Universidade de Lisboa

Faculdade de Farmácia

Nanodelivery strategies for bone targeting

Bone pathologies and nanoparticle treatment approaches

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Monografia orientada pela Professora Doutora Ana Francisca Bettencourt, Categoria: Professora Auxiliar com Agregação

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Resumo

A esperança média de vida teve um aumento de mais de 6 anos entre 2000 e 2019, passando de 66.8 anos em 2000 para 73.4 em 2019. Porém, a esperança de vida saudável não acompanhou este aumento de forma proporcional, tendo o impacto e incidência das doenças e distúrbios ósseos aumentado, o que constitui um problema para os doentes e para a saúde pública, visto que as suas eventuais repercussões, como o progressivo agravamento da saúde física e mental, têm um grande impacto na qualidade de vida dos doentes e afetam negativamente os recursos dos sistemas de saúde, levando à morte em casos mais graves.

As alterações nos tratamentos têm resultado num aumento da sobrevivência dos doentes, porém estas estratégias terapêuticas têm ainda pouca eficácia, fracos *outcomes* e muitos efeitos adversos, o que leva a elevada morbilidade e mortalidade.

A nanomedicina tornou-se uma realidade nos últimos anos. Nos últimos 20 anos, cerca de 80 formulações farmacêuticas da área da nanomedicina foram aprovadas, pela Food and Drug Administration (FDA) e pela Agência Europeia do Medicamento (EMA), para serem usadas em diversas áreas terapêuticas, tendo a maioria sido aprovada para as áreas de oncologia e hematologia.

O aumento da necessidade de desenvolver novas terapêuticas, eficientes e inovadoras, para tratar distúrbios ósseos, levou os investigadores a focarem-se nas terapêuticas de nanomedicina e na investigação de nanopartículas (NPs) que pudessem cumprir este objetivo.

Estes sistemas têm sido explorados nos últimos anos, devido à sua capacidade de transportarem diversas moléculas terapêuticas, de serem administrados por várias vias, de terem uma taxa de libertação controlada e de serem direcionados para alvos terapêuticos específicos que muitas vezes são difíceis de alcançar, como o osso.

Esta monografia apresenta uma revisão de alguns estudos recentes e do progresso, dos desafios e das expectativas associadas às terapêuticas de NPs, no que toca ao tratamento de doenças relacionadas com o osso, como as inflamações ósseas (osteomielite), as doenças degenerativas (osteoporose), as doenças genéticas (osteogenesis imperfecta), os distúrbios traumáticos (fraturas) e as doenças tumorais

ósseas, incluindo tumores ósseos primários, como o osteossarcoma e o sarcoma de Ewing, e também tumores ósseos metastáticos.

Palavras-chave: Nanomedicamentos; Nanopartículas; Distúrbios ósseos; Doenças ósseas; entrega in situ;

Abstract

The global mean life expectancy has increased by more than 6 years between 2000 and 2019, going from 66.8 years old in 2000 to 73.4 in 2019. However, healthy life expectancy has not proportionally accompanied this increase and the impact and incidence of bone injuries and diseases has risen, posing a problem for patients and public health since their potential repercussions, such as disability and downward spiral in physical and mental health, have a major impact on patients' quality of life and affect the resources of health systems, leading to death in more severe cases.

Although changes in treatment have improved patients' survival, these therapeutic approaches still have poor efficacy and outcome and various side effects, which lead to high morbidity and mortality.

Nanomedicine became a reality in recent years. Over the last 20 years, around 80 pharmaceutical nanomedicine formulations have been approved for use in various therapeutic areas by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), most of them for the oncology and hematology areas.

The increasing need for the development of effective and innovative therapies to treat bone diseases has led researchers to turn to nanomedicine approaches and the research of nanoparticles (NPs) that could fulfill this purpose.

These systems have been explored in recent years due to their ability to carry various therapeutic molecules, to be administered through different routes, to modulate release rate and to specifically target disease sites that are difficult to reach, like bone tissues.

This review presents a summary of recent studies and the progress, the challenges and the expectations associated with nanoparticle approaches as a treatment of skeletal-related disorders such as bone inflammation (osteomyelitis), degenerative disorders (osteoporosis), genetic disorders (osteogenesis imperfecta), traumatic disorders (fractures) and bone tumor diseases, which include primary bone tumors, such as osteosarcoma and Ewing's sarcoma, and metastatic bone tumors.

Keywords: Nanomedicine; nanoparticles; Bone disorders; bone diseases; in situ delivery;

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Abbreviations

AlPcS4@FNPs	Tetra-sulfonated aluminum phthalocyanine to poly-methyl methacrylate core-shell fluorescent NPs
Ag	Silver
ALN	Alendronate
ALP	Alkaline phosphatase
APRF	Advanced PRF formulation
ATRA	All-trans retinoic acid
Au	Gold
BMP	Bone morphogenic protein
BP	Bisphosphonate
BSA	Bovine serum albumin
BTZ	Bortezomib
Ca	Calcium
Ca-RISNPs	Calcium-BP risedronate NPs
CC	Core-cone
CeO2	Cerium oxide
Cis	Cisplatin
Cis@Fe3O4@MSNs	Cisplatin was loaded into MSNs
CS	Carboxylate chitosan
CT	Computed tomography
CTC	Circulating tumor cell
Cu	Copper
CXCR4 C-X-C	C-X-C motif chemokine receptor 4
DOX	Doxorubicin
DTX@Cap/HP	Calcium phosphate-polymer hybrid NP
ECM	Extracellular matrix
EGCG	Epigallocatechin gallate
EwS	Ewing's Sarcoma

GA	Gallium
GP	Glycerol Phosphate
GSP-2	Charged sulfated polygalactan
EMA	European Medicines Agency
HA	Hydroxyapatite
IP	Ipriflavone
MBGs	Mesoporous Bioactive Glasses
MIC	Minimum inhibitory concentration
MiRNA	MicroRNA
mPEG-PCL	Methoxy polyethylene glycol-poly(caprolactone) block polymers
MSCs	Mesenchymal stem cells
MS	Mesoporous silica
MSNs	Mesoporous silica nanoparticles
nHA	Nano-hydroxyapatite
NanoMBGs	MS-CaO nanospheres
NanoTLZ	Nanoformulation of talazoparib
NF	Nuclear Factor
NFATc1	NF of activated T-cells cytoplasmic 1
NPs	Nanoparticles
OB	Osteoblasts
OC	Osteoclast
OI	Osteogenesis imperfecta
OM	Osteomyelitis
OS	Osteosarcoma
PAA	Polyacrylic acid
PAMAM	Polyamindoamine
PARP	Poly-ADP ribose polymerase
PCL	Polycaprolactone
pDNA	Plasmid-DNA
PEI	Polyethyleneimine
PLGA	Poly(lactide‑co‑glycolide)
PRF	Platelet-rich fibrin
PSC	Polyglucose-sorbitol-carboxymethyl ether
PTH	Parathyroid Hormone

PTX	Paclitaxel
PTX-PLGA@[143B-RAW] NPs	PTX loaded PLGA NPs coated with 143B-RAW hybrid membrane
RANK	Receptor activator of nuclear factor kappa
RANKL	Receptor activator of nuclear factor kappa ligand
rBMSCs	rat marrow MSCs
RGD	tripeptide arginine–glycine–aspartic acid
ROS	Reactive oxygen species
SA	Sinapic acid
SCT	Salmon calcitonin
SDF1	Stromal cell-derived factor 1
siRNA	small interfering RNA
TMZ	Temozolomide
TRAP	Tartrate-resistant acid phosphatase
Zn	Zinc
ZOL	Zoledronic acid

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1. Introduction

Nanoparticles (NPs) are nanosystems with a size between 1 and 100 nm that can be used as therapy for a variety of disorders by themselves or in combination with different micro and macromolecules, including drugs. (1,2)

These carriers can establish stable interactions with different ligands which gives them the ability to target a specific tissue or cell line, controlling the delivery of loaded molecules. (2)

In addition to being able to effectively carry molecules of interest, being stable enough to pass through the biological barriers and being able to act on a specific target, the ideal NP should also be nonimmunogenic and biodegradable.(3)

Taking into account these properties, several formulations have reached different stages of clinical trials and some, like the SARS-COV-2 vaccines Spikevax® and Comirnaty®, have even been approved by EMA and FDA. (2)

Bones are in constant remodel due to resorption of old or damaged bone by osteoclasts (OC) and synthesis by osteoblasts (OB). (4)

This physiological process maintains bone quality, however, when there is an imbalance, bone mass and microarchitecture get compromised.

These bone disorders can be classified as genetic, metabolic, degenerative, traumatic, malignant, metastatic and primary, and myeloma-related. (2)

Although bones are highly vascularized, drug penetration and accumulation in the tissue is conditioned by the dense extracellular matrix (ECM) of bone tissues and by the protective nature of the bone marrow microenvironment. (5)

The lack of drug accumulation in the bone is also conditioned by the lack of targeting ability that current treatments present which leads to non-specific biodistribution and creates the need of high drug dosage which eventually leads to adverse effects and multidrug resistance. (6) (5)

Therefore, there is still a pressing need to develop new therapeutic options that are safer and more effective. (2)

Since NPs are controlled drug delivery systems with high targeting ability and a small size they could be used for transmembrane transport, cell labeling or gene therapy, or associated with implant surfaces, making them promising alternatives to current treatment options. (3)

To date, there is only one nanosystem approved by EMA as a therapy for a bone disorder.

Mepact® (mifamurtide) was approved in 2009 for the treatment of osteosarcoma (OS). Its addition to standard chemotherapy improved the overall survival from 70% to 78% and resulted in a reduction of 33% in the risk of death. (7,8)

2. Methods

The redaction of this thesis was based on the concepts in the field of nanomedicine with a focus on the use of NPs to treat bone diseases, analyzing studies that are developing novel therapies.

The writing of this review took place from December of 2021 to September of 2022. The articles mentioned in this thesis were gathered through web-based searches of main databases such as MDPI Open Access Journals, PubMed, Science Direct, Wiley Online Library, Elsevier, NCBI, Researchgate, BMC Public Health, The Royal Society of Chemistry's and Google Scholar. The European Medicines Agency (EMA) website was also assessed.

Search was conducted resorting to different words with the prefix nano-, including 'nanocarriers(s)', 'nanoparticle(s)' and 'nanotherapy(ies)', in combination with terms like 'bone diseases' and 'bone disorders'.

3. Bone inflammation/ infection

Nanoparticles for the treatment of Osteomyelitis

Osteomyelitis (OM) is the term used to describe bone and bone marrow infection, which affects an estimated 1 in 4000 people annually and can be classified based on the mechanism of infection, hematogenous versus non hematogenous, and the duration of illness, acute, sub-acute or chronic. Due to the release of inflammatory cytokines, OM can induce bone defects, osteonecrosis, and lead to a high recurrence of secondary infection. (9)

This disease develops mostly due to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus* spp., *Klebsiella* spp., *Escherichia coli* and *Staphylococcus epidermidis* (10)

Current approaches to treat OM such as administration of antibiotics, hyperbaric oxygen and surgery take in consideration issues related to debridement, management of infected foreign bodies, antibiotic selection and duration of therapy. (11)

Antibiotics have some limitations regarding their effectiveness which include poor bone penetrability, that prevents the drug from reaching the minimum inhibitory concentration (MIC) level in the tissue, poor blood circulation in the bone infected area, antimicrobial resistance and, in terms of pharmacokinetics, short half-life and systemic toxicity which limit the application of high doses of drugs.

When infections reach advanced stages, poor blood circulation in the infected area affects the drug concentration that reaches the tissue since necrosis leads to a shortage in blood supply which restricts the intravenous delivery of antibiotics.

This creates the need to administer high doses of drugs systemically and leads to high serum levels of antibiotic in extended periods that can induce serious side-effects such as ototoxicity and nephrotoxicity. (9,11)

Given these limitations and to reduce systemic toxicity, there is a clear need for local administration of antibiotics and the development of delivery carriers for controlled

drug release. (9,11,12) Since this controlled and targeted drug delivery can be achieved with NPs, extensive research is being conducted (Table 1).

Table 1. Examples of different studies evaluating the potential use of nanoparticles for Osteomyelitis treatment

Type of NP	Associated to	Main Findings		Ref
		In vitro	*In vivo*	
Magnetic gelatin (Gelatin, genipin, and magnetite)	Gentamicin	Gentamicin was slowly released on the first few hours GMGNPs 9.5 ± 2.8% of gentamicin was released in 2h GMGNPs released 100 ± 0.7% of gentamicin in 41 h	After six doses of GMGNPs treatment, abscess began to heal bone started to gain integrity NPs heal the infection more effectively compared to free gentamicin	(12)
Silk Fibroin	Vancomycin	Maximum loading of 18.84%; entrapment efficiency of >90%; The antibiotic's release was continuous Close to 100% of the drug was released in pH 4.5 after 3 days, although pH did not affect the bioactivity of the released antibiotic	Reduced bone infections at the defect site after 6 weeks	(13)
Poly(propylene sulfide)	Diflunisal	-	NPs accumulated in infected femurs and effectively mitigated OM-induced bone destruction, providing efficacious treatment of diflunisal	(14)
Ag	-	No change in the release profiles of Ag ions were observed *in vitro* between the implanted screws and the screws without the implantation	Strongly adherent NPs implants can effectively treat OM without any toxicity in major organs	(15)
Quaternary ammonium chitosan and CS	Vancomycin	Drug Release over 26 days NPs were cytocompatible After treatment with NPs, the ALP activity of the OB cells showed a significant increase Showed prolonged activity against *S. aureus* of more than 20 days	Progressive increase in bone volume at the end of the 8th week post-treatment VCM-NPs/Gel showed bone regeneration promotion and anti-infection properties	(9)

GMGNPs = gentamicin-loaded magnetic gelatin NPs; VCM-NPs/Gel= vancomycin NPs incorporated into the chitosan)-based thermosensitive hydrogel; CS = carboxylated chitosan; Ag = Silver

Table 1. Examples of different studies evaluating the potential use of nanoparticles for Osteomyelitis treatment (cont.).

Type of NP	Associated to	Main Findings		Ref
		In vitro	*In vivo*	
Ca alginate	Clindamycin	During the 12 h and 24h period, the highest percentage of the drug was released at pH 7.4 ½ MIC NPs, biofilm formation in the treated tubes was the lowest NPs treated *E. cloacae* showed reduced *exopolysaccharides* up to 55%	High loading and sustained release of clindamycin in NPs showed adequate viability on OB bone-like cells	(16)
MS with Arabic gum and colistin	Moxifloxacin and colistin	high affinity toward *E. coli* biofilm matrix thanks to the Arabic gum coating no cytotoxicity on OB, OC and macrophages reduction Less microcolonies adhered on the bone surface	Nanosystem was able to eradicate more than 90% of the bacterial load within the infected bone as well as absence of organ damage	(17)
CeO2	-	CeO2 NPs are radical scavengers and can inhibit the ABTS+ radical formation in a dose-dependent manner Growth inhibition toward *E. coli, S. typhimurium, L. monocytogenes, S. aureus, and B. cereus*	-	(18)
Calcium phosphate bioceramic	Tetracycline + ibuprofen	Better tetracycline release rate compared to a biphasic system of HA The CTP system to be biocompatible with significant antibacterial and anti-inflammatory activity	Rat cranial defects showed greater bone healing and new bone formation in the drug loaded CTP system compared to control (no carrier) at the end of 12 weeks	(19)

CeO2 = cerium oxide; CTP = CDHA/TCP system; CS = carboxylated chitosan; Ca = Calcium;

Table 1. Examples of different studies evaluating the potential use of nanoparticles for Osteomyelitis treatment (cont.).

Type of NP	Associated to	Main Findings		Ref
		In vitro	*In vivo*	
MS	ROS-scavenger nanoceria	Nanoceria was successfully incorporated into the pores of the MSNs Interacted directly with the mitochondria where ROS are produced Ce@MSNs are osteogenic, induce mineralisation and increase ALP activity *in vitro* Increase bone-like ECM production and OC activity Significant increase in RANKL expression Protective role of the Ce@MSNs against ROS production	-	(20)

Ce@MSNs = ROS-scavenger nanoceria encapsulated within MS NP; RANKL= Receptor activator of nuclear factor kappa ligand; ROS=Reactive oxygen species;

Antibiotic treatment is the traditional choice for treating OM so the majority of the NP that are being developed are loaded with different classes of antibiotics, such as aminoglycosides, glycopeptides, lincosamides, fluoroquinolones or tetracyclines.

Among the different NP systems that were studied for the delivery of vancomycin, silk fibroin has been explored as a carrier. (13) Silk NPs showed suitable biocompatibility, low immunogenic response, low bacterial adhesion, degradability, mucoadhesiveness, which can enhance the particles retention time at the infectious area, and functionality. In this study, the release of antibiotic from the NPs was higher in acidic pH, which can be favorable for the treatment of infections since the environment where bacterias such as *S. aureus* are found is often characterized by acidic conditions because of the degradation of products such as lactic and butyric acid that affect the pH.

In another study incorporated vancomycin NPs, prepared with quaternary ammonium chitosan, carboxylated chitosan (CS) and vancomycin, in a thermosensitive hydrogel composed of CS and α and β-glycerol phosphate disodium salt (GP). (9) This combination has it advantages since thermosensitive hydrogels can spontaneously turn

into semi solid gels, acting as sustained release depots of drugs that fully fill the damaged area, and since CS is structurally similar to glycosaminoglycans and has antibacterial, pain relief, and hemostasis properties.

CS gels usually have a cumulative release time that only reaches 5 days but clinical antibiotic treatment of OM takes longer than 4 weeks. This drug delivery system extends antibiotic release time to over 26 days which makes this strategy a promising one.

Another antibiotic that was used in previous studies was gentamicin.

Gentamicin is an aminoglycoside with broad spectrum activity. Researchers developed gentamicin-loaded magnetic genipin cross-linked gelatin NPs that are biodegradable, biocompatible and that, contrarily to other magnetic NPs, can be administered via intravenous route and don't require intense invasive applications. (12) This nanosystem showed therapeutic benefits since magnetic NPs generally allow the accumulation of drugs at a defined target site and prevent the elimination of the drug, especially from the reticuloendothelial system, with the assistance of an external magnetic field.

Another appropriate choice of antibiotic for OM treatment is clindamycin. Clindamycin loaded calcium (Ca) alginate NPs where crosslinked with phosphorylated polyallylamine to combine the alginate's excellent biocompatibility, biodegradability, bioavailability, gelation ability and low cost with the crosslinking benefits which include creating more complex structures that allow higher drug loading and prolonged drug release. (16)

Mesoporous silica NPs (MSNs) loaded with moxifloxacin with broad spectrum activity and further functionalized with arabic gum and colistin were also developed. (17) Colistin has a disaggregating effect and the arabic gum is biocompatible, has high affinity toward *E. coli* biofilm matrix and its degradation by secreted bacterial enzymes improves the retention of MSNs on the biofilm.

According to the researchers that developed this NP, this was the first time that MSNs were engineered to carry antibiotics with different molecular weights at the same time, instead of just one antibiotic.

MSNs can also be associated with other compounds besides antibiotics. MSNs incorporate cerium in the form of oxide (ceria) NPs (nanoceria) which has antioxidant properties and can mimic multi-enzyme activity and work as a free radical scavenger against *E. coli*. These NPs stimulate bone regeneration through release of osteogenic silica and reduce OC activity via the Reactive oxygen species (ROS) scavenging property of the nanoceria, combining antioxidant, osteogenic and anti-osteoclastogenic effects. (20)

In another study the antibacterial properties of cerium oxide (CeO2) NPs were also investigated but this time against a wider range of pathogens such as *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus* and *Bacillus cereus*. (18) This study showed through confocal microscopy images that the inhibitory effect of CeO2 NPs is present at a lower concentration, with respect to gentamicin (the standard drug), evidencing the potential antibacterial property of these NPs. However, MIC is only lower when the NPs were used against *Escherichia coli* and *Listeria monocytogenes* which may be related to the different structure between Gram-positive and Gram-negative pathogens.

Other types of metal NPs can be used as an alternative treatment for OM. Strongly adherent silver (Ag) NPs which were deposited using an electrodeposition process in stainless steel implants for *in situ* treatment *in vivo*. These offered a promising result in terms of eradication of infection in rabbit OM model without any toxicity in major organs such as heart, kidney, and liver on both 21 and 42 day time points. (15)

Most of the current NP research for OM is more focused on local antibiotic delivery than treating inflammation, which is crucial to speed up the healing process and arrest bone loss. Nano calcium phosphate ceramics deliver an antibiotic, tetracycline, and an anti-inflammatory drug, ibuprofen. In comparison to the system without an anti-inflammatory drug the combined drug system showed a more controlled drug release profile and *in vivo* implantation studies on rat cranial defects showed greater bone healing. (19)

Although some studies explore therapies involving the combination of antibiotics and anti-inflammatory drugs, others investigate anti-inflammatory drugs as adjunctive treatment strategies that focus on inhibiting bacterial virulence pathways. Diflunisal‐loaded poly(propylene sulfide) NPs accumulated at infected femurs in a

murine model of post-traumatic OM, effectively decreasing *S. aureus*-mediated bone destruction by limiting production of numerous virulence factors including cytolytic toxins. (14)

4. Bone degenerative disorders

Nanoparticles for the treatment of Osteoporosis

One of the most common metabolic diseases that affects the bone tissue is osteoporosis. (21)

Bone homeostasis is conditioned by the balance between OCs and OBs' activity and the process of bone resorption and formation. (22)

Osteoporosis is associated with increased bone resorption rate due to the increased number or activity of OCs which results in low bone mineral mass density and changes the microarchitecture of the bone. (21–24)

According to statistics, approximately 200 million people worldwide suffer from this disorder which is more common in post-menopausal women and can easily lead to bone fragility and consequently to fractures of the hips and backbone, which increase morbidity. (21, 23)

This disorder can be categorized into either primary or secondary osteoporosis.

Primary osteoporosis stems mainly from aging and postmenopausal response and secondary osteoporosis is associated with prolonged use of anti-inflammatory and immunosuppressive drugs, like glucocorticoids. (21)

Current treatments aim at bone healing and repair and involve the administration of anabolic drugs that induce bone formation, effectively increasing bone mass and reversing bone deterioration, or antiresorptive drugs that act mainly by suppressing OC activity, preserving bone mass and increasing bone strength. The most common involve bisphosphonates (BP), the Parathyroid Hormone (PTH) or estrogen. (22,24,25)

However, these conventional treatment options have some limitations including low bioavailability and ability to reach the target site due to their short half‑life and poor

absorption, which make high dose administration a requirement and consequently lead to long term safety concerns. (24,25)

Taking these limitations in consideration, the development of effective treatments, preferably non-invasive ones, is needed and could provide a radical intervention for improved therapy of osteoporosis.

Table 2. Examples of different studies evaluating the potential use of nanoparticle treatment for Osteoporosis.

Type of NP	Associated to	Main Findings		Ref
		In vitro	*In vivo*	
Au	EGCG	NPs showed better anti-osteoclastogenic effect than free drug Remarkable stability, significantly rapid cellular uptake, and excellent *in vitro* antioxidant activities	EGCG-GNPs greatly reversed bone resorption was more effective than applying free EGCG, specifically in inhibiting the number of OCs, improving bone density, and preventing bone loss	(26)
Liposomes	Antagomir-188	NPs increased ALP OBs formation	NPs tended to accumulate in the bone marrow and release antagomir-188 which promotes osteogenic differentiation, inhibits adipogenic differentiation and reverses the age-related bone loss	(27)
Ca-RISNPs	-	Higher drug deliverability and have lower cytotoxicity compared to free risedronate Potential application in treatments in bone metastatic cancers or other cancers Inhibiting tumor development and migration	-	(28)

Au = Gold; EGCG-GNPs = Epigallocatechin gallate-capped Au NPs;

Table 2. Examples of different studies evaluating the potential use of nanoparticle treatment for Osteoporosis (cont.).

Type of NP	Associated to	Main Findings		Ref
		In vitro	*In vivo*	
HA	SCT	98% of SCT was released from the SCT solution within 1 hour	Rats treated with sublingual SCT-HAP-NPs exhibited significantly lower ALP levels and serum Ca compared to the OVX control group These rats also revealed improved bone density and mechanical bone strength and a decrease in the number of resorption pits compared to control	(29)
SiO2-Pep	SCT	SiO2-Pep@SCT presented better properties than SiO2@SCT. Sustainable release of SCT Have stronger negative charge for higher SCT loading efficiency than silica NPs	NPs can remarkably promote osteogenic differentiation SiO2-Pep@SCT can efficiently prolong the half-life of SCT the SiO2-Pep@SCT complex was non-immunogenic The sustained hypocalcemic effect induced by drug duration has the benefit of stabilizing physiological activities *in vivo* and reducing the Ca loss from bone	(30)
Mesoporous SiO2-CaO	IP	Under less favorable hydric physiological conditions most of the drug remains retained within the NP core Increased macrophage response Induced a significant decrease of OC proliferation and resorption activity after 7 days in coculture with OBs, without affecting OB proliferation and viability	-	(31)
MS	Au	The NPs have good biocompatibility Stimulate an anti-inflammatory response and promote the secretion of osteogenic cytokines by macrophages resulting in the enhancement of osteogenic differentiation	AuMSNs could accelerate new bone formation in a critical-sized cranial defect site in rats based on CT analysis and histological examination	(32)

SCT = salmon calcitonin; SiO2 = silica; SiO2-Pep@sCT = pentapeptide-decorated silica NPs loaded with sCT; SiO2@sCT = sCT-loaded SiO2; AuMSNs = Au NP-loaded MS NPs; CT = computed tomography; GA = Gallium; IP = Ipriflavone;

Table 2. Examples of different studies evaluating the potential use of nanoparticle treatment for Osteoporosis (cont.).

Type of NP	Associated to	Main Findings		Ref
		In vitro	*In vivo*	
MBG	ZnO Osteostatin	Enhanced MC3T3-E1 cells viability The simultaneous additions of osteostatin and Zn2+ ions provoked higher differentiation capacity in MC3T3-E1 cells, compared with either raw BL or MBGs supplemented only with osteostatin or Zn2+	-	(33)
MBG	GA	Decrease of H+ concentration in because of the ionic exchange with Ca2+ from the MBGs ALP activity was higher when cells were cultured in the presence of MBG-58SGa and MBG-85SGa, when compared to their Ga-free analogues	-	(34)
Polysaccharide-based iron oxide	Iron	Induced antioxidative MC3T3-E1 and Raw 264.7 cells to scavenge ROS Promoted osteogenic differentiation and inhibited OC differentiation	Fe2O3@PSC NPs can protect mice from IA-induced osteoporosis increased ability of osteogenic differentiation and inhibition of OC differentiation *in vivo*. NPs are biosafe and did not delay osteoporosis in the postmenopausal mice	(35)
PLGA capsules	PEI: RANK siRNA complex	Significant reduction in RANK mRNA levels in OC precursor cells. Caused suppression of OC differentiation and activity	-	(36)

MBG = Mesoporous bioactive glasses; Fe2O3@PSC = PSC-based iron oxide NP; IA = iron accumulation; PEI:RANK = RANK siRNA complexed with PEI and loaded into biodegradable PLGA; Ca = Calcium; GA = Gallium;

Mesoporous Bioactive Glasses (MBGs) are oxide-based glass systems with a big surface area that can host drugs inside their mesopores and stimulate bone regeneration, yielding quicker *in vitro* responses when compared with other bioactive materials.

Their pore volume and highly ordered structure enable the incorporation in the glass network of bioactive metal ions such as zinc (Zn) 2+ ions, which stimulate bone

growth, as shown in a study by Pérez and researchers. (33) In this study, MBG with a composition of 80% SiO2–15% CaO–5% P2O5, 4% or 5% of ZnO and impregnated with osteostatin were developed and investigated.

These NPs enhanced pre-osteoblastic MC3T3-E1 cells viability and differentiation capacity regardless of the ZnO percentage and it was demonstrated that osteostatin enhanced the *in vitro* osteogenic capacity of Zn2+-enriched materials.

MBG can also be loaded with gallium (Ga) cations since they reduce resorption activity, differentiation and formation of OC without cytotoxic effects in a dose-dependent manner.

Considering ALP activity, viability of RAW 264.7 and inhibited expression of TRAP reported in the study, it was shown that MBGs maintained their bioactive behavior despite being doped with Ga and disturbed osteoclastogenesis while enhancing the early differentiation towards OB phenotype. (34)

Gold (Au) NPs capped with epigallocatechin gallate (EGCG), the main active compound of green tea, were synthesized to compensate EGCG's low aqueous solubility, (26) that leads to poor bioavailability and adverse effects. (37) This study demonstrated that NPs not only suppressed the mRNA expression of genetic markers of OC differentiation but also inhibited MAPK signaling pathways, which may lead to the production of ROS.

Iron in the form of Fe2 was combined with polyglucose-sorbitol-carboxymethyl ether (PSC) to create Fe2O3@PSC NPs that inhibit the generation of excess ROS that occurs when excess free iron ions sinks into bone tissues.

In vitro Fe2O3@PSC NPs induced scavenge of ROS by MC3T3-E1 and Raw 264.7 cells, which activated Akt-GSK-3β-β-catenin promoting osteogenic differentiation and inhibited OC differentiation by inhibiting the MAPK and NF-κB pathways. Researchers verified that when iron was supplied by the Fe2O3@PSC NPs *in vivo* there was no iron accumulation-related osteoporosis. (35)

Au based NPs have been prepared with various shapes and structures due to their stability and low toxicity. (38) Modulatory effects of Au NP (AuNP)-loaded MSNs (Au-MSNs) on macrophages and the subsequent effects on OB were investigated.

These NPs increased the osteogenic capability of pre osteoblastic MC3T3 cells due to increased expression of osteogenic markers, ALP production and ca deposition. (32)

MSN can also be loaded with anti-resorptive molecules, that inhibit OC activity and genesis, to treat osteoporosis such as ipriflavone (IP) or salmon calcitonin (SCT).

In a study by Casarrubios and researchers, cultures of OB and OC were used to inquire the effects of MS-CaO nanospheres (NanoMBGs) loaded with IP since this isoflavone is highly insoluble in aqueous environments, like human plasma, and has low bioavailability. (31) The entrapment of IP in the NP core allowed a controlled release from the nanospheres. NanoMBGs-IPs significantly reduced resorption activity and the number of OC that were in coculture with OB, without affecting OB proliferation and viability.

SCT is a polypeptide that binds to OCs through calcitonin receptors present on the cell membrane, exhibiting antiresorptive activity. Calcitonin was incorporated into HA NPs (SCTHAP-NPs) to be delivered by the sublingual route, a more simple and noninvasive way to administer drugs. (29) According to this study there was an improvement in bone microarchitecture, mass density and strength and the permeation of intact SCTHAP-NPs through the sublingual mucosa was comparable in terms of efficiency to subcutaneous administration at the same dose.

SCT was loaded into pentapeptide-decorated silica NPs (SiO2-Pep@sCT) to compensate for SCT's short half-life due to its rapid clearance *in vivo*. Biomarkers such as ALP and intracellular Ca showed that osteogenic differentiation was promoted *in vitro*. *In vivo* the half-life went from 30.5 to 69.3 min thanks to the NPs and micro-CT analysis showed enhanced trabeculation and faster bone repair. (35)

Another strategy that could be used to promote osteogenic differentiation is by inhibition of microRNA (miRNA) miR-188. Targeting ability of exosomes was improved by displaying C-X-C motif chemokine receptor 4 (CXCR4) on their surface. CXCR4acts as a ligand for stromal cell-derived factor 1 (SDF1) predominantly expressed by MSCs.

To allow delivery and encapsulation of antagomir-188, an exosome-liposome hybrid delivery system was developed. These hybrid NPs accumulated in the bone marrow reversing the age-related bone loss. (38)

Risedronate is a third-generation BP with low toxicity that can inhibit the mevalonate pathway that is essential for OCs, inhibiting bone resorption. Like most BPs, risedronate has a very short half-life so many studies have tried to deliver them via NPs.

NPs synthesized with calcium ions (Ca-RISNPs) that had lower cytotoxicity to bone cells compared with free risedronate and effectively reached and bounded to deeper areas of bone tissue. (32)

siRNA, to inhibit RANK, was complexed with polyethyleneimine (PEI) and loaded into biodegradable Poly(lactide-co-glycolide) (PLGA) to address one of the underlying causes of OP. These nanocapsules reduced RANK mRNA levels in 47% consequently suppressing OC activity. (36)

5. Bone genetic disorders

Nanoparticle Treatments for Osteogenesis imperfecta

Deranged bone metabolic activity due to genetic mutations can be associated with altered activity of bone cells, of ECM proteins, of bone microenvironmental regulators and of calciotropic and phosphotropic hormones.

Osteogenesis imperfecta (OI) is the term used to describe one of the rarest congenital skeletal disorders, with an incidence of 1 in 15 000 to 20 000 live births and it is mainly associated with altered ECM proteins although some phenotypes show reduced function of OBs and/or bone matrix mineralization. (39–41)

According to new classification systems there is a broad range of clinical phenotypes of OI, at least eighteen, whose outcomes range from mild to moderate to severe and can be linked to the mutation of several genes. The most common is caused by autosomal dominant mutations in either the COL1A1 or the COL1A2 gene, which encode the chains of type 1 collagen. This enables defective collagen synthesis, structure, folding, post translational modifications or processing that lead to defects on the bone matrix and mineralization and reduce bone quality. Defects directly in type I collagen structure or quantity are related to 80% to 85% of cases of OI. (39,41,42)

Regardless of the underlying mutation and despite the clinical forms being distinguished by their clinical severity, bone characteristic features commonly overlap. OI increases throughout life the risk of fragility fractures, connective tissue malfunctions, and skeletal deformities. (40–42)

The current treatments for this pathology try to prevent fractures, control symptoms and increase bone mass but these therapies present some disadvantages since they show weak effectiveness, lack of effects in some patients or cytotoxic side effects. (41)

Table 3. Examples of different studies evaluating the potential use of nanoparticle treatment for Osteogenesis Imperfecta.

Type of NP	Associated to	Main Findings	Ref
		In vitro	
nHA	BMP-2	Significant increase in Ca deposition within the nHA-BMP2 transfected cells Enhanced osteogenesis	(43)
MBG	siRNA	Inhibited the expression of RANK, and the subsequent biological functions, including the inhibition OC-mediated bone resorption and regulation of excessive bone loss	(44)
Au	ALN and pamidronate	Significant decrease in cell viability No effect on OB viability except when treated with the 100% treatment which corresponds to 1.63x10^11 particles and 3 μM ALN No effect on RANKL expression	(45)
GS-AgNP prepared from marine macroalga		No cytotoxicity; Upregulation of ALP, mineralization, and osteocalcin and BMP-2 expression	(46)
MSN-CC-PEI	(rno)-miRNA-26a-5p	Stable binding of the miRNA to the NPs led to successful delivery and cellular uptake miRNA-NP complexes were still functional after freeze-drying and storage rBMSC transfected by rno-miRNA-26a-5p/MSN-CC-PEI exhibited a significantly higher level of Runx-2, ALPL, CoL1A1, OCN, BMP-2, and IBSP compared to the control groups Amount of collagen produced was significantly higher miRNA-26a-5p was effective for osteogenic differentiation NPs were a suitable vector for the functional delivery of miRNA	(47)

MBG = Mesoporous bioactive glass nanospheres; Au = Gold; ALN = Alendronate; GS-AgNP = polygalactan-based bioactive Ag NP; MSN-CC-PEI = lyophilized MSN NPs with core-cone structure and coated with PEI; rno = *Rattus norvegicus*; IBSP = Gene that codes Integrin Binding Sialoprotein; BMP = Bone morphogenic protein; Ca= Calcium; RANKL= Receptor activator of nuclear factor kappa ligand

Hydroxyapatite (HA) is the main biomineral component found in human teeth and bones. (48) Since nHA is similar to biological HA, nHA has been investigated as a therapy for bone disorders.

HA NPs were combined with bioactive and biodegradable collagen scaffolds to develop a vector for delivery of plasmid-DNA (pDNA) encoding bone morphogenetic protein 2 (BMP2). These highly porous, mechanically stable, osteoconductive and

osteoinductive scaffolds induced MSCs mediated bone formation, increasing Ca production. (43)

Another study by Hosseinpour and associates also uses a non-viral vector but for transfection of miRNA to regulate MSCs proliferation. Large pore sized lyophilized MSNs with core-cone (CC) structure and coated with polyethylenimine (MSN-CC-PEI) were used as a system for delivering *Rattus norvegicus* (rno)-miRNA-26a-5p. A significantly higher level of expression of seven genes associated with osteogenesis, Runx-2, ALPL, CoL1A1, OCN, BMP-2 and IBSP, and enhanced ALP activity were exhibited in rat marrow MSCs (rBMSCs) treated with the NPs. (46)

The suppression of OC activity through localized release of small interfering RNA (siRNA) can also be a promising therapy for bone diseases such as osteoporosis. (44) In this study, the expression of the receptor activator of nuclear factor kappa B (RANK) was inhibited by siRNA that was delivered through silica-based mesoporous bioactive glass nanospheres (MBG), an inorganic biocompatible and degradable nanocarrier. The gene silencing effect led to a decline of 29% on RANK-expressing cell population and a down-regulation of osteoclastogenesis-related genes such as cathepsin-K, tartrate-resistant acid phosphatase (TRAP) and nuclear factor of activated T-cells cytoplasmic 1 (NFATc1).

Metallic NPs such as Au and Ag ones have been studied recently. Au NPs (AuNPs) were functionalized with alendronate (ALN) and pamidronate, two different BPs. (45) It was reported that the biocompatible and lowly toxic AuNPs that were studied, improved the delivery of pamidronate and ALN and reduced OC viability without affecting OB receptor activator of nuclear factor (NF)-jB ligand (RANKL) expression.

Ag particles were implanted altering the structure of polysaccharides isolated from the marine macroalga *Gracilaria salicornia*. The Van der Waals force of interaction between the Ag ions and oppositely charged sulfated polygalactan (GSP-2) trapped the Ag particles and increased the overall stability of the NP.

Upon treatment with the polygalactan-based bioactive Ag NPs, alkaline phosphatase (ALP) activity was significantly elevated in human MSCs and the percentage of osteocalcin (78.64%) and BMP-2-positive cells (46.10%) increased. (46)

6. Bone traumatic disorders

Nanoparticle Treatments for Fracture Treatment

Bone fractures are mainly caused by mechanical impact or stress but can also be secondary to age and/or certain pathologies such as osteoporosis, some types of cancers, diabetes and OI which affect significantly the quality and self-healing potential of bones (49,50)

The previously mentioned leading causes for bone fractures require special attention and quick intervention for a rapid healing.

Although routine treatment, which often involves setting and immobilizing the bones to give them time to heal, may be enough to heal simple fractures, the gold standard treatment for severe and extensive fractures are autogenous bone grafts, also known as autografts, which have several drawbacks despite its advantages, such as the limited quantity of graft available to make an autograft and 8.6% rate of major complications (infection, prolonged wound drainage, large hematomas and reoperation). (51,52)

Seeking proper alternatives is the subject of various studies in order to avoid complications such as risk of mal-union, delayed union, non-union or even osteonecrosis. (53)

Bone's self-healing ability is mainly conditioned by the stability of the fracture site and the patient's bone quality.

When a fracture occurs, the healing process begins with the differentiation of osteoprogenitor cells and bone mesenchymal stem cells (MSCs) adjacent to the fracture line into chondrocytes and OBs which eventually leads to the formation of woven bone with a trabecular structure.

Since bone remodeling depends on OB activity and mesenchymal stem cells' proliferation, they can be a target for future therapies to promote bone regeneration to treat fractures.

Some of the compounds that have been extensively investigated to promote bone healing are calcium phosphates, such as HA that has been used in bone repair applications due to their similarity to the mineral phase of natural bones, which confers them an excellent biocompatibility. (50,54)

Table 4. Examples of different studies evaluating the potential use of nanoparticle treatment for Fracture Treatment.

Type of NP	Associated to	Main Findings		Ref
		In vitro	*In vivo*	
APRF+	-	No cytotoxic effects on stem cells after 7 days The combination of AuNPs with APRF+ gel was found to increased cell viability rate in a paracrine manner compared to the non-treated control group Promote ALP activity	-	(55)
Cu-doped Ca silicate	-	Presence of Cu can improve the release and deposition of Ca into the bone tissue and enhanced OB formation OB were shown to begin propagation and regeneration of new bone tissue in the presence of critical concentrations of Si and Ca ions in within 48 h Complete healing of the tibia bone with normal architecture of bone tissue	The results for the *in vivo* studies were in line with the *in vitro* findings	(56)
Niosomes	BMP-7 gene	MSCs transfected with niosomes showed increased growth rate, enhanced ALP and ECM deposition which suggested the formation of OB-like cells	-	(57)

ALP = alkaline phosphatase; Ca = Calcium; Cu = copper; ECM = extracellular matrix; A-PRF+ = Advanced-platelet-rich fibrin modified by Au;

Table 4. Examples of different studies evaluating the potential use of nanoparticle treatment for Fracture Treatment (cont.).

nHA microspheres	BMP-2	the effective rhBMP-2 loading rate of the nHA microspheres was significantly higher than that of the HA microspheres The nHA has a larger specific surface area and pore size and has more opportunities to interact with BMP-2, thereby increasing the amount of BMP-2 loading Effective loading of the target protein, which reduces its initial burst release	Induce OB differentiation and affected its application, having a better ability to induce osteogenesis *in vivo* than HA microspheres loaded with rhBMP-2, contributing to bone formation and regeneration, especially for bone defects with an irregular shape	(58)
HA	Au	The HAAu NPs showed good cytocompatibility and internalized into hMSCs The increased level of ALP production, deposition of Ca mineralization, as well as the expression of typical osteogenic genes, indicated the enhancement of osteogenic differentiation of hMSCs. The incorporation of Au could activate the Wnt/βcatenin signaling pathway, which seemed to be the molecular mechanism underlying the osteoinductive capability of HA-Au NPs	-	(59)
nHA	Lithium ions	The hASCs proliferation was faster without undergoing apoptosis. 5 mol% Li+:nHA and Li+ ions improved osteogenic differentiation of hASCs and decreased expression of GSK3β while increasing β-catenin mRNA level. Li+, nHA and 5 mol% Li+:nHA improved mitochondrial dynamics and enhanced expression of neural differentiation marker genes	-	(60)

rhBMP-2 = recombinant human bone morphogenetic protein-2; HAAu = Au NPs-loaded HA; hMSCs = human bone marrow derived MSCs; hASCs = human Adipose Tissue-derived Stem Cells; GSK3β = glycogen synthase kinase 3β;

Table 4. Examples of different studies evaluating the potential use of nanoparticle treatment for Fracture Treatment (cont.).

NAB	HM69	HM69 could bind with MSCs with high specificity, while having minimal cross-reactivities with other cells HM69 could capture MSCs with a purity of >89%. NAB could bind and capture MSCs effectively, whereas it did not cause obvious cytotoxicity	*In vivo*, serum OPN, BGP, and ALP levels in the NAB group of rats were increased, indicating the repair and osteogenesis generation. The healing of bone defects in the NAB group was significantly better than control groups, the defects became blurred, and local trabecular bone growth could be observed in X-ray	(61)
Chitosan	Sinapic acid	No significant change in cell viability or number The quantification of ALP activity showed a significant increase in calcium phosphate deposits Treatment of mMSCs with SA stimulated luciferase activity	Increased bone formation and significantly increased bone volume More deposition of collagen was clearly observed	(62)

HM69 = 66-based DNA aptamer; NAB = Nano-Aptamer Ball; OPN = Rat osteopontin; BGP = osteocalcin; SA = Sinapic acid; mMSCs = Mouse MSCs; SBE = Smad binding element; BMSCs = ; ALP = alkaline phosphatase; ECM = extracellular matrix; NAB = Nano-Aptamer Ball

BMPs are signaling molecules that influence survival, proliferation and differentiation of multiple cells that are a part of various organ systems. Although they are known for improving osteogenic differentiation of MSCs, which is essential for fracture repair, their full potential is hampered by their short half-life.

A novel niosome formulation combined with polysorbate 80 was developed to deliver and transfect BMP-7 plasmid in MSCs. These NPs induced osteogenic differentiation and Ca deposition which was analyzed through ALP activity. (57)

Recombinant human BMP-2 was loaded into conventional HA and nanostructured HA (nHA) microspheres. Through 3D micro-computed tomography (CT) and histomorphometric observations it was possible to conclude that nHAs were able to carry more BMP-2, improving osteogenesis in a more significant way compared to conventional HA, and reduced initial burst release. This demonstrates that these NPs are a promising carrier for this protein. (58)

HA can also be loaded into metallic NPs such as Au ones. The synergy between AuNPs properties and HA properties lead to the activation of the Wnt/βcatenin

signaling pathway, which seemed to be the molecular mechanism that increased mineralization, ALP, Runx2, osteopontin and osteocalcin which indicates that these NPs promote osteogenesis and osteogenic differentiation and may be potential candidates for bone repair. (59)

Platelet-rich fibrin (PRF) is a family of platelet concentrate that is processed into a clot and widely used in dentistry to fill cavities or to be mixed with bone materials.

Studies indicate that although there is a lack of clinical evidence that makes a correlation between PRF and bone restoration and healing, PRF is likely to stimulate osteogenesis at the target sites.

AuNPs were added to the Advanced PRF formulation (APRF) in order to improve its regenerative potential. This combination led to significant difference in released ALP content compared to AuNPs or A-PRF alone which led to accelerated osteogenic differentiation of human MSCs. However, the role of AuNPs in the stability and resistance of growth factors and cytokines trapped in the clots still needs to be evaluated. (55)

Another metal that has been combined with other molecules is copper (Cu). These ions could be added to synthetic apatite to grant solubility, resorption and bone bonding ability. (56)

Three samples were prepared containing different concentrations of Cu and only 3 and 5% Cu-doped Ca silicate presented significant results. The tibias treated with the 3% or 5% samples had great rigidity, affording significant pressure to fracture, however only the 5% one increased bone density.

Part of the problem to treat fractures and other bone disorders is the lack of specificity for bone tissues and affinity with its cells. DNA aptamer HM69 was used to functionalize NPs, termed as NAB, for active targeting and recruitment of MSCs. *In vitro* these NPs were able to capture MSCs and *in vivo* this ability led to the enrichment of the bone defect area with MSCs, resulting in high levels of OPN, BGP, and ALP that are indicative of osteogenesis and bone repair. (61)

Sinapic acid (SA) is a phenolic acid derived from plants which is known for its antioxidant, anti-inflammatory, antimicrobial and anabolic properties that has limited bioavailability.

To study the anabolic effect of this acid on bone metabolism, chitosan (CS) NPs were loaded with SA and incorporated into polycaprolactone (PCL) fibers, a synthetic polymer that gives the NP structure and the ability to be degradable.

The release of SA from PCL/nCS/ SA promoted MSCs differentiation towards OB and bone regeneration *in vivo*. (62)

7. Bone Tumor Diseases

7.1 Primary Bone Tumors

Primary bone cancers are relatively rare, with an overall annual incidence of 1 case per 100,000 adults in Europe representing 2% of all human neoplasms.

They include bone, cartilage and connective tissue tumors that range from indolent to very aggressive that can eventually be metastatic. (63)

Two of the foremost common primary bone tumors are OS and Ewing sarcoma, which primarily occur in children, adolescents and young adults. (64)

Bone cancers are characterized by regional or localized pain associated with overlying tenderness and decreased range of motion, which mimic common musculoskeletal injuries. These nonspecific symptoms and the fact that the pain often begins after minor physical trauma make timely diagnosis challenging. (65)

OS is the most common primary bone neoplasm, being responsible for nearly two-thirds of all cases, mainly developing in the distal femur, proximal tibia, and proximal humerus. It is more common in the pediatric population, being the third most common childhood malignancy, although it can occur at any age (64–66)

It is believed that OS tend to occur mainly during childhood since it is a time with more active bone formation and elongation, which enable cell mutations.

It originates from malignant mesenchymal cells, which aren't associated with the expression of specific oncogenic markers, that later differentiate into OBs, which in turn produce a malignant osteoid matrix. (64,67)

As mentioned previously, OS is not associated with any specific clinical signs. It’s characterized by pain and in more advanced cases spontaneous fracture. (66)

The primary mode of treatment for 80% of OSs is surgical resection and they are generally not treated with chemotherapy. (67)

Ewing sarcoma is the second most common bone sarcoma and it appears mainly in bones but also in soft tissues in 15% of cases.

The tumor is in 90% of cases genetically characterized by a specific chromosomal translocation that fuses a member of the FET family of proteins, which are RNA-binding proteins involved in transcription and splicing, with different members of the ETS family of transcription factors. (63,66)

As previously mentioned, it is similar to OS in terms of presenting symptoms and age at occurrence but a major difference between these primary bone tumors is the anatomic locations in which they typically develop. Although it is also possible to find this tumor in long bones, Ewing sarcoma is commonly seen in the pelvis, ribs, and scapula. (64)

Current standard treatment is multimodal and includes multidrug chemotherapy, radiation therapy and/or tumor surgical resection.

Despite progress in therapies for OS and Ewing sarcoma, 30 to 40% of patients still succumb to their disease five years later, OSs having the worse survival, mainly due to refractory and/or recurrent disease so new therapeutic approaches are necessary and research is targeting different actors of the bone microenvironment. (66,67)

7.1.1 Nanoparticle Treatments for Osteosarcoma

Table 5. Examples of different studies evaluating the potential use of nanoparticle treatment for Osteosarcoma.

Type of NP	Associated to	Main Findings		Ref
		In vitro	*In vivo*	
Zn oxide and CeO2	-	Loss of cell membrane integrity, oxidative stress, and apoptosis was observed in treated MG-63 human OS cells	-	(68)
Lipid-polymer NPs with CD133 aptamers	ATRA	ATRA reduced the formation of colonies compared with control	CD133+ cells possessed markedly increased ability in OS formation ATRA-PLNP-CD133 treatment showed the best inhibitory effect towards tumorsphere formation ATRA treatment led to a markedly decreased proportion of CD133+ OS initiating cells than the saline control	(69)
Hydrophobic Poly(ester amide)	Apa	Apa NPs showed better *in vitro* therapeutic efficacy than free Apa Apa NPs showed superior anti-OSC effect on both OS cell lines	Apa NPs had a longer *in vivo* circulation, which ensured Apa NPs more prone to reach tumor sites and allowed the considerable uptake by OS cells Apa NPs were capable of accumulating more effectively in OSC-derived tumors Significantly inhibits the OS stem-like cells-derived tumor growth in contrast with free Apa, with minimal side effects	(70)

HA-PEG = Hyaluronic acid/polyethylene glycol; MIONP = magnetic iron oxide NPs; Apa = Apatinib; OSC = Osteosarcoma stem-like cells; PTX = Paclitaxel ; OCN = osteocalcin; HA–BSA–PTX = ; PDA = ; BSA = bovine serum albumin; ATRA = All-trans retinoic acid; OS = Osteosarcoma

Table 5. Examples of different studies evaluating the potential use of nanoparticle treatment for Osteosarcoma (cont.).

HA and BSA	PTX and Ca ions	Sustained release properties of PTX and Ca2+ and low cytotoxicity to human fetal OB *in vitro* The NPs group had the ability of longtime drug sustained release NPs had the ability of entering the cells Low cytotoxicity, inhibited tumor cell growth and induced cancer cell apoptosis Could reduce the migration ability of 143B cells, which effectively prevented the metastasis of tumor cells The ALP and OCN activity increased with the release of PTX and Ca2+	The tumors treated with HA–BSA–PTX NPs were significantly lighter than the control group The survival time of nude mice after intratumoral injection of HA–BSA–PTX NPs was greatly prolonged Effectively inhibited tumor metastasis	(71)
MS–coated bismuth sulfide	DOX	Controllable release of DOX Actively OS cells targeting capability Cell biocompatibility and hemocompatibility Enhanced therapeutic effect by targeted photothermal therapy–chemotherapy enhance tumor cells eradication effect through the mitochondrial apoptosis pathway	NIR irradiation, the active targeting effect, burst drug release, and hyperthermia together could efficiently kill the tumors, leading to efficient suppression on the malignant sarcoma No obvious changes in body weight The tumor edge was seriously damaged and no metastases were observed in the peritumoral tissues, which indicates efficient prevention of recurrence Good biocompatibility	(72)
tetra-sulfonated aluminum phthalocyanine and poly-methyl methacrylate core-shell fluorescent	-	induced high level of OS cells death in the 2D co-culture in the 3D co-culture, a substantial decrease of both MSCs and OS cells viability was observed	Were able to decrease OS growth The NPs had a larger distribution, thus supporting the tumor targeting effect	(73)

HA-PEG = Hyaluronic acid/polyethylene glycol; MIONP = magnetic iron oxide NPs; Apa = Apatinib; OSC = Osteosarcoma stem-like cells; PTX = Paclitaxel ; OCN = osteocalcin; HA–BSA–PTX = ; PDA = ; BSA = bovine serum albumin; ATRA = All-trans retinoic acid; OS = Osteosarcoma

Table 5. Examples of different studies evaluating the potential use of nanoparticle treatment for Osteosarcoma (cont.).

PLGA	143B-RAW hybrid membrane	Potent ability to induce apoptosis of 143B cells NPa coating with macrophage membrane had more tendency to inflammatory sites than the ones without macrophage membrane modification	NPs could effectively reach the tumor site 24 h after administration Exhibited a greater decrease of tumor volume There wasn't a significant body weight reduction No negative effect on lung, heart or spleen but slight injuries in the liver and kidney	(74)

Metallic oxide-based NPs have been reported as intrinsic therapeutic agents for the treatment of OS since they can be effective without carrying chemotherapy drugs.

ZnO and CeO2 NPs were phytosynthesised using the leaf extract of Rubia cordifolia L.

These NPs were tested on cultured MG-63 human OS cells in which they induced cell death (apoptosis and necrosis) through the generation of ROS. (68)

Paclitaxel (PTX)-loaded PLGA NPs coated with 143B-RAW hybrid membrane (from the OS cell membrane and macrophage cell membrane) NPs (PTX-PLGA@[143B-RAW] NPs) were formulated and tested on OS cell line 143B *in vivo* and *in vitro*. (74)

Researchers observed that in both essays PTX-PLGA@[143B-RAW] NPs promoted the uptake of PTX by 143B cells and induced their apoptosis.

In vivo, PTX-PLGA@[143B-RAW] NPs exhibited chemotactic effects on pre metastatic niches in inflammatory environments and better targeting efficacy, inhibition of tumor growth and lower toxicity than free PTX.

NPs that also contained PTX were prepared with HA, bovine serum albumin (BSA) and tested on OS (143B) cells by Liu and collaborators for post-surgical cancer treatment of OS in situ. (71)

The main purpose of this formulation was to allow the sustained release of this antineoplastic agent and of Ca ions in a fetal osteoblastic (hFOB 1.19) cell culture *in vitro* and against 143B cells *in vivo*.

In this study researchers concluded that *in vitro* these NPs had low cytotoxicity to hFOB 1.19 cells and promoted osteogenic differentiation of BMSCs while inducing higher apoptotic rates in 143B cells and reducing their proliferation, migration and invasion abilities when compared with PTX by itself.

The sustained drug and Ca release, the osteogenesis effect, the antitumor effect and the inhibition of tumor metastasis activities were also observed *in vivo* with lower toxicity when compared with the PTX treated group.

Another drug that is used as an antineoplastic is doxorubicin (DOX).

This drug was encapsulated in MS–coated bismuth sulfide NPs that were covalently conjugated with arginine-glycine-aspartic acid (RGD) peptide. (72)

The presence of bismuth sulfide in this formulation enabled X-ray CT imaging and NIR-responsive photothermal therapy–chemotherapy. This is a major advantage for OS treatment since the NIR laser irradiation only applies to the tumor site, controlling the release of DOX and reducing side effects to normal tissues. (75)

Highly malignant OB line cells UMR-106 were used *in vitro* and injected into mice *in vivo*.

These NPs actively targeted OS cells thanks to RGD and the therapeutic effects were enhanced by targeted photothermal therapy–chemotherapy in comparison to only chemotherapy or PTT.

In vivo, UMR-106 tumor-bearing mice exhibited shrinked and damaged tumors and no metastasis were observed.

During the 2 weeks of treatment with NPs, no obvious damages in major organs were observed. However, without more studies this isn't enough to assume with certainty that these NPs have good biocompatibility.

Other drugs besides antineoplastics can be loaded into NPs to treat OS, like antiangiogenics. These prevent tumor growth and eventual metastasis. However, due to drug resistance and poor accumulation in OS cells, the use of these drugs can lead to apoptosis resistance.

Apatinib, an antiangiogenic tyrosine kinase inhibitor, was encapsulated in hydrophobic poly(ester amide) NPs in order to improve accumulation of the drug in the tumor. (70)

This formulation increased apoptosis and necrosis to a greater extent when compared with apatinib by itself both *in vitro* and *in vivo* with no histological changes in the main organs of mice.

Lipid-polymer NPs were loaded with an active metabolite of vitamin A, ATRA, and combined with CD133 aptamers that target CD133+ OS cells. (69)

Both *in vitro* and *in vivo* essays used Saos-2 cells, a human OS cell line.

In vitro, researchers evaluated if OS initiating cells were indeed the ones with CD133 marker and were able to conclude that tumor-sphere formation was increased in CD133+ OS cells in comparison with CD133- cells.

In vivo, Saos-2 subcutaneous xenografts were transplanted into mices which after 10 days were treated with the formulated NPs. Proportion of CD133+ OS initiating cells decreased with NP treatment.

Other studies also resorted to SaOS-2 and to Saos-2 subcutaneous xenografts to test the effect of formulated NPs. (73)

These NPs were formed by adding tetra-sulfonated aluminum phthalocyanine to poly-methyl methacrylate core-shell fluorescent NPs (AlPcS4@FNPs) and loaded into human MSCs to make the most of their ability to migrate and infiltrate the tumor site.

Thanks to photosensitive tetra-sulfonated aluminum phthalocyanine, photodynamic therapy effect is achievable in the near-infrared light with an LED source.

AlPcS4@FNPs were internalized and retained by MSCs without losing or altering their motility both *in vitro* and *in vivo* and in both essays they induced a high death rate in SaOS-2 cells.

7.1.2 Nanoparticle Treatments for Ewing's Sarcoma

Table 6. Examples of different studies evaluating the potential use of nanoparticle treatment for Ewing's Sarcoma.

Type of NP	Associated to	Main Findings		Ref
		In vitro	*In vivo*	
AgCl-NPs and Ag/AgCl-NPs	-	The RPE-1 cells, which were used as a model of nontumor cells, were less affected by the administration of AgCl-NPs or Ag/AgCl-NPs A673 tumor cells had significantly reduced number and viability levels when treated A673 cells also showed a significant increase in ROS production and loss of mitochondrial membrane potential, which culminated in an increase in the percentage of apoptosis among the population	-	(76)
mPEG-PCL	ML111	NPs have the required size and nearly neutral charge, which should result in extended blood levels, enhanced tumoral accumulation via passive targeting, minimal renal clearance, and impaired detection by macrophages Spontaneous leakage of ML111 from this NP formulation is unlikely, and the preparation appears to exhibit inherent sustained release properties Is nontoxic to non-malignant HEK293 cells	The NP was used for *in vivo* efficacy studies of ML111 Reduction in tumor growth was observed in treatment groups Control groups were euthanized before 28 days due to the limitation of allowable tumor volume while the treated groups survived until day 48, at which point the mice were euthanized for further histological and toxicity studies. Mice treated with ML111-NP did not exhibit loss of body weight	(77)

RPE-1 = non tumor human cells; EwS = Ewing's Sarcoma; NanoTLZ = nano talazoparib; CTC = Circulating tumor cell; CPT = camptothecin; MDA-MB-231 = breast cancer cells; ALN = Alendronate; BTZ = bortezomib; pSiNP = porous silicon NPs; AgCl-NPs = silver chloride NPs; Ag/AgCl-NPs = silver/silver chloride;

Table 6. Examples of different studies evaluating the potential use of nanoparticle treatment for Ewing's Sarcoma (cont.).

Polymeric	ML111	NPs compromise the viability of EwS cells without affecting non-malignant cells. NP exhibit strong synergistic effects in combination with vincristine on EwS cells, while this drug pair exhibits antagonistic effects towards normal cells	NP efficiently accumulate in orthotopic EwS xenografts after intravenous injection and provide superior therapeutic outcomes in a combination with vincristine without evident toxicity	(78)
	TLZ	-	NanoTLZ preferentially accumulates in tumors, likely through the EPR effect, and presents a pronounced target inhibition effect	(79)
Au	topoisomerase I inhibitor SN-38	The uptake of the NPs by Ewing sarcoma cells was more than 99% The viability of all cells treated with the NPs was significantly diminished when compared to the no treatment control The Ewing sarcoma cells also showed diminished viability when treated with the NPs, while the viability of U-2 OS, which lacks the mRNA in study, was not significantly affected NPs with SN38 also significantly inhibited the growth of Ewing sarcoma cells in long-term clonogenic growth assays	Over 60% of Ewing cells were positive for fluorescently labeled Au-NPs 24 hours after a single intratumoral injection The viability of the tumors treated with the NPs was significantly diminished in comparison to the tumors injected with PBS or NPs lacking SN-38	(80)

RPE-1 = non tumor human cells; EwS = Ewing's Sarcoma; NanoTLZ = nano talazoparib; CTC = Circulating tumor cell; CPT = camptothecin; MDA-MB-231 = breast cancer cells; ALN = Alendronate; BTZ = bortezomib; pSiNP = porous silicon NPs; AgCl-NPs = silver chloride NPs; Ag/AgCl-NPs = silver/silver chloride;

Part of the management of ES requires systemic treatment like chemotherapy, the most common agents being vincristine, DOX and cyclophosphamide, however this strategy leads to cumulative toxicity. (81)

To try to avoid said toxicity, polymeric ML111, a novel drug against EwS cells, NPs (ML111-NP) were developed and combined with pre-existing drugs for this disease. (78)

After developing eight different formulations researchers reached the conclusion that NPs exhibited a strong synergistic effect with vincristine.

Encapsulated ML111 had a more efficient accumulation and retention and was 2.4 times more potent than ML111 by itself.

Both *in vitro* and *in vivo* studies confirmed that this combo led to a decrease in the IC50 value of vincristine by ten to several hundred-fold in different EwS cell lines.

Another study also used ML111 but to avoid said solubility issues they chose to deliver the drug via methoxy polyethylene glycol-poly(caprolactone) block polymers (mPEG-PCL). (76)

SK-N-MC, a Ewing's sarcoma (EwS) cell line, presented really low viability *in vitro* which was consistent with *in vivo* experiments since mice bearing subcutaneous SK-N-MC xenografts had a reduction in tumor growth of 67% and 85% to doses 4.5 and 15 mg/kg, respectively.

Previous studies reported that Poly-ADP ribose polymerase (PARP) had an effect in the regulation of the oncogenic EWSR1-FLI1 fusion protein. Since the fusion of EWSR1 gene with FLI1 is associated with 85% of ES tumors, inhibiting PARP activity could be a promising therapy. However, PARP inhibitors haven't performed as desired in *in vivo* studies. (82,83)

A nanoformulation of talazoparib (NanoTLZ), a potent PARP inhibitor, was developed to potentiate the neoplastic effect of temozolomide (TMZ). (79)

At the end of the study 4 out of the 10 mice treated with this combination had no palpable tumors. However, one of the other 6 mice did not tolerate the combination therapy.

SN38 was anchored to Au NPs via oligonucleotides that only allowed the release of the drug in the presence of complementary mRNA only found in ES cells which allowed a more controlled and selective release of SN-38, an irinotecan used as an antineoplastic agent. (80)

The growth of ES cells was inhibited *in vitro* and tumor viability significantly decreased *in vivo*.

Ag-based NPs have shown potential to treat other bone disorders, as previously mentioned.

The antineoplastic effect of AgCl-NPs, produced by microalgae, and Ag/AgCl-NPs, produced by yeast, was evaluated against a coculture of human RPE-1 cells (non-tumor cells) and A673 cells (EwS). (76)

Both NPs practically didn't affect RPE-1 cells while exhibiting cytotoxicity since they reduced the number and viability of A673 cells by increasing ROS production which led to a rise in the percentage of apoptosis. Comparing these NPs, AgCl-NPs induced higher lysosomal damage on A673 cells.

7.2 Bone metastases

Bone can host numerous types of cancers that are either primary or secondary, the second being the most common malignancy of the bone and evolving mainly from advanced-stage cancers. (84,85)

It is a common site for metastases since the bone marrow environment is rich in growth factors, cytokines and adhesion receptors and because the blood flow is relatively slow. (86)

Metastatic bone disease represents a common complication in patients with advanced breast and prostate cancers, occurring in 65 to 80% of them having a higher incidence in multiple myeloma whose incidence ranges from 70 to 95%. Other advanced carcinomas also metastasize to the bone such as thyroid, lung, and renal carcinomas with an incidence of 35 to 42%. (84,87–89)

This pathology can lead to bone fractures, hypercalcemia, spinal cord injury and pain. The spine is the most common region for metastatic bone lesion being followed by the pelvis. (84,86,87)

As medical management improves the overall survival of patients with cancer the number of patients with this type of metastasis is increasing but the current therapeutic scenarios for this pathology by resorting to chemotherapeutics, surgical methods or radiotherapy are still suboptimal, since patients often have a poor therapeutic response.

This scenario creates a need for novel palliative options for bone metastasis. (85)

Table 7. Examples of different studies evaluating the potential use of nanoparticle treatment for Bone Metastases.

Type of NP	Associated to	Main Findings		Ref
		In vitro	*In vivo*	
Ca phosphate-Polymer hybrid	zoledronate and docetaxel	Good targeting ability Great binding affinity with bone tissue Accumulated in bone and tumor tissues for a long time Inhibited the growth of tumor cells in in-vitro 3D bone metastases model of bone metastases Reduced the number of prostate cancer cell and significantly reduced the number of bone lacuna in in-vitro 3D model of bone metastases of prostate cancer by reducing the number of OC	Stronger anti bone metastases of prostate cancer activity *in vivo* as compared with the same dose of DTX+ZOL	(90)
Fluorescenc e-labeled magnetic	Cis	Could effectively suppress the migration of CTC-derived cells CTC survival was more effectively inhibited by the NP	Pulmonary metastasis was significantly inhibited Tumor growth was suppressed to the greatest extent by treatment with NPs compared with other groups CTC survival could be inhibited effectively	(91)
ALN and poly(amidoa mine)	Docetaxel	decreased the number of cancer cells as compared with control group and free DTX group (enhanced anticancer activity) Inhibited the formation of OCs	More NPs accumulated in cancer tissue Suppression of bone resorption, pain response and growth of bone metastases	(92)
Dendrimer conjugated with catechol and PEG groups	BTZ	Efficient internalization of the BTZ complex by breast cancer cells such as MDA-MB-231 cells The targeting and pH-responsive properties of the BTZ nanomedicine are beneficial for increasing the therapeutic outcome and reducing adverse effects of BTZ during cancer therapy	The animals treated with the targeted BTZ had the smallest tumors among the groups Low toxicity of the NPs Depressed the progression of metastatic bone tumors and significantly inhibited the tumor-associated osteolysis	(93)

ALN = Alendronate; Cis = Cisplatin; Ca = Calcium; CTC = circulating tumor cell; ZOL= Zoledronic acid; CuS = Copper monosulfide; DOX = Doxorubicin; MDA-MB-231 = breast cancer cells; pSiNP = porous silicone NP; BT-isMOF = bone targeting immunostimulatory metal–organic framework

Table 7. Examples of different studies evaluating the potential use of nanoparticle treatment for Bone Metastases (cont.).

superparamagnetic iron oxide	RD6 furin inhibitor peptide	Furin is important for OC formation and bone resorption Furin is required for the invasive and metastatic potential of breast cancer cells and that the RD6 peptide is a non-cytotoxic yet potent and efficient peptide inhibitor of Furin function Inhibitory effect on cellular migration High affinity binding to the HA component of bone	Effectively target to the tumor-bone site where it exerted its inhibitory effect on target cells (MDA-MB-231) thereby reducing the tumor size and therefore T2 MRI signal Seen accumulating on the trabecular bone surface of the tibial bone Tumor size was significant reduced	(94)
Porous silicon	Camptothecin	Capable of killing triple-negative BCa cells Specific binding to EGFR-overexpressing cells	The anti-EGFR targeting Ab did not provide an advantage in facilitating the internalization of pSiNP into cancer cells compared to non-targeted pSiNP probably due to the biological barriers present in the *in vivo* tumor microenvironment Reduced tumor growth and metastatic spread Reduce metastasis to lung, liver and murine bone	(95)
Metal-organic framework with ZOL	Immunostimulatory cytosine–phosphate–guanosine (CpG) oligonucleotides	enhanced the cellular uptake of CpG by endocytosis exhibited the strongest inductive effects on macrophage polarization to M1 phenotype favorable effect of BT-isMOF in suppressing OC formation and preventing bone resorption binding capacity of ZOL with calcium phosphate	The ratio of M1 macrophages in the bone marrow was significantly elevated Favorable effect of BT-isMOF in suppressing OC formation and preventing bone resorption specific deposition and accumulation of BT-isMOF on bone tissues Very little targeting or accumulation of the functionalized MOF NPs were seen in the kidney and liver The morphological structure of the tibial bone did not change at 4 and 8 weeks after treatment, clearly indicating the inhibition of bone resorption Polarization of macrophages to the pro-inflammatory M1 phenotype within the tumor-bone microenvironment	(96)

ALN = Alendronate; Cis = Cisplatin; Ca = Calcium; CTC = circulating tumor cell; ZOL= Zoledronic acid; CuS = Copper monosulfide; DOX = Doxorubicin; MDA-MB-231 = breast cancer cells; pSiNP = porous silicone NP; BT-isMOF = bone targeting immunostimulatory metal–organic framework

Table 7. Examples of different studies evaluating the potential use of nanoparticle treatment for Bone Metastases (cont.).

CuS	DOX	DOX was effectively loaded 90% of DOX was released from CuS@MSN-DOX with laser irradiation in 24 h (15% more than without) Apoptosis could be observed among U87 cells incubated with the NPs Full tumor elimination was achieved by the intra-tumor injection of the NPs with a low laser irradiation dose	Tumor growth was efficiently inhibited	(97)

ALN = Alendronate; Cis = Cisplatin; Ca = Calcium; CTC = circulating tumor cell; ZOL= Zoledronic acid; CuS = Copper monosulfide; DOX = Doxorubicin; MDA-MB-231 = breast cancer cells; pSiNP = porous silicone NP; BT-isMOF = bone targeting immunostimulatory metal–organic framework

Bortezomib (BTZ) is an antineoplastic drug authorized by EMA to treat multiple myeloma, in combination with other drugs, and mantle cell lymphoma.

When tested by researchers against Giant Cell Bone Tumor this drug had the ability to induce tumor cell apoptosis and inhibit OC recruitment evidencing its potential to treat bone tumors. (98)

This drug was loaded on a RGD-targeted dendrimer conjugated with catechol and PEG groups in order to reduce its side effects and poor penetration in the solid tumors. (93)

The catechol linkage enabled controlled and pH sensitive release of the antineoplastic to metastatic bone tumors and RGD, and integrin ligand, allowed efficient internalization since integrin is highly expressed by these metastases. (99)

MDA-MB-231 cells (breast cancer) were cultured with these NPs. *In vitro* results showed that BTZ release occurred when NPs were in contact with the cell's endolysosomes leading to cancer cell death.

In vivo studies showed that these NPs led to 20-fold increased tumor accumulation of BTZ when compared to the drug by itself, inhibiting metastatic bone tumor growth.

RGD was linked to copper monosulfide (CuS)-based NPs loaded with DOX for active targeting. (97)

DOX release was triggered with NIR light. This type of treatment is classified as chemo-photothermal therapy (chemo/PTT) and eventually leads to thermal ablation of tumor cells.

Since it isn't an oxygen dependent method it can be used to overcome the problems of tumor metastasis caused by hypoxia.

The combination of Chemo/PTT with these NPs led to a therapeutic effect *in vivo* 3.53 times higher than therapy approaches without this combination.

Researchers also found that this strategy could inhibit tumor liver metastasis.

Other studies also created pH responsive NPs.

Ca phosphate-polymer hybrid NP (DTX@Cap/HP) were formulated so they could deliver, in an acidic and high in GSH environment, Zoledronic acid (ZOL) to bone metastases tissue and DTX and ZOL in cancer cells. (90)

In vitro 3D model DTX@Cap/HP inhibited metastasis that resulted from prostate cancer cells and bone lesions and *in vivo* significantly decreased the metastases compared to free DTX and ZOL.

DTX was loaded into polyamidoamine (PAMAM) that was connected to ALN (DTX@ALN-PAMAM) via pH-sensitive bond in order to treat lung cancer bone metastases. (92)

In vitro, DTX@ALN-PAMAM had better cytotoxicity and ability to inhibit OC formation than DTX by itself. This was also verified in treated mice since NPs accumulated at bone metastasis site suppressing its growth.

Cisplatin (Cis) was loaded into magnetic silica NPs (Cis@Fe3O4@MSNs) that were modified with PEG-linked peptide that targeted CXCR1 and polyacrylic acid (PAA), a pH-responsive material. (91)

CXCR1 is a receptor for IL-8 that is overexpressed on circulating tumor cell (CTC), so by linking a targeting peptide to the NPs researchers were able to precisely deliver Cis, inhibiting metastasis of OS in both an orthotopic OS model and patient-derived tumor xenograft model.

Bone metastasis are the most common form of metastasis associated with breast cancer, therefore researchers are always studying new NPs to target these tumor cells. (100)

Metal-organic framework (MOF) NPs had their surface modified by addition of ZOL and were loaded with immunostimulatory cytosine-phosphate-guanosine (CpG). (97)

OC activity and macrophages have an important role in the bone tumor environment so when *in vitro* essays showed that the NPs inhibited OC formation and induced macrophage activity researchers were able to see the potential of this approach.

The intratibial murine model of breast cancer bone metastasis showed specific targeting by NPs, due to strong binding to calcium phosphate, and suppressed OC-mediated bone destruction.

Another strategy that was developed against bone metastasis of breast cancer was the development of porous silicon NPs (pSiNP) loaded with camptothecin (CPT), a chemo drug, displaying cetuximab, an antibody. (95)

This antibody targets epidermal growth factor receptor (EGFR) which is found in the triplenegative BCa cells that were cultured with the NPs.

These study’s results showed that although these NPs didn't really influence primary tumor growth reduction, they significantly impaired the metastatic burden in humanized bone and in other organs such as lung and liver.

8. Conclusions and Future Perspectives

Nanomedicine is a relatively new strategy to treat bone disorders that has better outcomes compared with traditional approaches, thanks to the many properties of NPs.

However, despite all the research and development of new NPs, most of them don't reach clinical trials, not going past the preclinical stage that consists of *in vitro* and animal testing.

There are some challenges that need to be addressed so that NPs for bone disorders could move to other stages.

Some of the NPs that were recently developed accumulate near the target site but still lack bone targeting ligands that are specific for the disorder in question. This happens with NPs for primary and secondary bone tumors that instead of binding to neoplastic cells end up binding to bone tissues, for which they present more affinity. This poses a challenge for NP accumulation in the tumor.

NPs also present some concerns related to their safety since some of them are small enough to pass through biological barriers, such as the blood–brain barrier one.

Despite all the issues, in the future studies *in vivo* could be long term to explore NPs behavior and biocompatibility.

Active targeting strategies should also be pursued using antibodies or acid/enzyme responsive linkers. This would allow a more controlled release of carried molecules, improving therapeutic efficiency and reducing side effects.

In the future, combinations of different therapies, such as PTT and NPs that carry antineoplastic agents, should also be explored. These have a synergic effect that allows prolonged therapeutic window and reduction of drug dosage that leads to less side effects due to chemo drugs and reduces the probability of drug resistance.

NPs as therapeutic agents for bone disorders hold great promise and with the authorization and introduction worldwide of the SARS-CoV-2' vaccines containing lipid-mRNA NPs, the field of nanomedicine is entering a new era.

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