

Advances of the Nanotechnology in Targeted Nanomedicines for Treatment of Bone Cancers and Diseases

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Abstract: The ever-evolving field of nanotechnology has been applied over the years as an amazing potential tool in bone disorders through the development of targeted drug nanosystems. Bone diseases can be referred as any bone condition able to cause morbidity or even mortality to the host. Osteosarcoma has been most investigated condition for management and treatment by nanomedicine, and several nanomaterials such as nanoparticles, nanocapsules, nanospheres, nanodiamonds are being used for drug delivery. Likewise, other bone diseases, such as osteoporosis, osteonecrosis, osteoarthritis, bone tuberculosis have received nanotherapy with a large rate of success. The nanomedicine seems to make up a targeting lack by conventional therapy and chemotherapy, besides appointing to a trend of conservative clinical treatment, especially for severe disorders. Additionally, nanomedicine has advanced over the years, therefore, there is a strong need to accelerate its application in bone diseases reducing the mortality rate related to these conditions. This review article provides an overview on the advances of nanotechnology over the last two decades and highlights the pathways of investigations for targeted delivery nanoensembles.

Keywords: Nanotechnology, Nanomedicine, Nanodrugs, Bone Cancer, Osteosarcoma, Bone Infection, Osteoarthritis, Targeted Delivery.

INTRODUCTION

The first idea of nanotechnology has been launched by the physicist Richard Feynman in 1959, who suggested the development of a molecular machine; while the term “nanotechnology” was first used in a scientific paper, in 1974 [1]. Currently, this term means a multidisciplinary field that integrates the engineering with the biology, chemistry and physics in a unique phase of the scientific field at a nanoscale, and branching to the health area among others. So, the well-known term bionanotechnology refers to the application of nanotechnology in organism microenvironment.

The nanomaterials are produced in a range of less than 100 nanometers (nm), as called above “nanoscale”, corresponding to the size of cells and molecules. To achieve the performance at cellular or molecular level. This characteristic is extremely beneficial as so-called nanomedicine concerns to the precision of bioaction and high effectiveness by drugs with minimal or no side effects. This hypothesis is leading the nanotechnology for wide interest of researchers across the world with a large progress made during the past decades.

Bone cancer or diseases such as osteoarthritis, rheumatoid arthritis, bone tuberculosis, osteoporosis, and osteomyelitis remain a serious challenge for

physicians and a trouble for their patients. The growing attention on nanomedicine is driven to improve the treatment of such disorders as well as to prevent them [2,3].

Nanodrugs and drug delivery nanocarriers present improved pharmacokinetics and consequently offer several advantages, such as carrying the therapeutic agents to the target site while keeping a safe plasma drug concentration, protecting other molecules and cells, and improving the drug circulation with a stable drug retention time in body. Besides, these drug nanosystems improve the cell tolerability and the therapeutic effects accelerating the treatment and minimizing the immunogenicity [4,5], and are expected to revolutionize the pharmaceutical manufacturers, shortly. Aside from treating bone diseases, endowing the nanosystems with the ability to recognize bone injuries is of great interest.

These biological properties are designed not only to treat such bone issue, but also help in preventing the bone tissue destruction, contributing to a fast and accelerated bone repair, and finally restoring a better quality of life for patients. In this regard, some authors have reported the employment of some drug-loaded nanosystems such as nanocapsules, nanoclusters, nanospheres, and what seems the most advanced use is to direct the nanoparticles straight toward the bone cancer or bone metastasis thus inhibiting its development and growth of metastatic cells *in vitro* and *in vivo* while preserving the surrounding healthy tissue [6-8]. The same effect is being tried to be achieved in

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bone infections *in vitro* and *in vivo*, with investigation on nanoparticles, nanopellets and nanodrugs loaded or coated with antibiotics to eradicate the infection and induce the anti-inflammatory effects [9-11].

In this review, the advances in the application of nanotechnology for the treatment of bone cancers, metastasis and diseases are highlighted focusing on the main trends and progress of the nanomedicine during the past two decades and the advantages of applying nanosystem in targeted drug delivery within the biological microenvironment. Also, the role of various nanomaterials and nanosystems with strong potential for medical applications has been discussed.

BONE TISSUE MICROENVIRONMENT

The bone is a mineralized and vascularized connective tissue with a highly dynamic metabolism and an active remodeling, a process called the bone-turnover, although macroscopically it seems inert.

The bone tissue is organized into an extracellular matrix with about 35-40% organic content (type I collagen fibers, glycoproteins and proteoglycans) and 60-65% inorganic content (specially calcium and phosphorous originating calcium phosphate); the bone cells such as the osteoblasts or bone-forming cells raised from osteoprogenitor cells, which in turn come from mesenchymal stem cells lineage (MSCs), the osteocytes with their dendritic processes that produce an interconnected canaliculi network, and the osteoclasts arising from hematopoietic stem cells (HSCs) to carry out the bone resorption [12].

Osteoblasts are single-nucleus bone cells responsible to produce the bone matrix or the osteoid [13]. This matrix is comprised of a combination of extracellular proteins, such as sialoprotein (calcium-ligand), osteocalcin (vitamin K-dependent; binds calcium to bone), osteopontin (cellular-adhesion), alkaline phosphatase (ALP; signaling the osteoblastic activity), and mostly collagen type I [14,15] which represents about 90% of bone matrix. The cell differentiation is signaled by bone morphogenetic proteins (BMPs) and growth factors (GFs), while their functions are orchestrated by endocrine organs, too. Osteocytes are in majority in the bone tissue trapped within the osteoid matrix and secrete sclerostin to activate osteoclasts and to inhibit osteoblasts [16]. Besides, they contribute with endocrine functions by secreting hormones to regulate mineral homeostasis and hematopoiesis [17]. While trapped into the bone matrix the cellular processes of these osteocytes give

rise to a canaliculi network. Bone remodeling refers to the dynamic bone metabolism achieved by removal of old bone tissue by osteoclasts and a simultaneous bone neoformation by osteoblasts [18]. Thus, any disturbance in this mechanism may lead to in a bone disorder.

Bone-forming osteoblast is typically 25 nm in size [19]; hydroxyapatite (HA), the inorganic component of bone matrix formed by the union of calcium and phosphorous ions, has about 20-80 nm in length and 2-5nm in width [20]; type I collagen, the major organic component of bone tissue has an average of fibrils with 300 nm in length and 0.5 nm in width [12]. These ranges explain the concept of scaling of the nanotechnology mentioned above. The bone matrix and its compounds are the starting point for investigating the range of nanomaterials that may be applied in bone cancer and disease; the goal of bionanotechnology in nanomedicine is to enable the nanosystems for interactions at cellular level, with high precision and effectiveness.

Regarding the microarchitecture of the bone, this tissue comprises of a large system of vascular canals in of a mineralized tissue; the central canals surrounded by bony lamellae with many canaliculi and transversal canals giving rise to the osteon so known as Havers system (Figure 1). All this canaliculi network is interconnected ensuring the nutrition and hemodynamic balance of the bone tissue. The physiological processes such as bone turnover, remodeling and repair, are dependent on this microenvironment of bone tissue.

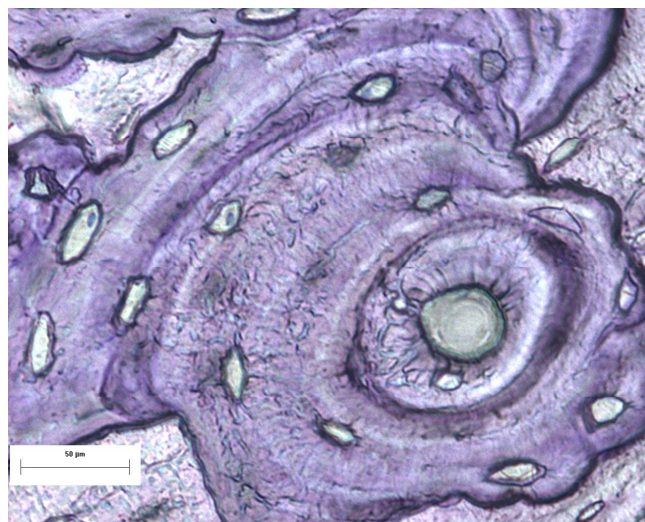


Figure 1: Light microscopy (H/E) showing an osteon (Havers system), osteocytes, and all vascular channels interconnected, which includes the canaliculi and the central canal, into bone tissue.

For the past two decades the nanotechnology has been emerging in the field of health through the so-called nanomedicine as an efficient tool to treat cancer and diseases of the bone. The drug nanosystems are promising to attack tumor cells with high efficacy and minimal damage to the surrounding non-ill cells, to be beneficial in bone metabolic conditions, and to fight the infection and decrease the inflammation. The nanosystems are produced from various types of carriers, and they can deliver the therapeutic agents to targeted niche in high concentration and high specificity to sick cells with non-toxicity to healthy cells. The hope of cure through nanomedicine also arises from the fact that bone disorders with large destruction of the bone tissue can clinically lead to a deformation or amputation of parts of the human body restricting the quality of life, or the metastatic conditions may become potentially fatal.

NANOMEDICINE FOR BONE CANCER

Cancer in general seriously affects the development of social care at all levels [21] and it is becoming a major public health problem in several countries. Many cancer-related deaths also could be due to metastasis from a primary tumor. The bone tissue is the predominant niche to developing metastasis commonly from prostate and breast cancer, two of the most common cancer types affecting people worldwide [22,23].

Sarcomas are a group of malignant tumors that can arise both from hard or soft tissue, such as bone, cartilage and muscle, is aggressive and massively destructive and may be lethal. Among bone sarcomas, the osteosarcoma (OS) is the most common primary tumor that originates from the malignant proliferation of mesenchymal cells to produce bone-like matrix [24] with high recurrence and low survival. Several therapies and methods in nanotechnology have been investigated to manage these tumors. The Ewing sarcoma is a metastatic bone cancer in children and young adults characterized by a genetic abnormality of chromosomal translocations [25]. Multiple myeloma is a rare bone cancer, but the second most common hematologic malignancy in elderly people with a considerable morbidity pathological plasma cell leukemia, extramedullary myeloma and end-organ destruction [26].

Nano-hydroxyapatite (nano-HA/nHA) has a microstructure similar to natural bone which has been investigated over years due to some well-known features such as its probability to be carried with anti-

cancer drugs and having the same compounds as the inorganic content of bone matrix, thus able to allow the cell adhesion and proliferation for bone remineralization [11,27].

Nano-HA has been employed in two sizes (20 and 80 nm) for biological response of OS cells where it inhibited the cell growth via inducing the caspase-9 dependent apoptosis. The inhibition of cell growth and apoptosis were size-dependent where the larger nanoparticles were more effective than smaller ones [27]. The nHA may induce the osteoblast adhesion, proliferation and the alkaline phosphatase synthesis thus promoting the hard tissue repair. Moreover, the smaller nHA was more similar in features to the natural HA during the process of biomineralization than the larger ones [27]. It can be stated that this potential of biomineralization from calcium phosphate nanomaterials may be applied for bone replacement as supporting of repairing on osteosarcoma treatment.

Currently, the targeted delivery drug for bone diseases is achieved mainly by using bisphosphonates (BP), such as alendronate (ALE) or zoledronate (ZOL), which is the gold pattern drug to decrease the bone lysis. This type of drug has affinity to bone and its time of staying in organism could be decreased when it is a part of a drug delivery nanosystem, because of its stability to lead out the medicament towards the targeted niche. Also, doxorubicin (DOX) is an active antitumor drug which inhibits the tumor cells proliferation, and is widely applied for bone tumor treatment. Nanomedicine or delivery nanosystem depends not only on the physiochemical and pharmacological properties of the drugs, but also on the stability and physical characteristics of nanosystems that are required for a good working with high effectiveness.

Targeted nanoparticles (TNPs) made up of alendronic acid (ALE) and poly(lactic-co-glycolic acid) polymeric core (PLGA) encapsulating doxorubicin (DOX) with hydrophilic phosphate groups on its surface, had been acting as a drug reservoir in an *in vitro* assay against mouse osteosarcoma KM7M2 cells [28]. The DOX-loaded TNPs when compared with free DOX TNPs *in vitro* showed an increased cellular cytotoxicity in a time- and dose-dependent fashion. The exterior phosphate groups of TNPs were attracted by protein tyrosine phosphatases receptor on surface of OS cells and facilitated the accumulation of TNPs in the targeted cancer cells leading to the increase in intracellular drug concentration and consequently higher cytotoxicity due to the rapid cellular uptake from

DOX-loaded TNPs. This nanosystem strongly binds to mineral bone aiming to target the bone microenvironment and had been shown active accumulation into the OS cells with a dose-depending toxicity. Meantime, the higher dose occurred by an increased incubation time and led to an enhancement of cellular uptake, since this feature is a time-dependent uptake. Higher dose concentration revealed that TNPs deliver a higher dose on cells, boosting the nanocarriers in a promising and effective way for osteosarcoma treatment. This mechanism occurred due the cell membrane specific interaction with the phosphate moiety of ALE in a typical endocytosis-mediated receptor uptake [28].

The use of DOX in targeted-drug nanosystems seems to be unanimous in the scientific community to treat OS. So, DOX has been incubated with lipid-modified dextran derivative to form NPs which were non-cytotoxic, played an anti-proliferative activity and induced the apoptosis of OS cell lines by intracellular drug accumulation. All results of DOX-NPs overcame the results from DOX alone [29]. This research has shown the expected effectiveness of the DOX-loaded nanosystems.

The aforementioned report leads to the hypothesis that the targeting specificity of nanomedicines for most cancer cells is an important characteristic that needs to be further investigated in *in vitro* experiments and applied *in vivo* because these cells show a progressive and aggressive profile.

A drug delivery nanosystem including HA, bovine serum albumin (BSA) and paclitaxel (PTX), consisting of nanoparticles 90 nm in size, for OS cells (143B) and osteosarcoma treatment was investigated recently by Liu *et al.* [30]. The HA-BSA-PTX NPs had high drug loading efficiency and sustained drug releasing ability. The *in vitro* assay showed significant cytotoxicity for the OS cells, which is a drug concentration-dependent effect of NPs with inhibition of tumor cell growth followed by cell apoptosis. Another important discovery of this study was that this drug delivery nanosystem reduced the migration ability of the tumor cells, greatly weakening the cell invasion on healthy tissue and consequently preventing a tumor metastasis; HA properly promoted the osteogenic differentiation of bone marrow stromal cells (BMSCs). Firstly, the inoculation of 143B cells was performed on a nude mouse model to simulate tumor *in situ*, then the *in vivo* treatment with HA-BSA-PTX NPs gradually decreased the tumor revealing a good therapeutic effect and controlling the growth rate of osteosarcoma as

compared to the control group (HA-BSA NPs) which showed signs of tumor metastasis distantly into lungs, liver and intestines [30]. This study reveals the effectiveness of a nanosystem loaded with an anticancer drug that may be further investigated for clinical application.

Besides the composition of nanodrug systems, it has been reported above that the content and size of NPs are essential to stimulate the biomineralization. In other words, a nanosystem is ideal when it carries the anticancer agent to targeted bone and simultaneously promotes the biomineralization instead, only stopping bone lysis. This mechanism could be reached by the use of calcium phosphate aggregated into nanosystems. Other attractive feature of NPs is their accumulation in solid tumors resulting from their enhanced permeability and retention effect [31,32]. Also, the polymeric NPs are interesting due their high biodegradation ability at the targeted site which would facilitate the local drug releasing.

Scientific community has a mutual consensus to apply the nanodrugs in bone cancer treatment. The human osteosarcoma cell line MG63 and myocardial H9C2 cell lines were cultured with a drug releasing Dox-loaded exosome (Exo-Dox). This nanosystem maintained the intact structure after it entered in tumor cells, revealing an efficient cellular uptake to induce cell death; the acidic microenvironment from lysosomes of cancer cells accelerated the Dox releasing making it a properly targeted nanomedicine in cancer treatment. Besides killing the cancer cells, Exo-Dox also demonstrated an excellent cytocompatibility and low cytotoxicity to normal cells which makes it a promising nanocarrier to load chemotherapeutic drug, Dox [33]. The growth of a tumor is conditioned to vascular proliferation to provide the nutrition to the tumor and apatinib is a known drug inhibiting this feature. So, apatinib NPs have been developed to suppress OS stemness and enhance osteosarcoma stem-like cell apoptosis due to the improved cellular uptake *in vitro* and *in vivo*. On the other hand, the apatinib alone didn't affect the cell death, which means a resistance of OSCs (osteosarcoma cells) to this isolated drug, increasing the chances of a metastasis. Apatinib was rapidly eliminated by circulation. The apatinib NPs avoided side effects on healthy cells despite enhancing the drug accumulation in OS cells [34].

A new generation of recombinant protein-based nanodrug carrier was developed to deliver the hydrophobic prodrug aldoxirubicin (ALD), which has a significant anti-tumor effect in OS. Positively charged

proteins were supramolecularly complexed with polyethylene glycol (PEG) and ALD leading to well-defined NPs (PCP-PEG-ALD) with a high efficiency of encapsulating and an optimized drug bioavailability. It revealed a long-acting anti-tumor effect due to enhanced half-life, and an excellent inhibition of lung-metastasis and cardiotoxicity in treatment of OS *in vivo*. Likewise, the bioavailability of PCP-PEG-ALD nanoensemble was greatly improved and inhibited the proliferation of OS cells *in vitro*. Pharmacokinetically, there was the releasing of ALD only after cleavage with a consequent decrease of side effects *in vivo*, contrary to non-formulated ALD [35].

To understand the histopathology of the osteolytic damage by OS, a high expression of the receptor activator of NF- κ B ligand (RANKL) on the surface of OS cells lead to an enhancement of the differentiation and activation of osteoclasts. Zolendronic acid (ZA) plays a substantial role in reducing of RANKL expression in OS cells [36]. This anti-osteolytic effect is the main reason to aggregate ZA into a drug nanosystem to treat OS. A core ZCD, wherein calcium ions (Ca^{2+}) and ZA form a metal-organic framework loaded with DOX, and the shell (v-R), vascular endothelial growth factor (VEGF) ligand-modified red blood cell membrane nano-vesicle gave rise to the V-RZCD nanosystem, with biocompatibility and biodegradability, which was applied to attack OS *in vitro* and *in vivo* [37]. This nanosystem revealed a large effect of OS cell apoptosis, meaning a good antitumor effect, and a significantly increased accumulation of V-RZCD on tumor site with a clearly low concentration in lungs, liver, and kidneys of mice, meaning the reduction of side effects. Whereas AZ has poor stability and rapid elimination *in vivo* [38], this nanosystem came out with an improvement for drug pharmacokinetics. Its degradation releases calcium ions to supply blood calcium and play a part in the synergistic anti-osteolysis effect with ZA, which means that the V-RZCD not only offers targeted OS treatment but also inhibits OS-mediate osteolysis [37].

Investigations are under way to find out an efficient treatment for Ewing sarcoma which is the second most common primary bone tumor; it is an aggressive form in children with metastasis in lungs and bone marrow, in a quarter of cases [39], corresponding to the five years survival rate in only 50% [40]. NPs of nanocapsules (NCs) and nanospheres (NSs) with antisense oligonucleotides (AONs) were tested in Ewing Sarcoma (EWS) tumor subcutaneously cultured in nude mice. Both NPs inhibited the xenografted tumor after an intratumor injection for AON administration

[25]. In other situations, small interfering RNAs (siRNAs) NCs were administrated in nude mice by intratumor injection to deliver siRNA and there was a significant inhibition in the tumor growth from a specific silencing inhibition of EWS-Fli1 RNA expression [41]. siRNA is a powerful tool for specific inhibition of gene expression, and has also been carried by diamond nanocrystals (50nm in mean size), being an attractive candidate for intracellular delivery with cationic polymer into EWS cells. These nanodiamonds (NDs) were coated with polyethylenimine (PEI) and polyallylamine hydrochloride (PAH), vectorized with siRNA by physical adsorption to the cell surface, and were designed to target the oncogenic EWS-Fli1 junction in the chimeric mRNA (nucleotides 822-842). Polymer coating plays role in determining the siRNA:ND uptake mechanisms used and the biological activity of the siRNA, which was efficiently delivered to cells and induced the degradation of the target mRNA by micropinocytosis, destructing the tumor cell [42]. The siRNA and AONs could be an additive for cancer therapy as a nanosystem reduces the size of tumor to an acceptable level which could be far less harmful than the conventional cancer therapy. Silver-based nanoparticles (Ag-NPs) when applied in EWS cells had an antitumor potential by the releasing of silver ions against EWS cells leading to a reduced number and viability of tumor cells. The cellular apoptosis was a consequence of lysosomal damage and changes in reactive oxygen species (ROS) production with loss of mitochondrial membrane. However, the same NPs when applied in RPE-1 cell line (pigmented retinal epithelium) didn't present any damage which means no side effects in regular cells [40].

Multiple myeloma (MM) is an incurable condition of cancer that affects the bone marrow, and by this reason some therapeutic strategies must be envisaged. Injectable and biodegradable systems based on incorporation of anti-estrogens (AEs) in NPs were synthesized to be investigated in multiple myeloma RPMI8226 xenograft model besides the breast cancer MCF-7 cell xenograft model, which induced apoptosis. AEs-NPs strongly affected MM cells growth by blockage of cell cycle progression with induction of apoptosis. However, the researchers highlighted that the pathway by which AEs exert their effects in MM cells has not been established; they indicated these NPs as a potent new therapeutic strategy for anti-estrogen administration not only for estrogen-dependent pathologies such as breast cancer but also for other diseases where estrogens receptors are needed to be targeted by anti-estrogens [6].

Biocompatibility and biodegradation allied to low cytotoxicity make polymers good candidates to carry drugs in targeted bone. The biodegradation is important to help drug releasing. ZOL has a strong affinity towards bone what makes it a perfect ligand for bone targeting in bone cancers and hard tissue metastasis, and when combined to docetaxel (DTX) it could be a good synergist in the management of bone metastasis. Thus DTX-loaded PLGA-PEG-ZOL NPs has been tested in BO2 and MCF-7 cell lines, and the DTX from NPs coupled with ZOL revealed a majorly improved therapeutic potency for the cellular apoptosis, providing an effective delivery vehicle in bone metastasis and cancer. There was a prolonged blood circulation half-life *in vivo*, less liver uptake, and high ratio of NPs concentration in bone tumor with enhanced tumor retention. This targeted efficiency is due to ZOL affinity toward bone disorder attracting more NPs to the tumor site and getting internalized by endocytosis. These conjugated NPs could be used to deliver therapeutics successfully in conditions such as bone tumor, bone metastasis or other diseases [43].

DOX-conjugated BP-NPs were engineered with PEG to increase their blood half-life and to be tested *in vitro* and *in vivo*. These NPs reached the tumor area revealing the potential to be used in both diagnosis and treatment for primary tumor or bone metastasis as an active tumor targeting. The conjugation of DOX into BP-NPs increased the anticancer activity of the drug against Soas-2 osteosarcoma cells by the uptake of DOX into cell, when compared to BP-NPs. DOX-BP-NPs revealed high affinity to osteosarcoma cells in a short-term assessment [7] providing a stronger anticancer effect to treat OS.

Nanosystem formulated with ALE/DOX Ag₂S demonstrated good blood clearance rate, a relative low release and low cytotoxicity with delivery of DOX to induce cancer cell apoptosis and inhibits the osteolysis, *in vivo* and *in vitro*. The clearance rate could be attributed to high bone affinity *in vivo*. Mineralization nodules were revealed that means potential for bone homeostasis modulation. The nanosystem was rapidly deposited in bone tissue and DOX was released in tumor site on-demand triggered by the acidic tumor microenvironment. The effective chemotherapy of bone tumor was accomplished by inhibition of osteolysis with minimal side effects and toxicity, besides promoting the on-site killing of cancer cells [8].

Another anticancer drug widely used is bortezomib (BTZ), the first proteasome inhibitor clinically approved

for the treatment of multiple myeloma, besides also being effective for osteosarcoma. When conjugated with ALE in polymeric NPs (PLGA, PEG) it has presented a good behavior towards the targeted bone with high retention, accumulation and bone homing accompanied by the decreased tumor burden of MM in mice, and extremely beneficial in prevention of cancer progression. BTZ has the ability to alter the microenvironment and prevent tumor growth via mechanisms that increase the bone volume, trabeculae number, or osteoid thickness. It may also be used as pretreatment for modifying the bone microenvironment and enhancing the bone strength and volume [44]. In another study, BTZ also has been used in polymeric NPs, which were stable and efficiently internalized by breast cancer cells with liberation of BTZ to kill cancer cells *in vitro*. BTZ nanomedicine had a long blood circulating time and a high level of apoptosis inside tumor, inhibiting the growth of bone tumor and the osteolysis in a metastatic bone tumor model *in vivo* [45]. Both studies showed the efficacy of polymeric carriers for targeted delivery of BTZ in treatment of bone tumor. The NPs with no BTZ-loading presented a minimal cytotoxicity in tumor cells with low cellular uptake indicating that the presence of anticancer agent in nanosystems is important to achieve the tumor cells apoptosis and consequently, break the cancer growth cycle.

Phytic acid (PA) is a natural compound in seeds, beans, cereals, vegetables and fruits and is a potent anticancer [46], bone targeting [47], and anti-osteoclastogenic agent [48]. It was prepared with cisplatin (CDDP), the first-line anticancer drug, to synthesize CPPA NPs, a new type of carrier-free nanomedicine for treatment of malignant bone tumors. The *in vitro* releasing kinetics of CPPA showed minimal cytotoxicity and high penetration capability, while the *in vivo* assessment revealed low hematological and liver toxicity with an inhibition of the tumor growth. These features could characterize the NPs as potent anticancer nanomedicine with highly reduced adverse effects. An important point is that the nanosystem also inhibited the osteoclastic differentiation of primary bone marrow monocytes which has an important role in the prevention of osteoclastogenesis to stop osteolysis. It is an ideal feature hoped from anticancer nanomedicine, especially for tumors or metastasis characterized by bone destruction [49].

Worldwide, many patients need to receive the implantation of an orthopedic material after the surgical bone resection, a common strategy to treat bone

cancer and removing large cancerous tissue in all of its edges. It is a way to avoid the recurrence of tumor and warranty a good quality life for patients. Based upon it, not only anticancer drug delivery systems are developed to act at bone site, but the chemotherapy can also be applied through the surface of orthopedic material to prevent the recurrence of cancer. So, to impart an anticancer potential to an orthopedic material, the selenium (SE) is introduced with about 25 selenoproteins containing antioxidant properties into the defense system of human body consequently preventing diseases [50], and even inhibiting carcinogenesis. SE-NCs coated titanium orthopedic

implant had suitable dose dependent release of SE, decreased the density of cancerous osteoblasts in mouse OS cells with greater number of healthy osteoblasts and ALP activity, but with no toxic effects on these cells. Nanosurface roughness presented as an efficient factor for enhancing healthy bone cell adhesion and osteoblasts function, likewise inhibiting cancerous cells densities [51].

Tumor cells from bone cancer may destroy the bone marrow and stimulate the osteolysis, meanwhile, the bone degradation further promotes the tumor growing with metastases [52,53]. In spite of drug nanosystems

Table 1: Range of Nanomaterials and its Applications in Bone Cancer and Bone Metastasis Over Two Past Decades

Year	Nanomaterial	Role of Nanomaterial (Assay)	Bone disease	Reference
2003	AON-loaded NPs	To inhibit the growth of an EWS-Fli-1 dependent tumor, which is responsible for Ewing sarcoma (<i>in vivo</i>)	Ewing sarcoma	[25]
2005	AEs-NPs	Arrest the growth of tumor (<i>in vitro</i> e <i>in vivo</i>)	Multiple myeloma	[6]
2006	siRNA-loaded NCs	Anticancer activity, inhibition the growth of EWS-Fli-1 (<i>in vivo</i>)	Ewing sarcoma	[41]
2009	DOX-loaded NP system	Anti-proliferative effects (<i>in vitro</i>)	Osteosarcoma	[29]
2010	Nano-HA	Inhibited and led to apoptotic cell death of the tumor cells (<i>in vitro</i>)	Osteosarcoma	[27]
2010	SE-NCs coated titanium orthopedic material	It is chemopreventive, inhibited cancerous cell functions, and promoted healthy osteoblast functions (<i>in vitro</i>)	Bone cancer resection	[51]
2012	PLGA-PEG-ZOL NPs	To deliver therapeutics towards bone and tumor site (<i>in vitro</i> e <i>in vivo</i>)	Bone tumor, bone metastasis or other bone disease	[43]
2012	siRNA-loaded NDs	To carry siRNAs into cancerous cells (<i>in vitro</i>)	Ewing sarcoma	[42]
2014	Polymeric ALEBTZ-NPs	Released the drug to target cancer, decreased tumor burden (<i>in vivo</i>)	Multiple myeloma	[44]
2016	ALE-PLGA-DOX TNPs	To carry DOX in targeted bone microenvironment (<i>in vitro</i>)	Osteosarcoma	[28]
2016	DOX-conjugated BP NPs	Increase the efficacy of the anti-cancer drug (<i>in vitro</i> and <i>in vivo</i>)	Primary and metastatic bone cancer	[7]
2017	ALE/DOX-Ag2S Nanodrugs	Inhibits the osteolysis and it kills the cancer cells (<i>in vivo</i>)	Bone metastasis	[8]
2018	BTZ nanosystem	Depressed the progression of metastatic bone tumor and inhibited the tumor-associated osteolysis (<i>in vivo</i>)	Metastatic bone tumors	[45]
2019	Nanodrug DOX	Effect anti-tumor (<i>in vitro</i>)	Osteosarcoma	[33]
2020	Nano-Apatinib (nanodrug)	Inhibits the osteosarcoma stem-like cells-derived tumor growth (<i>in vitro</i> and <i>in vivo</i>)	Osteosarcoma	[34]
2020	HA-BSA-PTX nanoparticles	To inhibit the ability of proliferation, migration and invasion of malignant cells (<i>in vitro</i>)	Osteosarcoma	[30]
2020	CPPA NPs	To ensure high bone targeting and to inhibit osteoclastogenesis (<i>in vitro</i>)	Bone metastatic breast cancer	[49]
2021	Ag-NPs	Anti-tumor activity with minimal effects against healthy cells (<i>in vitro</i>)	Ewing sarcoma	[40]
2021	PCP-PEG-ALD nanosystem	Anti-tumor effect enhanced and inhibited pulmonary metastasis and cardiotoxicity (<i>in vivo</i>)	Osteosarcoma	[35]
2021	Multifunctional nanodrug system	Inhibits osteolysis induced by tumor (<i>in vitro</i> and <i>in vivo</i>)	Osteosarcoma	[37]

for cancer therapy, bone targeting is arousing a progressive interest for scientific investigations, a scenario which was not a reality ten years ago. A range of nanosystems indicated for the most common bone cancers and bone metastasis, which have presented promising results over the past two decades, is presented in Table 1.

Whereas bone diseases or bone cancers could seldom be cured because of poor drug distribution at the bone niche, this subject of targeted delivery is catching the sights of scientific world. It is well known that some tumors have predilection to metastasize in bone, especially lung, breast and prostate cancer; the conventional treatment for both primary and metastatic bone tumor usually includes a combination of surgery, radiotherapy and chemotherapy, some of these procedures are damaging to healthy tissues surrounding the tumor. Thus, the nanoensembles in the field of nanomedicine present a strong trend to suppress the growth of tumor with a less toxic chemotherapy and less invasive removal procedures, thus offering a better quality of life to patients. These nanosystems have some properties, well documented in the literature, to make them eligible and promising for tumor targeting, such as their biodegradability, drug delivery ability, accumulation in tumor, and reduced side effects. A wide range of nanomaterials and nanodrugs are available to be applied in bone cancer treatment. The effective drugs, such as DOX, ALE, ZOL, BTZ in combination with others substances, such as AES, SE have been widely investigated and commonly loaded in nanosystems with positive results. These drugs have been chosen because of their affinity towards tumor cells or bone cancer niche, showing effectivity to target bone conditions. Interestingly the NPs based on calcium phosphate are promising to contribute for bone replacement, and the polymeric compounds may accelerate the biodegradation to promote the rapid release of anticancer drug into targeted site. As important as curing cancer is ensuring a way to reconstruct the lost bone tissue. For this, the orthopedic materials/prosthesis coated with nanomaterials and anticancer nanodrugs, or antioxidant nanoproteins provide an intelligent way to bring these properties into bone microenvironment.

Further investigations are required to accelerate the application of nanomedicine in clinical practice to reduce the number of global deaths caused by cancer.

NANOTECHNOLOGY FOR BONE DISEASES

Human skeleton provides the basis for locomotion, support and protection of various vital organs, and is a

source of blood cells and mineral ions, both essential for basal metabolism [12]. Some of bone conditions are discussed that may cause serious morbidity and complications in humans; the role of nanomedicine in the treatment of these disorders is presented.

The Legg-Calvé-Perthes disease (LCPD) is a juvenile form of osteonecrosis that affects the proximal epiphysis of femur in children aged 2-14 years [54]; it is caused by a temporarily disrupted blood supply to bone epiphysis which becomes necrotic with an increased radiodensity and may culminate in collapse of bone [55]. This osteonecrosis is carried out by the osteoclasts. The osteoclastogenesis denotes the process of proliferation, differentiation and activity of osteoclasts. Alendronate, a class of bisphosphonate (BP) as inhibitor of osteoclastogenesis, is well-known for affecting osteoclast cell death and impairing the adhesion pattern of these cells *in vitro*. When compared to free ALE, the combination of ALE into GNP (modified gold-nanoparticles or BP-GNPs) showed very little osteoclasts cell viability, indicating that the combination induces cellular death. Possibly, the improved efficacy of BP-GNP could be due to its structure, which could facilitate the changes in the setting of the BP to act at the cellular level, as called nanodrug. Such changes allowed increase in the kinetics of ALE and farnesyl pyrophosphate synthase (FPPS) binding event, which is located in peroxisomes, mitochondria, and the cytosol of all mammalian cells. Another possible reason may include the surface-modified GNPs which allow the entry of higher concentration of ALE molecules into cells, since each nanoparticle has its own potential to conjugate with many molecules. These GNPs are potentially effective as localized nanodrug delivery vehicles for BP in the inhibition of the osteoclastogenesis by osteonecrosis [56].

The bone vascularization is not wide spread and thus may be easily overcome by an infection in the bone. The systemic administration of antibiotic may not approach the exact bony niche that contains the bacterial strain, and so this condition can quickly progress to a chronic disease. The bionanotechnology brings up a very promising drug delivery nanosystems to act precisely on targeted site.

The vast majority of osteomyelitis cases are caused by *Staphylococcus aureus*. Chronic osteomyelitis with methicillin-resistant *Staphylococcus aureus* (MRSA) can culminate in serious defects and remains a challenge for orthopedic surgeons. A critical infection in bone involves the debridement of necrotic tissue as a

part of the treatment; this procedure could lead to a massive bone loss depending of the extent of injury. Concerning that, nHA as vancomycin carrier (VAN-loaded nHA) has been investigated in chronic MRSA osteomyelitis. The VAN release *in vitro* was gradually decreased and it remained effective for 25 days leading to a decreased number of bacteria. Radiographically, most of the nHA-VAN pellets were reabsorbed and replaced by large areas of newly formed trabecular bone, and medullary cavity, becoming normal bone in six weeks, in proximal tibial metaphysis. The nHA exhibited a good release profile both *in vitro* and *in vivo*, and inhibited the progress of bone infection, overcoming the chronic osteomyelitis in a short period and preventing its recurrence. These NPs contributed to replace bone while acting as osteoconductive and osseous defect-filling with high solubility and calcium release. This way, nHA-VAN released vancomycin over a prolonged period which cured MRSA chronic osteomyelitis while replacing the bone simultaneously [57]. The association of a drug with the calcium phosphate NPs provides a novel way to treat bone infections and to convert such niche to a bone repair condition [12]. The n-HAs are known for their good absorption property and biocompatibility, and their surgical implantation in bone averts the requirement for a second surgery for removal of bony mass. In another nanosystem researchers aggregated VAN in PLA/nHA to treat osteomyelitis. The PLA/nHA/VAN nanoscaffold released VAN with its highly antibacterial activity, while the n-scaffolds promoted the adhesion and proliferation of osteoblasts leading to bone regeneration [58]. Smaller the size of NPs, greater is its half-life and circulation toward the targeted bone.

Silver ions (Ag) possess a well-known bacteriostatic property. The aggregation of this ion with nHA and others polymers is proposed to treat bone infections. Ag/nHA/PU (polyurethane) was synthesized for treating chronic osteomyelitis and presented a good bacteriostatic effect *in vivo* [11] explainable by two hypotheses; first, the microorganisms could be attracted to the HA surface by an electrostatic force giving rise to a direct interaction between Ag ions and bacterial membrane; second, the Ag ions released from nanoensemble could form a bacteriostatic environment around the whole implant [59]. Additionally, Ag/nHA/PU worked as a good delivery of silver with strong activity against *S. aureus*, meanwhile contributing to repair the bone [11].

Nanomedicine also has been applied in bone infections caused by enterobacteria, such as *Escherichia coli*. Silica NPs loaded with moxifloxacin

and coated with arabic gum and colistin (AG/CO/MX-loaded NPs) had high affinity toward bacterial biofilm matrix and showed non-cytotoxic to healthy cells, resulting in eradication of the *E. coli* osteomyelitis [60].

Bone tuberculosis (BT) is the most common form of extrapulmonary TB [61]. While isoniazid (INH) is the first-line therapy for bone tuberculosis (BT), it has limited clinical benefits due to severe side effects after long-time administration. However, a derivative of INH, called DINH, was loaded in a nanoensemble due its hydrophobicity as well as its better antibacterial activity and biosafety. To achieve a sustainable drug delivery, the antibacterial effect of DINH loaded liposomes were combined with the self-healing property of PLGA-PEG-PLGA based hydrogel (DINH/Lip/PLGA-PEG-PLGA). It is worth noting that this nanosystem demonstrated an initial burst of drug release *in vitro* with no cytotoxicity to healthy cells, which is a desirable feature for effective antitubercular activity and rapid release of DINH *in vivo*, compared to INH [62].

An imbalance among bone cells with a high recruitment or formation of osteoclasts can trigger some metabolic diseases, such as postmenopausal osteoporosis, rheumatoid arthritis, multiple myeloma or even lytic bone metastases. Gold NPs (AuNPs) are biocompatible and can inhibit the osteolysis in some bone disorders through the inhibition of osteoclast differentiation. The AuNPs restrained the osteoclast maturation by suppressing pre-osteoclast fusion and of macrophage colony-stimulating factor, launching up this type of nanoensemble as a good therapeutic agent for osteoclast-related bone metabolism disease. Not only the maturation, but the osteoclast differentiation was highly suppressed by AuNPs, although through mechanisms yet unknown [63]. Additionally, the AuNPs and Au nanoclusters with glutathione (GAC) as a template presented profound anti-inflammatory effects by inhibition of secretion of lipopolysaccharide-induced proinflammatory mediators. On the other hand, larger NPs have presented poor absorption with accumulation in peritoneum and liver due to their larger molecular weight, after intraperitoneal injection. Smaller sized NPs showed high absorption [10].

The osteoarthritis (OA) is one of the most disabling joint disease in older adults worldwide [64], characterized by degeneration of joint tissues. In the most severe cases, the pain and the restricted movements are remarkable as resulted from the cartilage degradation or joint bone erosion. As it is an incurable clinical condition, the treatment is generally carried out by the administration of anti-inflammatory

drugs to control the disease progression and minimize the acute stages of pain for patient.

The boronate-stabilized polyphenol-polyoxamer (PPNP) assembled dexamethasone (DEX) nanodrug has been prepared to be applied for OA treatment. DEX is a corticosteroid with a well-known anti-inflammatory and immunosuppressive action, and is widely applied in joint disorders. The angiogenesis and inflammation had been controlled by these NPs with remarkable reduction of both the angiogenesis and the cartilage degradation. The biosafety of DEX-PNPP was also a remarkable feature with no effects on heart, liver, spleen, lung, and kidney in mice [65].

Osteoporosis is clinically well-known and a common disorder of skeletal system especially in women after menopause; it turns the bone in a structure of lower-

mineral density with significant reduction of its mechanical strength. OS can also affect men although, less frequently. As sequelae of these condition, the bone becomes weak and very susceptible to fractures what interferes negatively on quality of life of these people. Since current osteoporosis medical treatments can cause side effects, the new techniques based on the nanosystems have been widely investigated for clinical applications.

Regarding that, Ryu *et al.*, in 2016, carried out the first *in vitro* and *in vivo* research in bone-targeted delivery based on ALE-loaded NDs (nanodiamonds). Once this drug is bounded to bone in the presence of ALE, it remains incorporated for a longer period increasing the mechanical strength of the bone, while both ALE-PLGA and NDs had low affinity to pre-osteoblastic cell line (MC3T3-E1) with lower cellular

Table 2: Range of Nanomaterials and its Applications in Several Bone Diseases Over Two Past Decades

Year	Nanomaterial	Role of Nanomaterial (Assay)	Bone disease	Reference
2011	ALE-GD-NPs	Enhanced effect on inducing osteoclast apoptosis and impairing osteoclast function (<i>in vitro</i>)	Legg-Calvé-Perthes disease	[56]
2012	VAN-loaded nHA	The released VAN remained effective (<i>in vitro</i>). Repaired bone defect and inhibited the infection (<i>in vivo</i>)	Chronic osteomyelitis (methicillin-resistant <i>S. aureus</i>) with bone defects	[57]
2016	ALE-NDs	Enhanced the synergistic ALP activity (<i>in vitro</i> and <i>in vivo</i>)	Osteoporosis	[66]
2018	Nano-silver (AgNPs)	Effectivity on pathogens (<i>in vivo</i>)	Osteomyelitis and soft tissue infections	[9]
2019	GNPs (AuNPs)	Reduced osteoclast bone absorption (<i>in vitro</i>)	Osteoclast-related bone metabolism diseases	[63]
2019	DINH loaded liposome into nanodrug system	Prolonged drug release (<i>in vitro</i>); beneficial for localized bone infection treatment (<i>in vivo</i>)	Bone tuberculosis	[62]
2019	Au nanoclusters	Inhibit the production of LPS-induced proinflammatory mediators, inducing profound anti-inflammatory effects (<i>in vitro</i> and <i>in vivo</i>)	Rheumatoid arthritis	[10]
2019	Ag/n-HA/PU	Antimicrobial activity and favorable repair of bone defects (<i>in vivo</i>)	Chronic osteomyelitis	[11]
2019	PLA/nHA/VAN n-scaffolds	Rapid release, anti-bacterial activity against <i>S. aureus</i> and increased adhesion and proliferation of osteoblasts (<i>in vivo</i>)	Advanced osteomyelitis	[58]
2020	RLX-Vit.D-NLCs	Enhanced the RLX bioavailability and Vit D metabolite (<i>in vitro</i> and <i>in vivo</i>)	Post-menopausal osteoporosis	[69]
2021	AG/CO/MX-loaded silica NPs	Antibacterial effect (<i>in vitro</i> and <i>in vivo</i>)	Bone infection by <i>E. coli</i>	[60]
2021	DEX-PPNP nanodrug	It enables an efficient repolarization of macrophages for anti-inflammation, and reduces the cartilage degradation and osteoarthritis progression (<i>in vitro</i> and <i>in vivo</i>)	Osteoarthritis	[65]
2021	MSNPs combined to an osteogenic peptide and siRNA plasmid	Increased the expression of osteogenic-related genes improving the bone microarchitecture (<i>in vitro</i> and <i>in vivo</i>)	Osteoporosis	[67]

uptake. The ALE-loaded NDs showed an effective performance for cellular uptake and ALP activity *in vitro*, which has been attributed to the aggregation of ALE in NDs. Differently of NDs with not ALE, the ALE-ND nanosystems presented a low charging in organs (liver, kidney, and spleen) when intravenously administrated, and a properly accumulation in bone, meaning to be a promising potential of ALE/NDs for bone targeted delivery [66]. Another type of nanosystem based therapy is mesoporous silica NPs (MSNPs) combined to osteostatin and siRNA to treat osteoporosis. The *in vitro* experiment revealed a proper cellular uptake with non-cytotoxic effect to MC3T3-E1. The presence of ALE enabled the accumulation of NPs inside the HA matrix. The *in vivo* experiment revealed an increase in the improved microarchitecture of osteoporotic bone [67]. The affinity of ALE to bone increased the cellular uptake and allowed the bounding of nanosystems to bone, probably supporting an improvement in the treatment of osteoporosis.

Postmenopausal osteoporosis can occur as a consequence of a physiological process called menopause in senile women. A primary ovarian failure secondary to apoptosis leads to a drop in estrogen level resulting in an imbalance in bone turnover, which is characterized by an increased resorption bone with low bone formation. Ovarian function declines with aging [68] thus, weakening the skeletal system predisposing it to fractures.

Thus, the raloxifene (RLX) is a selective estrogen receptor modulator, and the vitamin D (Vit.D) is an important fat-soluble vitamin which is usually administered to treat postmenopausal osteoporosis, have been conjugated in nanostructured lipid carriers (NLCs) in order to overcome the low solubility and low bioavailability in microenvironment. *In vitro* and *in vivo* the RLX-Vit.D-NLCs improved the RLX bioavailability with an increased level of vitamin D; a complete level of adsorption of RLX and Vit.D was revealed, making this nanosystem available as a promising therapeutic for osteoporosis treatment [69].

The production of nanosystems to treat bone infections has been worth because they bring great advantages, such as specific accumulation in targeted sites, higher effectivity, low toxicity, as well as decreasing of severe side effects, which means better safety for administration [70]. In lieu of the fact the larger particles could host and entrap more drug, otherwise, the smaller ones could reduce the probability for low absorption and accumulation in organs, besides accelerate the releasing of drugs by rapidly resorption of NPs, and even provide higher safety in its administration. A range of nanosystems for most common bone diseases, which have presented promising results over past two decades is presented in Table 2; while a summary of all nanosystems developed for bone disorders and discussed in this article is represented in Figure 2.

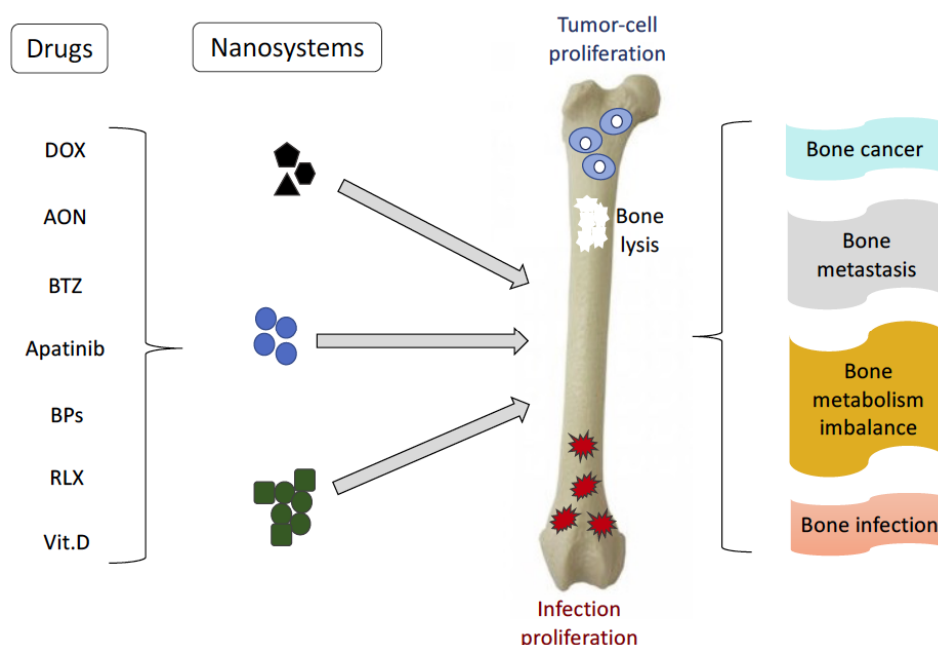


Figure 2: A schematic representation of different drug-loaded nanosystems mediating targeted delivery for application in bone disorders.

CONCLUSIONS AND FUTURE PERSPECTIVES

Bone cancer and bone diseases are serious concerns worldwide, and in advanced stages may lead to loss of hard tissue and posing limitations for the patient, or may even lead to prove fatal outcomes. Over the last two decades bone targeted delivery has been successfully investigated and tested with applications in medicine, especially for therapeutic purposes. Different materials like silver or gold NPs or even nHA have been aggregated to some drugs to treat bone cancer or diseases. Nanosystems positively could act as carrier drugs or genes for therapy when present a high biosolubility which result in effective drug release. The biodegradation of the targeted delivery nanoensemble has been reached with the aggregation of polymers in its formulation, which besides favoring the drug releasing also help in achieving the high cellular concentration of the therapeutic agent to suppress the growth of tumor cells. The acidic or infection tumor microenvironment is what triggers a deposition of the drug from a nanosystems into the lesion site, a process known as targeted therapy. The production of a nanosystem positively increases the half-life of drug with a high therapeutic efficacy due the improved cellular uptake and a slow elimination from systemic circulation. The affinity of drugs to bone such as the bisphosphonates further increases the effectiveness of nanotherapy. These features and the on-demand drug release are crucial to minimize toxicity in normal tissue. Overall, the researches in nanomedicine have been noticeably advanced over the last two decades, regarding nanosystems and loaded-drugs *in vitro* and *in vivo* assays. These studies have considered the nanoparticle size, biodegradability, biosolubility, cellular uptake, half-time in blood circulation, accumulation in organs, therefore, there is a strong need to accelerate the efforts to apply nanotherapy in human being, which seem promising with trending for a conservative treatment of bone cancer and bone diseases consequently decreasing the severity of morbidity worldwide.

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