = arginine–glycine–aspartic acid; SPECT = single-photon emission computed tomography.

* denotes imaging modality used in the article but is not an exhaustive list of the potential diagnostic applications.

with SPIONs were used to detect tumor boundaries in bone metastasis compared with untargeted controls in a breast cancer metastasis animal model [19]. Following the idea of tissue targeting, theranostic alendronate-functionalized iron-doped poly(dopamine) NPs were used to image tumor shrinkage via MRI [95]. Compared with untargeted controls, the NPs suppressed tumor growth and reduced osteolytic bone damage in an orthotopic bone tumor model because of combined chemo-photothermal therapy of iron and the therapeutic efficacy of the loaded drug, 7-ethyl-10-hydroxycamptothecin [95]. BP-functionalized, PEGylated ^{99m}Tc-SPIONs exhibited enhanced accumulation at bone compared with untargeted controls [77] and leveraged multimodality to overcome SPION sensitivity issues, enabling successful longitudinal monitoring in animal studies. In the absence of the radiolabel, improved BP–poly(ethylene glycol) (PEG)–SPION sensitivity was attributed to the small NP size (~20 nm), hydrophilicity of BP and PEG that allows for proton relaxation at the iron oxide NP surface, and the presence of the PEG coating, which prevented aggregation. This approach may be useful to enhance diagnostic capabilities of MRI for detecting osteoporotic bone and bone metabolic activity.

Emerging technologies such as photoacoustic (PA) and near-infrared (NIR) imaging combined with biomaterial diagnostics and theranostics present unique opportunities for orthopedic applications. PA imaging can reach tissue depths up to 5–6 cm with outstanding spatial resolution of ~5 μm. Improved capabilities of PA imaging were exploited using PEGylated gold nanorods

targeted with osteosarcoma-specific peptides to visualize neovascularization with high contrast via PA imaging, enabling differentiation between tumor and healthy tissue [23]. To diagnose and treat bone metastasis in an animal model of breast cancer, gold nanorods were incorporated within MeSiNPs functionalized with zoledronic acid and imaged photoacoustically [83]. MicroCT revealed tumor size, and osteolysis was reduced compared with untreated controls. NIR imaging allows for deep tissue penetration with minimal tissue autofluorescence. To diagnose and treat bone metastases, zoledronic acid–targeted MeSi-coated UCNP doped with gadolinium were injected in a bone metastatic breast cancer model [32]. UC luminescence and NIR imaging revealed that loaded UCNP reduced tumor size compared with empty UCNP controls. The theranostic effects of UCNP were further verified using microCT, which revealed reduced osteolysis [32]. Similarly, QDs have been incorporated within cell-targeted NPs to characterize the heterogeneous bone marrow cellular repertoire [79]. To achieve dual modality, arginine–glycine–aspartic acid–functionalized liposomes co-loaded with iron oxide and CdSe QDs were developed [21]. In a prostate cancer bone metastasis model, QD MRI signal was 1.5-fold higher than iron oxide NPs alone and tumor fluorescence was higher than untargeted liposomes. QDs have also been used to detect prostate cancer–related delta/notch-like epidermal growth factor–related receptor expression, which has prognostic value for bone metastases [97]. Core–shell silica NPs (C dots) are emerging as alternative materials to QDs [98]. This class of biomaterials covalently integrates organic fluorophores into the core

of core–shell silica NPs, resulting in enhanced brightness ($\sim 2\text{--}3\times$ vs. QDs) and photostability. For example, C dots were recently used to label cancer cells to study early-stage bone metastasis [99].

Local diagnostics and theranostics

Implantable biomaterials are adapted to local diagnostics or theranostics through the addition of therapeutics or contrast agents (Figure 1). There are several recent examples of bone diagnostics incorporated within traditional tissue engineering scaffolds. Electrospun poly(caprolactone) scaffolds encapsulating porphyrin-based sensors for oxygen tension, which is correlated with healing, have been used to monitor bone regeneration using two-photon microscopy [16]. A limitation of this approach is that sensitivity may decrease in humans in which scaffold placement will be deeper versus murine models. In an alternative system, MRI was used to image gadolinium-doped hydroxyapatite (HA) NPs incorporated into electrospun poly(caprolactone) scaffolds to track *in vitro* bone regeneration [100] as well as nano-hydroxyapatite–alginate–gelatin scaffolds incorporating SPIONs for detection of cellular infiltration and scaffold mineralization [87]. Bone morphogenetic protein 2 was immobilized within iron oxide core mesoporous silica beads and incorporated within calcium phosphate cement, enhancing contrast and improving bone regeneration for up to 8 weeks after theranostic implantation [84]. Finally, gold NPs entrapped within gelatin methacrylate hydrogels were developed to enable imaging during the process of bone regeneration within a condyle defect [20], enabling greater resolution of bone microarchitecture than gelatin methacrylate hydrogels alone. The ability to assess bone regeneration and/or integration using engineered scaffolds can improve monitoring and enable earlier detection of treatment failures.

Future directions

Biomaterial-based diagnostics and theranostics have great promise for orthopedic applications. However, significant opportunities and challenges remain. Refinement of biomaterials designed for diagnostics and theranostics is necessary to meet the demands of orthopedic applications. In general, conventional theranostic and diagnostic biomaterials are combinations of existing technologies. This modular approach that integrates therapeutic, diagnostic, and targeting moieties enables a high degree of tunability in the resulting diagnostic/theranostic at the cost of complexity. This principle also applies to diagnostic agents embedded within biomaterials for local applications, which allows independent tuning of the scaffold properties and detection modalities. Complementary to this trend of

isolating functionalities is that of more holistic design tailored to orthopedic diagnostics/theranostics. As opposed to adapting existing formulations for additional diagnostic/theranostic functionality, inherently multifunctional biomaterials allow highly integrated designs with fewer components that may ultimately allow for greater reproducibility in design, manufacturing, and testing. An example of holistic design is ^{64}Cu -porphyrin technology, where the biomaterial complexes with Cu and is also fluorescent. Not only will holistic design likely provide greater efficacy but may also streamline Food and Drug Administration approval because of its simplicity.

Several difficult to detect orthopedic conditions are well suited for diagnosis and treatment with high-contrast and high-specificity NPs, including tumors, osteomyelitis, and osteoporosis. Nevertheless, myriad challenges remain for systemically administered biomaterials including NPs. For example, bone possesses unique barriers to NP delivery including limited vascularization, large volume, and high density. Bone can also have disease/disorder-specific challenges to delivery, including sub-micrometer canaliculi, which can serve as a reservoir for bacteria [101,102]. More generally, NP protein adsorption reduces targeting efficacy and shifts biodistribution to the reticuloendothelial system, which contributes to poor bone accumulation [70]. Improvements in surface chemistry to modulate protein adsorption and maintain targeting specificity, which have recently been reviewed elsewhere [103–105], are critical to improve diagnostic/theranostic resolution as well as safety and efficacy.

For local applications, the addition of diagnostic or theranostic capabilities to conventional orthopedic implants/treatments also has great potential benefit. In particular, augmenting local biomaterials or implants would enable rapid intervention and/or minimize the need for additional invasive procedures. Incorporating sensing functionalities within surgical implants may enable early detection of complications such as poor graft integration, infection, nonunion, or implant loosening. Vascularization and host cell infiltration are important metrics for successful tissue integration/regeneration [106,107] and valuable to inform clinical decision-making regarding need for revision surgery. However, current clinical diagnostics lack resolution to enable imaging of microvessels and cells *in vivo* [108,109]. Similarly, implant-associated bacterial infections are difficult to detect because of limited specificity of existing diagnostic modalities versus normal postsurgical inflammation [110]. Incorporating bacteria-responsive materials within

implants or antibiotic poly(methyl methacrylate) (PMMA) beads could allow for enhanced infection detection and treatment postoperatively [111]. Although these technologies have not yet been developed, locally delivered theranostics represent an important area of exploration in the future.

Diagnostic requirements differ between research and clinical applications. Many of the biomaterials described here have fluorescent detection modalities, which provide unique diagnostic information including bacterial detection [37] and metabolite or protein sensing [16,86]. Although efficacious for research purposes, fluorescence is not a standard clinical detection modality and is limited by tissue penetration. Rather, x-rays/CT, magnetic imaging, and ultrasound are clinical mainstays that can be augmented to improve contrast and specificity for orthopedic applications through development of diagnostic and theranostic biomaterials.

Clinical translation of the orthopedic diagnostic and theranostic biomaterials discussed here will be challenging. NP diagnostic agents have been used clinically for other applications and provide a road map for orthopedic applications [112]. However, the newness (>95% of publications to date with 'theranostics' as a keyword in Web of Science are from 2012 or later) and complexity of theranostics make their path to regulatory approval unclear. Early theranostics relied on relatively simple radioactive payloads and chelating agents and do not provide good models for the translation of more complex biomaterial approaches necessitated by orthopedic applications. Nevertheless, for successful translation of orthopedic diagnostics or theranostics, demonstration of safety and efficacy in large animal models is critical because of the difficulties associated with imaging in bone due to depth, complexity, and size. As the biomaterial field continues to grow, the goal is to integrate the advantages offered through the systems described herein to advance orthopedic diagnostics and theranostics and ultimately improve patient quality of life.

Conclusions

Early diagnosis and treatment of orthopedic pathologies is important to treat diseases and injuries effectively and limit undue morbidity. However, traditional diagnostic modalities are subject to limitations, prompting the need for advances in orthopedic diagnostic and theranostic biomaterials. Bone presents unique challenges for diagnostics and theranostics compared with other tissues because of its unique composition and limited accessibility. Several promising biomaterial strategies have emerged for bone diagnostic and theranostic applications, although work in this area is still relatively new. Although current clinically approved applications are limited, the rapid

development and expansion of diagnostic and therapeutic modalities within biomaterials offers tremendous promise for clinical use.

Author contributions

All authors contributed to manuscript planning, writing, editing, and revision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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