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APPLIED NANOTECHNOLOGY IN ORTHOPAEDIC ONCOLOGY

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Abbreviations

BPs: Bisphosphonates

BSA: Bovine Serum Albumin

CaP: Calcium Phosphate

CM: Cell Membrane

CNFs: Carbon NanoFibers

CNTs: Carbon Nanotubes

CS: Chitosan

CT: Computed Tomography

CTAB: Cetrimonium bromide

CUR: curcumin

DOX: Doxorubicin

DTPA: Diethylenetriamine Penta-Acetic Acid

EMA: European Medicines Agency

EPR: Enhanced Permeability and Retention

ESB: European Society for Biomaterials

FDA: US Food and Drug Administration

G: Graphene

GdNPs: Gadolinium Nanoparticles

GNPs: Gold Nanoparticles

GO: Graphene Oxide

HAp: Hydroxyapatite

IARC: International Agency for Research on Cancer

ICG: Indocyanine green

IO: Iron oxide

LDH: Layered Double Hydroxides

LPNs: Lipid Nanoparticles

MRI: Magnetic Resonance Imaging

MSC: Mesenchymal Stem Cell

MSNs: Mesoporous Silica Nanoparticles

MWCNTs: Multiwall carbon nanotubes

NDs: Nanodiamonds

NIRF: Near-infrared Fluorescence

NMs: Nanomaterials

NPs: Nanoparticles

PAH: Polyallylamine hydrochloride

PEEK: Polyetheretherketone

PEG: Poly(ethylene glycol)

PEI: Polyethylene-imine

PET: Positron Emission Tomography

PLA: Poly(lactic acid)

PLeu: Polyleucine

PLGA: Polylactic-co-glycolic acid

PMMA: Polymethylmethacrylate

PTT: Photothermal therapy

QDs: Quantum Dots

ROS: Reactive oxygen species

SEM: Scanning Electron Microscopy

SLN: Silica loaded nanoparticles

SPECT: Single Photon Emission Computed Tomography

SPIONs: Superparamagnetic iron oxide nanoparticles

TEM: Transmission Electron Microscopy

ZOL: Zoledronate

Abstract

Nowadays, bone cancer is a major issue especially for young people. Common diagnosis and therapeutic methods are not entirely effective as they lack adequate precision and efficacy. Novel nanostructures have drawn widespread attention due to their broad applications in tumor diagnosis and therapy. In the present review, the impact of nanotechnology in diagnosis and treatment of bone cancer is discussed. A plethora of nanostructures and their applications in diagnosis, treatment as well as in theranostics for bone tumor diagnosis, treatment and regeneration of bone defects is described in order to provide an outlook for all nanoscientists. In addition, reference is made in toxicity of the nanostructures utilized in medical applications. The literature review revealed that nontechnology based methods for imaging and treatment of bone tumor could provide earlier diagnosis and more effective therapy compared with up-to now existing methods. However, the toxicity of nanostructures remains a longstanding challenge for the research community.

Nanotechnology in orthopaedics

Nanotechnology is the science for small with great effects. It can be defined as the science and engineering involved in designing, synthesizing, characterizing and using nano-scale materials and devices *[Tasker et al, 2007]*. Nowadays, the field of nanotechnology is quickly expanding and plays an important role in humanity, as in many fields, such as medicine, energy, environment, construction, and telecommunications, our lifestyles are changing. Both theoretical and experimental sciences face a complex challenge by nanotechnology, which also offers a major opportunity for the growth and well-being of human health *[Di Sia, 2017; Mostafavi et al, 2019]*.

In the last decades, research in medicine has been connected with materials science in order to apply materials to health care and as a consequence a new category of materials the so-called biomaterials has been developed. In 1976, at the first Consensus Conference of the European Society for Biomaterials (ESB), a biomaterial was defined as “a nonviable material used in a medical device, intended to interact with biological systems”; however the ESB’s current definition is a “material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body” *[Vallet-Regi 2011]*.

Nowadays, the use of orthopaedic implants is growing quickly worldwide. Bone defects with low regeneration potential resulting from tumor, trauma, infection or periprosthetic osteolysis need to be surgically treated. Studies have shown that there are more than 600.000 joint replacements per year in the United States alone, and about 80% of all transplants are bone autografts and allografts *[Shokuhfar et al, 2017]*. The selection of an optimal bone graft is based on various aspects such as tissue viability, defect size, graft size and shape, biomechanical features, graft handling, price, biological features, ethical issues, and related problems. Autografts, allografts, xenografts and synthetic and bio-logically based, tissue-engineered biomaterials and combinations of these substitutes are some of the materials used in bone grafting. Allografts and xenografts have osteo-inductive and osteo-conductive properties but lack the osteogenic properties of autografts. Autografts are the most common used implants for the reconstruction of small bone defects and present strong osteogenic

properties relevant to bone healing, modeling and re-modeling. Some of the disadvantages of autografts are pain and site morbidity, as well as other risks such as major vessels or visceral injuries during harvesting. An alternative option are allografts but with major disadvantages too. Some of them are rejection, transmission of diseases, cost and lower incorporating properties with the host healing tissues in comparison to autografts whereas, xenografts except from the drawbacks of allografts, carry the risks of transmission of zoonotic diseases *[Oryan et al. 2014]*. The longevity of common orthopedic implants is not perpetual and unfortunately, there have been recording many cases of implants' failure. Thus, more surgeries are needed and consequently, pain, cost of therapy and the added risk of post-surgery complications are increased. One of the major factors of failed implants is the reduced surface interaction between the implant and the host tissue which results in inadequate tissue regeneration around the biomaterials. Consequently, an orthopedic implant have to be inhabitable for osteoblasts to proliferate onto its surface and regenerate new bone tissue. Several ceramic, polymeric, metallic and composite scaffolds at the nano-scale have been developed for bone/cartilage tissue engineering applications (Table 1). Nanomaterials (NMs) can be categorized as nanoparticles, nanocrystals, nanotubes, nanofibers, nanowires, nanoclusters, nanorods, nanofilms, etc. The selection of the appropriate material as well as the production method are the two main factors affecting the success rate of bone tissue engineering products. *[Shokuhfar et al, 2017]*.

Table 1: *Nano-sized materials in orthopedic applications [Shokuhfar et al, 2017].*

Category	Material	Structures	Characteristics
Metals	Titanium alloys	Nanotubes, nanorods, nanoparticles, UFG	High corrosion resistance, osteoconductive
	Cobalt-chromium alloys	Nanostructures	Excellent corrosion resistance as well as friction resistance
	Silver	Nanoparticles, nanocoating	Anti-infection coatings, antimicrobial/antiviral properties
	Stainless steel	Nanostructures	Resistant to many corrosive

Category	Material	Structures	Characteristics
			agents, excellent fabrication properties
	Tantalum	Nanoparticles	Ductile, anticorrosive, biocompatible and cost effective
Polymers	Collagen	-	Poor mechanical properties, low immune response, increase cell adhesion, chemotactic
	Chitosan	2D/3D scaffolds, nanofibers, nanocoating	Promotes osteoconduction and wound healing and hemostatic properties
	Hyaluronic acid	-	Minimal immunogenicity, low mechanical properties, chemotactic in combination with suitable agents
	Silk	Nanofibers	High compressive strength, increase cell migration, vascularization, osteoconduction
	Polylactic-co-glycolic acid (PLGA)	-	Biocompatible, tunable degradation rates, sufficient mechanical properties, safety for clinical use, processability
	Poly(e-caprolactone)	2D/3D scaffolds	Bioresorbable, slow degrading, low chemical versatility, degradable by hydrolysis or bulk erosion
	Polymethylmethacrylate (PMMA)	2D/3D scaffolds	Using as bone cement, biocompatible, thermoplastic, low ductility brittle
	Poly(lactic acid) (PLA)	2D/3D scaffolds, nanofibers, nanocoating	Desirable mechanical properties, bioabsorbable, biodegradable, thermoplastic
	Polyetheretherketone (PEEK)	-	Poor osseointegration, excellent mechanical properties, physical and chemical stability,

Category	Material	Structures	Characteristics
			biologically inert and safe
Ceramics	Calcium phosphates	Nanoparticles	Improved cell differentiation; osteoconductive
	Hydroxyapatite	Nanorods	Good osseointegration, slow biodegradation rate, low fracture toughness
	Bioactive glass	Nanoparticles, nanocoating	Brittle and weak; enhanced vascularization
	Metallic oxides (eg, alumina, zirconia, titania)	Nanotubes, nanocoatings	High biocompatibility, desirable corrosion and wear properties
Carbon materials	CNTs/CNFs	Nanofibers, nanotubes	Excellent mechanical strength and electrical conductivity, low density
	Graphene/graphite	Nanosheets	Excellent thermal and electrical conductivity, high tensile strength, reflexivity
	Diamond	Nanoparticles, nanocrystals, nanorods	Higher tribological and mechanical properties
Composites	Ceramic nanophase in a ceramic or polymer matrix	Nanosheets	
	Metallic nanophase in a ceramic or polymer matrix	-	More support for cell activity, higher osteoconductivity, tailorable degradation rate, improved biological and mechanical properties

In order to overcome the difficulties related to the conventional implant biomaterials in the microscale, many scientists have been concentrated to nanomaterials for the production of orthopedic implants due to their advanced properties. Owing to the nano-scale dimension of natural tissue, the biomimetic feature and physical and chemical properties of NMs play an important role in cell growth and tissue regeneration *[Shokuhfar et al., 2017]*. Recent advances in NMs have introduced novel orthopedic implants with high potential for better osseointegration

to effectively mimic new bone development compared to conventional implants *[Simchi et al., 2015].*

Exceptional physicochemical properties and nanostructured NMs extracellular matrices (ECM) can reduce infection and enhance bone growth. Several researches have revealed that implants, constructed with materials at the nanoscale, with surface roughness that imitate the natural tissue enhance more the tissue growth than smooth implants. Furthermore, nano-sized materials have been shown to be advantageous in promoting bone growth, or to be disadvantageous in promoting inflammation or infection. Consequently, the design and production of nanomaterials is a new challenge in orthopedic applications, offering a novel types of implants and scaffolds that can imitate the complex and hierarchical structure of hard / soft tissues. One of the key factors in the design of materials for soft / hard tissue regeneration is controlling cell behavior. Nano-scale structures are reported to be able to control cell orientation and alignment and collagen matrix mineralization. Moreover, structures at the nano-scale give rise to mineral deposition of calcium (Ca) and phosphorus (P) by osteoblasts from the culture media *[Shokuhfar et al., 2017]*.

For several decades, nanotechnology has altered science and consumer products. Recently, nanotechnology's biology and medicine applications have improved the medical diagnostics, drug delivery and engineering. Recent research into this new technology has shown its potential with new forms of disease detection and intervention, especially in orthopedics. Through recent advances in bone tissue engineering, implantable materials, diagnosis and therapy, and surface adhesives, nanomedicine has altered orthopedics. Within the field of orthopedics, the potential for nanotechnology is vast and much of it seems to be untapped, but not without obstacles. Nanotechnology has gain access to an especially successful role in orthopedic surgery, where several applications for manipulating nanoparticles have been discovered by bench and translational research. These innovations enable improved clinical capabilities through many different avenues *[Garimella et al., 2017]*.

More specifically, nanotechnology in orthopaedic is used: (i) for the development of novel drug delivery systems for chemotherapeutic agents and antibiotics, (ii) for the surface modification of implants and prosthesis to enhance osteointegration and impede biofilm formation, (iii) for the development of controlled

drug release systems to hamper implant-related infections, (iv) in tissue engineering for scaffolds development in order to be used in bone and cartilage defects, and (v) in diagnosis and especially in the field of oncology and musculoskeletal infections. *[Gavaskar et al., 2018].*

Implants and scaffolds

Implantable biomaterials have become essential in orthopedic surgery, largely because of their ability to ensure osteointegration and to improve stimulation of healthy bone processes, particularly in comparison to their typical material counterparts. These fundamental improvements are of particular significance, since an increased number of orthopedic implants is needed in the aging population. Orthopedic implants are used in a variety of ways in several areas of the body, but in all areas, the function and purpose of implants are well served by the addition of nanomaterials. Nanoconstructed implants have overcome many of the risks associated with the use of allografts, autografts and xenografts, but yet they are still failing because they are not able to restore full functionality as well as they do not often have longevity over a decade or two at best. However, nanotechnology has proven advantageous in the construction of orthopedic implants, improving the therapy of many types of bone defects and orthopaedic traumas. Several materials such as gelatin, bioceramics, biodegradable polymers, and polysaccharides have been studied and applied, resulting in the use of a wide range of possible materials with their own unique properties and benefits *[Garimella et al., 2017]*. Both the physical properties and nanoscale features of these materials enable them to promote cell growth and tissue regeneration and thus to act well within the human body *[Bauer et al., 2013]*. The potential NMs to imitate the cellular environment is critical in the replication of cell mechanisms, which are also in nanometer scale and come together to form extra-cellular matrices. Moreover, the surface area of implants constructed of nanomaterials is greater than that of the conventional implants, which helps cultivate a healthy environment for bone growth and reduce infection rates *[Lin et al., 2014]*. Nanomaterials can be also used as coatings on the conventional scaffolds. It have been shown that extracellular adhesion proteins interact better with nanophase implant

scaffolds than conventional implant surfaces. Greater absorption of these proteins provides a well-suited environment for osteoblast adhesion, bone formation and implant-bone fusion (Fig. 1). In addition, the use of nanotechnology for implants' production has been presented to have many encouraging effects on clinical outcomes, including a reduction in the possibility of infection and an improvement in scar appearance *[Garimella et al., 2017].*

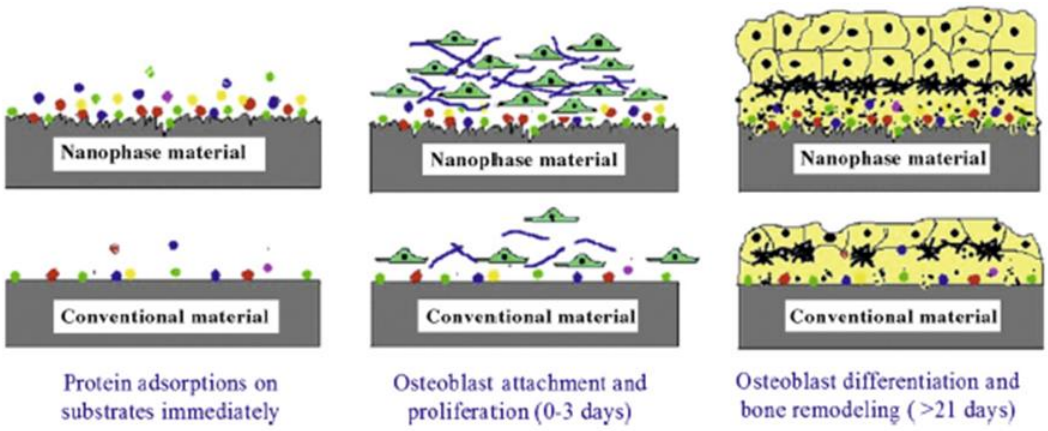

Figure 1: *"Schematic illustration of the mechanism by which nanomaterials may be superior to conventional materials for bone regeneration. The bioactive surfaces of nanomaterials mimic those of natural bones to promote greater amounts of protein adsorption and efficiently stimulate more new bone formation than conventional materials". [Zhang et al., 2009]*

Diagnosis

Nanotechnology can be also used in the field of diagnosis for bone diseases such as Paget's disease, osteoporosis and renal osteodystrophy. For this purpose, biosensors are often used. These sensors can be implanted and are available in many designs and forms. Several biosensors often employ carbon nanotubes (CNTs), because of their unique properties which make them strong and electrically conductive *[Yun et al., 2009]*. There is a diversity of nanotechnology-based detection products that revolutionize the field of orthopedics. For example, for osteoporosis, diagnostic techniques are of great importance in providing timely, affordable and non-invasive accurate data detection. There were few detection options before techniques using nanomaterials. However, new methods using nanotechnology enable osteoporosis to be detected with a handheld device. More specific, a biochip using

GNPs for the detection of a protein that is correlated to osteoporosis has been established. It has been shown that bone conditioning has been effectively evaluated and that the degree of damage to bones has been accurately detected and identified *[Singh et al., 2012]*. Additional application of nanotechnology in orthopaedics is the monitoring and updating of orthopaedic therapies. Some sensors have been equipped to detect bone growth and bone failure and to dispense additional therapeutic drugs as required *[Garimella et al., 2017]*.

Delivery of drugs

Nanotechnology has altered therapeutics by enabling higher accuracy in drug delivery, proving to be particularly useful in the field of orthopaedics. Like other organs, bones are prone to different orthopedic diseases, such as osteomyelitis, osteoarthritis, osteoporosis, osteosarcoma, bone metastasis, etc. *[Hasnain et al., 2017]*. Treatments for bone diseases have more than a few restrictions such as wide tissue excision when amputation surgery is used, ineffective targeting and negative effects on other tissues and organs during chemotherapy and radiation therapy, when biomacromolecular drugs are used, the physicochemical stability in biodistribution as well as plasma half-life are reduced, and inadequate bone graft sources as well as risk of infection and host immune responses. In order to overcome these limitations and to increase the therapeutic efficiency and reduce adverse effects, targeted drug delivery using nanomaterials is a potential solution *[Shokuhfar et al., 2017]*.

It is well known that nanomaterials are advantageous in applying in medicine. The drug-loading capacity is increased due to the high surface:volume ratios; the solubility and the stability of the drugs is also increased in conjugation with delivery vehicles; the targeted drug delivery and controlled release with stimuli-responsive functional groups and reduced systemic adverse effects on other organs and tissues; and the improved transfer ability in cell membranes, allowing intracellular drug delivery or delivery to specific organelles. As far as treatment of bone diseases is concerned, nanomaterials also present unique advantages. Since bone tissue consists of inorganic minerals and organic matrices assembled on the nanoscale, NPs can be assimilated into the bone microenvironment in order to approach and cure diseased bone. Furthermore, nanocarriers such as calcium phosphate (CaP) or gold

nanoparticles (GNPs), and nanodiamonds (NDs), are able to motivate new bone growth by stimulating mineralization or promoting bone cell activity. In addition, nanotechnology-based intracellular targeted drug delivery can improve the therapy effectiveness of bone disease by delivering drugs accurately to subcellular areas. Consequently, targeted intracellular drug delivery has excellent potential to address multidrug resistance, a long-standing challenge for cancer chemotherapy *[Yang & Webster 2009; Walmsley et al., 2015; Cheng et al., 2017]*.

Nanomaterials can be used in drug delivery applications due to their distinctive physical and chemical properties such as ease of functionalization with biological targeting molecules, high surface:volume ratio for efficient drug loading, and tiny size to overcome tissue barriers for more effective targeting. The development of inorganic and organic nanomaterials (Fig. 2) has resulted in new drug delivery methods for bone disease treatment *[Cheng et al., 2017]*.

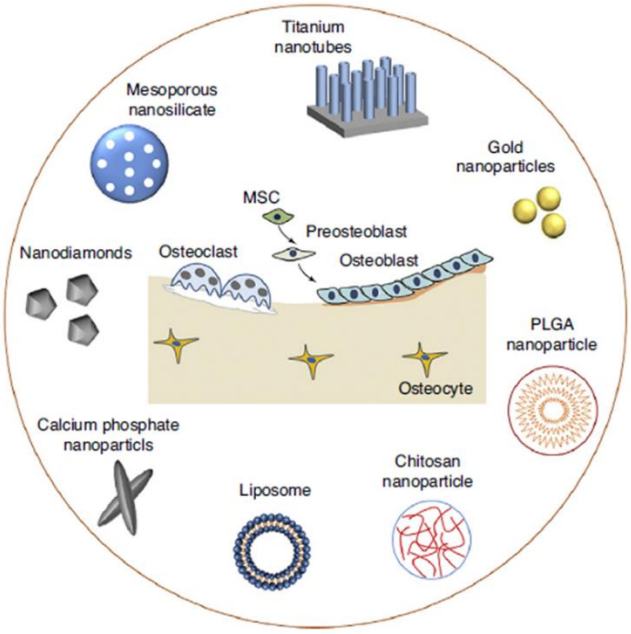

***Figure 2**: "Examples of nanomaterials for bone drug delivery. The inorganic nanomaterials include titanium nanotubes, gold nanoparticles, calcium phosphate nanoparticles, and mesoporous silica nanoparticles. The organic nanomaterials include chitosan nanoparticles, poly(L-lactide-co-glycolide) (PLGA) nanoparticles, and liposomes. These nanomaterials can selectively target bone tissues and cells to deliver drugs. Abbreviation: MSC, mesenchymal stem cell" [Cheng et al., 2017].*

Conclusions and future perspectives

Nanotechnology is a relatively newcomer to the forefront of orthopaedic research, diagnostics, and treatment. Nevertheless, it has been able to alter the science and practice of orthopaedic treatment. Many standard therapies are being substituted, as nanotechnology offers methods of treating the human body in ways that are more accurate, better for bone growth, and theoretically safer, at least in terms of infection rates and re-operation requirement.

Although nanotechnology, is a very promising field, it is not a panacea to many of the problems of orthopaedic oncology. Questions such as: "Is there long-term clinical safety?", should be answered prior to its acceptance and clinical implementation. Early studies have shown that there is a risk of cytotoxicity of the lungs and inflammation of the internal organs. It is therefore easy to understand that further research is needed. In order to better understand the role of nanotechnology in the future, it is necessary to better understand its positive and harmful long-term effects. There is also a need for answers to questions about the toxicity and viability of nanoparticles and sensors. Moreover, more head-to-head comparisons between nanomaterials and common materials will better elucidate the nanotechnology value proposition and guide future studies.

Problems related to manufacturing, regulatory, and cost obstacles should be explored and enhanced. Due to the nature and complexity of the products, the production process is difficult. The high cost of these products can decrease accessibility, and present regulatory processes can be sustained, restricting the immediacy of translating research into practice. Mitigating these issues will improve nanomaterials' availability and better encourage their use in the field of orthopedics.

To conclude, it is necessary to give further and continuous attention to the quality of existing biomaterials and to the research to identify and develop even better nanomaterials. The mean lifetime of up-to-date nanomaterials rages from one to two decades and undoubtedly has room for improvement. The regulatory and safety challenges will possibly continue as problems hampering its widespread and fast acceptance and use, but with additional research, it seems that nanotechnology has growing niche within the field of orthopaedics.

Nanotechnology in orthopaedic oncology

Cancer may be a genuine issue of our century and one of the foremost reasons of death, being responsible for one of eight deaths happening around the world. Based on real data the International Agency for Research on Cancer (IARC) gauges ~13.1 million deaths related to cancer by 2030. For many researchers, it becomes clear that the low survival rate resulted from the lack of satisfactory drug delivery systems and from the absence of effective, natural or synthetic antitumor agents *[Ficai et al., 2015]*.

Bone cancer includes primary bone tumors and metastatic lesions. Primary, is created in the bone and can be categorized as benign or malignant. They represent only 0.2% of all cancers, but their incidence has increased in the past decade. Bone metastasis which is more frequently, occurs when cancer cells spread from a primary, distant site and metastasize to the bone, where the cells may lay dormant before they proliferate to form a cancerous lesion. Metastatic bone cancer is frequently seen in many kinds of cancer and is especially common in patients with breast and prostate cancer, with metastatic spread to the bone at around ~70% *[Forde et al., 2017]*.

Primary bone cancer is the bone disease with the highest mortality; the statistical report on cancer in 2015, showed that there were about 1490 deaths happened in the 2970 cases of diagnosed bone and joint cancer [Chen et al., 2017]. The bone cells turn into cancelous giving rise to a disease that is heterogeneous in its origins and clinical indices. Osteosarcoma (OS) is the most common, primary malignancy of bone (36%), followed by chondrosarcoma (up to 30%) and Ewing sarcoma (16%) (Fig. 3) *[Marques et al., 2014; Evola et al., 2017]*.

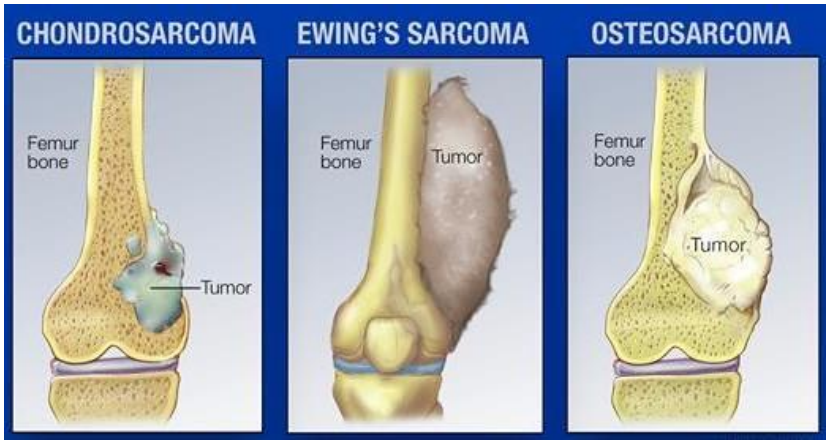

Figure 3: *Types of bone cancer.*

Osteosarcoma has a well-recognized double peak of incidence in early adolescence (75%) and elderly in the 7th and 8th decades (20%). Incidence worldwide is 3 cases per million annually. This type of cancer occurs primarily in large bones (90%). At the beginning of the 20th century, five-year survival was less than 20% with amputation being the only solution. The majority of deaths were due to pulmonary metastases. After the 1980s, the 10-year survival rate for localized osteosarcoma increased to 60-70% due to chemotherapy and surgical resection, while the five-year survival rate of metastatic osteosarcoma remained 25% to 30% *[Iwata et al., 2014; Diessner et al., 2019; Tempelaere et al., 2019]*. Osteosarcoma at older ages has increased due to the rapid aging of the population. Balducci et al. *[Balducci et al., 2005]* speculate that in 2030, 70% of all cancers will occur in patients over the age of 65. Some researchers state that the older the patient, the lower the survival rate *[Harting et al., 2014; Lee et al., 2009]*, while others found no association between age and survival *[Jaffe et al., 2002; Allison et al., 2012]*. While for young patients a typical therapy is well-defined, for elder patients no standardized strategy has been established. In addition, the efficacy of chemotherapy in older ages is still a controversial issue *[Tempelaere et al., 2019]*. Although the most commonly used therapeutic agents for the sarcomas' treatment are doxorubicin and ifosfamide, the therapeutic window they provide is narrow, with the exception of some responsive subtypes. Several sarcomas present chemical and radio resistance while in many cases the recurrent tumors are progressive, thus the additional chemotherapy is being toxic. Treatment with a combination of sequential single agents or variations in dose strength has not improved the therapeutic efficacy *[Susa et al., 2011]*.

Ewing's sarcoma accounts for almost 5% of all malignant bone tumors with more frequent patients being children or adolescents. While significant progress has been made in the therapy of Ewing's sarcoma through chemotherapy and local control measures (in the last 40 years the survival rate has increased from 10-15% to 65-70%), a noteworthy percentage of patients still die due to the disease progression. Currently, the therapy of Ewing's sarcoma of bone is based on combined treatment with neoadjuvant chemotherapy, radiation therapy and surgical resection of the primary tumor. Survival in primary metastatic Ewing's sarcoma is lower than 30% and consequently new therapies are required for these patients *[Balamuth et al., 2010; Fiorenza & Jeys 2010; Lin et al., 2019]*.

Chondrosarcoma, a cancer of cartilage cells, commonly affects long and flat bones (i.e ribs, pelvis, and scapula). In contrast to the above mentioned sarcomas, is most common in middle aged and elderly patients, with a peak diagnosis at >40 years, whereas it appears most frequently in adults *[Forde et al., 2017]*.

Metastatic bone cancer includes the displacement of cancer cells from the main site to a distant site(s) where they colonize and grow new lesion(s). Prostate, breast, lung, kidney, and thyroid are the most common sites of primary tumor origin. Bone metastasis is a significant cause of morbidity and is characteristic of aggressive cancer, reduced recovery time and bad prognosis. Bone metastases are divided into three categories, osteolytic (bone destructive), osteoblastic (bone forming) or mixed depending on the bone remodeling process impacted and radiological appearance (Fig 4). Whether the metastasis appears lytic or blastic, it can trigger severe skeletal complications called skeletal-related events (SREs); thus resulting in the need for radiotherapy and pathological fractures *[Forde et al., 2017; Jinnah et al., 2018; Coleman et al., 2020]*.

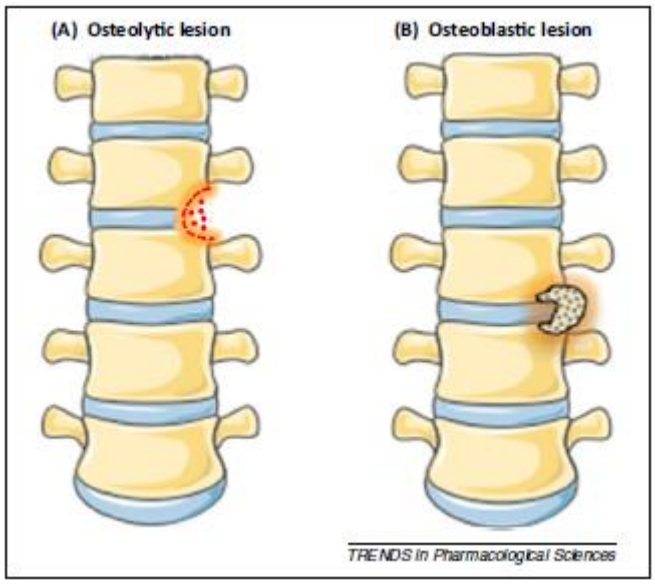

Figure 4: *"Illustration of osteolytic and osteoblastic bone metastatic lesions. Cancer causes predominantly two types of bone metastatic lesions based on the radiological appearance, with bone destruction being observed in osteolytic bone metastatic lesions (A), while bone-forming phenotypes are observed in osteoblastic bone metastatic lesions (B)". [Krzeszinski & Wan, 2015].*

Although several unconventional therapies are available such as photothermia and hyperthermia as well as the use of different nanoparticles due to their intrinsic antitumoral activity, in the majority of bone cancer cases the therapy includes surgery, radio- and chemotherapy. Researches have shown the option of combining surgery with chemotherapy *[Andronescu et al., 2013]*, surgery with hyperthermia [Andronescu et al., 2010] as well as surgery with hyperthermia and antitumoral nanoparticles (Fig. 5) *[Ficai et al., 2012]*.

In addition to the treatment of bone cancer, nanotechnology also holds a major role in diagnosis (Fig. 5). Various nanoparticles carrying ligands which can interact with specific molecules existing on the targeted cells' surface can be produced. Furthermore, a precise, imaging of tumors at cellular level can be accomplished by adding contrast agents to nanoparticles. For example, superparamagnetic iron oxide nanoparticles or quantum dot nanocrystals have been studied as contrast agents for tumor imaging. It is possible to produce nanoparticles which can absorb light and emit heat. These nanoparticles permeate selectively the tissue of concern and can be detected with laser technology. Thus, various intracellular procedures can be evaluated by delivery and detection of these nanoparticles, providing higher accuracy compared with conventional methods. The

application of nanotechnology in bone cancer is very promising as it has the potential to revolutionize the earlier detection of cancer, its metastases, as well as the ability to thoroughly assess the post-therapy response *[Savvidou et al., 2016]*.

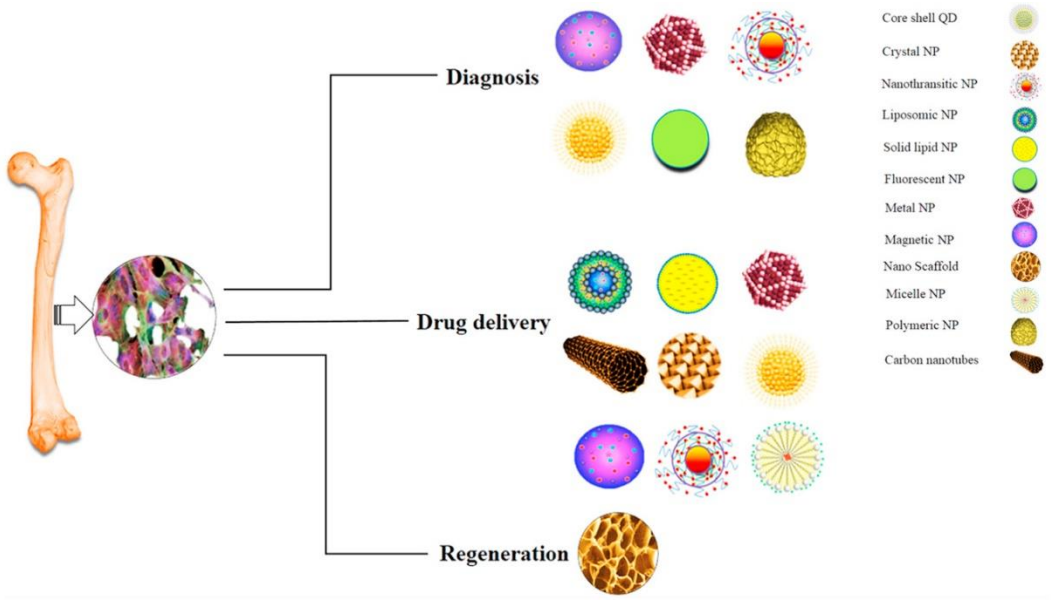

Figure 5: *Applications of nanoparticles in orthopaedic oncology.*

Nanotechnology in diagnosis of bone cancer

Cancer imaging has importantly improved the prognosis of sarcoma patients. Except from detecting the tumor, it is an important mean of recurrence and determining the therapeutic response after adjuvant therapies *[Susa et al., 2011]*.

The diagnosis of bone cancer is focused on the localization and characterization of the tumor by the use of a variety of distinct imaging methods such as radiographs, Computed Tomography (CT), Magnetic Resonance Imaging (MRI), (quantitative) 99Tc bone scans, Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT), Optical Imaging including fluorescence mediated tomography and near-infrared fluorescence reflectance (NIRF) imaging, as well as flow cytometry. These methods take advantage of utilizing different contrast agents to monitor and collect information about tissue anatomy, physiology, and metabolism. Novel enhanced and specific contrast agents are continuously being implemented for these and other imaging methods to improve clinical care. MRI is, up-to-date, the standard of care for primary tumor detection and of the pre-operative planning. Nowadays surgeons have the ability to resect the tumor with sufficient margins in order to avoid local or distant metastasis. These methods are also valuable for research where the objective is to identify patients with a metastatic spread. Spiral CT is presented more sensitive than the commonly thin cut CT for the detection of metastases. In addition, whole body MRI is also sensitive for the detection of metastases, though its use has been limited (mostly in paediatrics) because it is time-consuming. Now frequently used is PET with the use of 18F-labeled fluorodeoxyglucose (FDG), which is up-taken by cells showing increased glucose metabolism. It has been applied in some sarcomas, but for sarcomas with no increase in glucose uptake, it is not suitable. However, changes in skeletal morphology or radionuclide uptake may be discrete, non-specific or entirely absent at previous phases of the disease cycle. Furthermore, not all imaging methods are equally suitable for monitoring the progression of the skeletal disease or the therapeutic response, especially in patients with short survival periods. Several tumor biomarkers, such as prostate specific antigen (PSA) which is correlated with the degree of neoplastic tissue may be valuable in the clinical imaging of cancer as well as in therapy response.

However, with a few exceptions, these biomarkers do not give information especially to skeletal involvement *[Naomi and Womer 2010; Susa et al., 2011; Bone remodeling markers and bone cancer Markus J. Seibel; Aguilar, 2013;Wu et al., 2018]*.

Conventional bone cancer detection methods require time and many times are inaccurate which could delay the detection of cancer in the last stages of the disease, making it more difficult to rescue patients early *[Moradi et al., 2019]*. In addition, we have to overcome obstacles of optical imaging such as low depth penetration, absolute quantification, poor spatial resolution, as well as development, validation, and approval of relevant imaging agents for human use *[Vats et al., 2017]*. On the other hand, bio-imaging and bone cancer detection techniques based on nanotechnology provide quick and accurate techniques that give early cancer detection and could help doctors to provide timely and more active therapy *[Moradi et al., 2019]*. Nanomaterials have been proven to be very effective in the field of bone cancer diagnosis as several contrast agents can be encapsulated into nanoparticles or nanoparticles' surface can be functionalized with functional groups which are able to be coupled with imaging probes. In addition, nanoparticles' surface can be further modified with ligands that actively target tumor cells *[Susa et al., 2011]*. Due to their size, nanomaterials can be dispersed within tissues and within fluid pathways *[Love, 2017]*. For example, magnetic nanoparticles are very promising and useful for cellular and molecular imaging of several tumors including bone cancer. Their size should be between 10 to 100 nm in order not to be excreted by the kidney (<10 nm) and to not trigger immune system (>200 nm). In addition, magnetic nanoparticles can provide a better contrast in MRI, obtaining a more accurate depiction from the target and consequently having a better diagnosis. These nanoparticles have attracted the interest of the research community due to their optimum half-life and very low toxicity as well as due to the fact that they can be used either *in vitro* or *in vivo* enhancing the stability of the agents *[Moradi et al., 2019; Saji et al., 2010]*. Nanoparticles can enhance the resistance to photobleaching and to metabolic disintegration, they present high quantum yield and absorbency and near infra-red (NIR) emission. There are several categories of nanoparticles that have been employed as optical contrast agents, i.e quantum dots, gold nanoparticles, magnetic nanoparticles, liposomes, dye-doped silica etc. Metal nanoparticles such as silver and gold, which are not

fluorescence materials, can offer surface plasmon resonance in order to detect the tumor. Developing nanoparticles with multiple imaging modalities such as fluorescence, CT and MRI for cancer detection is also on demand *[Saji et al., 2010]*.

The contrast agents that are used in MRI are supra-magnetic and in CT are bismuthic *[Moradi et al., 2019]*. However they present low relaxivity values and have potential toxicity *[Saji et al., 2010]*. By functionalizing the surface of bismuthic nanoparticles with polymers the toxicity and the degeneration of the final material will be reduced. Nanoparticles with near-infrared (NIR) excitation and emission such as quantum dots have been proven useful for non-invasive tumor detection by *in vivo* studies *[Saji et al., 2010; Vats et al., 2017; Moradi et al., 2019]*. Table 2 presents studies on the diagnosis of bone malignancy using nanoparticles.

Table 2: *Studies on the diagnosis of bone malignancy using nanoparticles*

Study	Nano-structure	Production method	Results	Reference
Biodegradable bisphosphonate nanoparticles for imaging and therapeutic applications in osteosarcoma	Bisphosphonates (BP) nanoparticle		Efficient targeting of OS tumor tissue	*Rudnick-Glick et al., 2015*
Long-circulating iodinated albumin–gadolinium nanoparticles as enhanced magnetic resonance and computed tomography imaging probes for osteosarcoma visualization	Iodinated albumin–gadolinium nanoparticles	Protein-directed synthesis/ chloramines-T method	Potential for image-guided drug delivery and image-guided surgery.	*Wang et al., 2015*
Bull serum albumin coated Au@Agnanorods as SERS probes for ultrasensitive osteosarcoma cell detection	BSA (Bull Serum Albumin) coated gold–silver core–shell nanorods modified with Raman reporter 5,5-dithiobis 2-nitrobenzoic acid (DTNB)	Seed-mediated growth method	Efficient for cancer cell ultrasensitive detection with acceptable biocompatibility	*Yue et al., 2016*

Study	Nano-structure	Production method	Results	Reference
	(Au@AgNRs@BSA @Anti-MICA)			
Sensitive electrochemical cytosensor for highly specific detection of osteosarcoma 143B cells based on graphene-3D gold nanocomposites	Graphene-three dimensional nanostructure gold nanocomposites (G-3D Au)		A fast response, high sensitive and selectivity for cancer cells	*Wu et al., 2018*
Nano-confinement-driven enhanced magnetic relaxivity of SPIONs for targeted tumor bioimaging	SPIONs-PLGA-ALE lipid	Nanoprecipitation	Enhanced contrast ability of SPION based contrast agents in the diagnosis and treatment of cancer.	*Nguyen et al., 2018*

Wang et al. *[Wang et al., 2015]*, developed multimodal imaging nanoparticles for *in vivo* osteosarcoma imaging with the use of MRI and CT. Although MRI offers high-resolution images of the soft tissues, it is difficult to distinguish the tumor from the surrounding healthy tissue when unenhanced contrast agents are used. In addition, CT can visualize 3D reconstructions of the targeted tissue but the intrinsic low sensitivity of it, causes poor contrast among soft tissues. Consequently, the combination of these two imaging techniques may enhance the visualization of the tumor. However, the combination of two different contrast agents will be harmful for the patient, thus a multimodal probe must be able to overcome this problem and to be applied for the diagnosis via both MRI and CT techniques.

Hence, Wang et al., *[Wang et al., 2015]* used a biomimetic approach in order to develop, for first time, bovine serum albumin (BSA)-gadolinium nanoparticles (GdNPs) which subsequently iodinated via the chloramine-T method. BSA was used as a stabilizer for the biomineralization synthesis of GdNPs and as a molecule bearing several chemically active groups, which are able to iodinated to form an iodinated BSA-GdNPs (I-BSA-GdNPs) complex. The research group tested the stability and biocompatibility of the as-prepared I-BSA-GdNPs and the results were very promising

as the nanoparticles showed excellent chemical stability and biocompatibility. Furthermore, the MRI and CT tests both *in vitro* and *in vivo* showed intense X-ray attenuation coefficient, and good MR imaging ability. They compared the novel nanoparticles with a clinical available contrast agent namely Gd-DTPA and the measured r_1 value was 12.03 mM^{-1} s^{-1}, almost four times higher than the value of Gd-DTPA (3.19 mM^{-1} s^{-1}). In addition, I-BSA-GdNPs showed stronger T_1 signals than Gd-DTPA with the same Gd concentration (Fig. 6). The high relaxivity coefficient of I-BSA-GdNPs was attributed to the efficient longitudinal relaxation of the water protons on the gadolinium oxide nanoparticles' surface where a large amount of Gd^{3+} retaining high magnetic moments. X-ray absorption of I-BSA-GdNPs was compared to the absorption of commercial Ioversol *in vitro* and the results revealed that their behavior is similar.

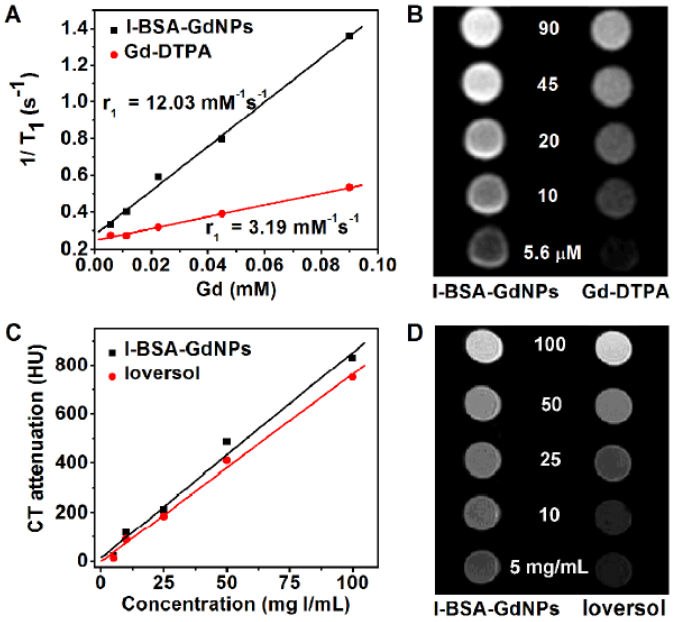

Figure 6: *"r_1 relaxivity curves of I-BSA-GdNPs and Gd-DTPA (A), T_1-weighted MR phantom images of I-BSA-GdNPs and Gd-DTPA at different concentrations (B), CT values (HU) of IBSA-GdNPs and Ioversol (C), CT phantom images at different concentrations of I-BSA-GdNPs and Ioversol (D)" [Wang et al., 2015].*

In vivo tests were also shown promising results, as a distinct enhanced signal of the tumor was detected within 24 h in MRI tests and in CT images. The exact profile of the osteosarcoma was designated in comparison with other tissues at the first 30 min whereas after 24 h, the CT signal were higher, indicating further applications in the

passive tumor targeting imaging. The results suggested that the novel I-BSA-GdNPs may accumulate for a long time within the tumor matrix via EPR effect compared to commercial available Ioversol which was mainly accumulated in the kidney and the bladder at 30 min due to its short time of circulation (Fig. 7).

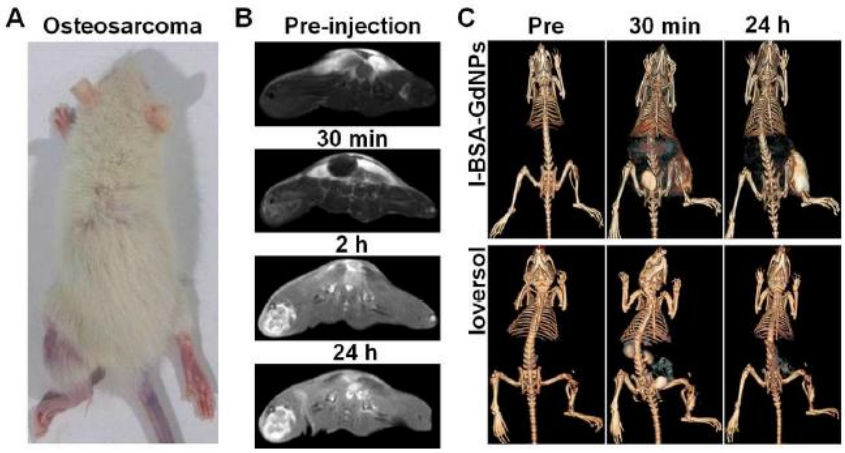

Figure 7: *"Orthotopic osteosarcoma animal models (A), In vivo T_1-weighted MRI images of orthotopic osteosarcoma rats before and at 30 min, 2 h, and 24 h after I-BSA-GdNPs injection (B), CT 3D images of orthotopic osteosarcoma rats after Ioversol and I-BSA-GdNPs injection (C)" [Wang et al., 2015].*

As it has already mentioned magnetic nanoparticles and more precisely superparamagnetic iron oxide nanoparticles (SPIONs) which are highly biocompatible are a very promising contrast agent in MRI. They can be developed by various chemical techniques including co-precipitation, reduction-precipitation, and hydrothermal methods, where the ions Fe^{3+} and Fe^{2+} react in aqueous solutions. Both Fe^{3+} and Fe^{2+} are already existed in human body and have clear metabolic pathways; hence, they have attracted widespread research interest in the field of diagnostics and therapy. However, the majority of the SPION-based clinical contrast agents are discontinued due to severe pain, low transverse magnetic relaxivity range (80–180 mM^{-1} s^{-1}), low circulation half-life, and lack of disease specificity. Thus, Nguyen et al., *[Nguyen et al., 2018]* developed a novel SPION based nanotag using a bone targeting ligand namely alendronic acid (ALE) and a biocompatible hydrophobic polymeric core namely PLGA (Fig. 8) which presented high transverse magnetic relaxivity of 625 mM^{-1} s^{-1} at 14.1T, which was extremely higher than that of clinical contrast agents such as Feridex® (r_2 = 120 mM^{-1} s^{-1}, 3 T) and Supravist® (r_2 = 57 mM^{-1} s^{-1}, 3 T) (Fig. 9). The results from a binding study, conducted using hydroxyapatite as a bone model,

revealed that the nanoclusters presented strong binding affinity to the bone. Furthermore, the nanoclusters presented biocompatibility after tested in an *in vitro* cellular study against K7M2.

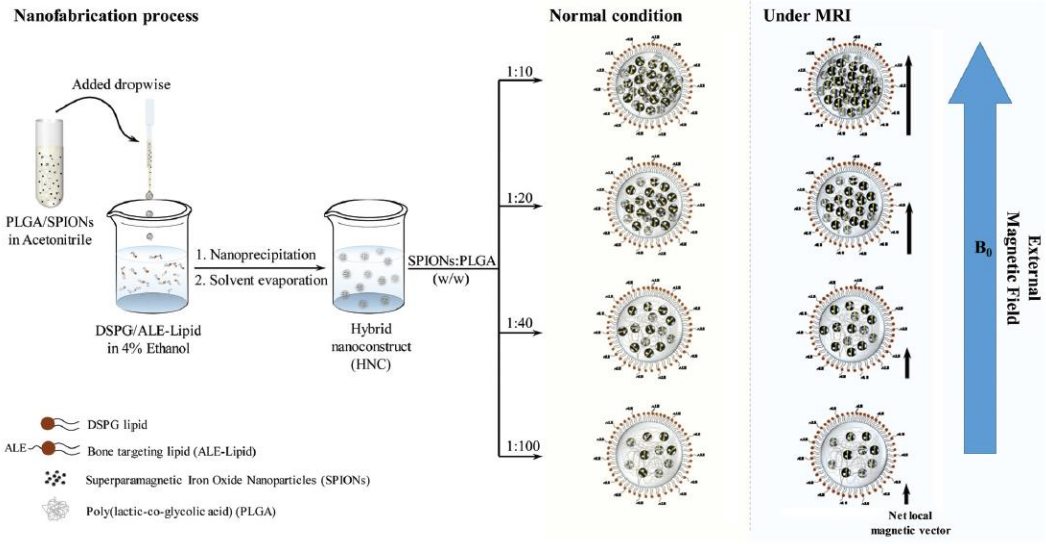

***Figure 8**: "Fabrication process of magnetic nanoparticles using nanoprecipitation. The clustering degree of HNC is tunable by changing the ratio (w/w) of SPIONs and polymeric matrix PLGA in the organic phase. In the designed experiment, the amount of PLGA was kept constant at 1 mg, while the amount of SPIONs was varied at 10, 25, 50, and 100 µg, corresponding to SPIONs:PLGA (w/w) ratio of 1:100, 1:40, 1:20, and 1:10, respectively. As a consequence, the interparticle distant of SPIONs reduced and they confined within the polymeric matrix" [Nguyen et al., 2018].*

Generally, T2 relaxation of water protons depends on the diffusion rate of water and the interaction time between water protons and local magnetic moment of nanoclusters, so the research team optimized the synthesis conditions in order to produce nanoclusters with reduced interparticle distant of SPIONs which eventually confined within the polymeric matrix. These clusters enhance the magnetic relaxivity, which could be attributed to the increase in net local magnetization due to proximal field inhomogeneity when an external magnetic field (B_o) is applied. So, they concluded that the MRI relaxivity of the developed nanoclusters have SPION density-dependent behavior, characterized by importantly increased relaxivity by increasing the packing density *[Nguyen et al., 2018]*. Several studies have shown that an effective way to enhance the relaxivity of SPIONs, is packing individual SPIONs into a cluster *[Ragheb et al., 2013; Smith et al., 2015; Lin et al., 2015]*. In addition, the use of a hydrophilic shell also enhances the interaction with water protons, thus the relaxivity

too. For example, Paquet et al. *[Paquet et al., 2011]* encapsulated a cluster of SPIONs into a hydrogel, resulting in 2 to 3 times boosting of relaxivity. It has also been shown that by encapsulating clinically available contrast agents, such as Resovist® or Endorem™, into liposomes higher relaxivity is presented, ranging from 150 to 200 $mM^{-1}\,s^{-1}$ *[Mikhaylov et al., 2011; Lorenzato et al., 2013]*.

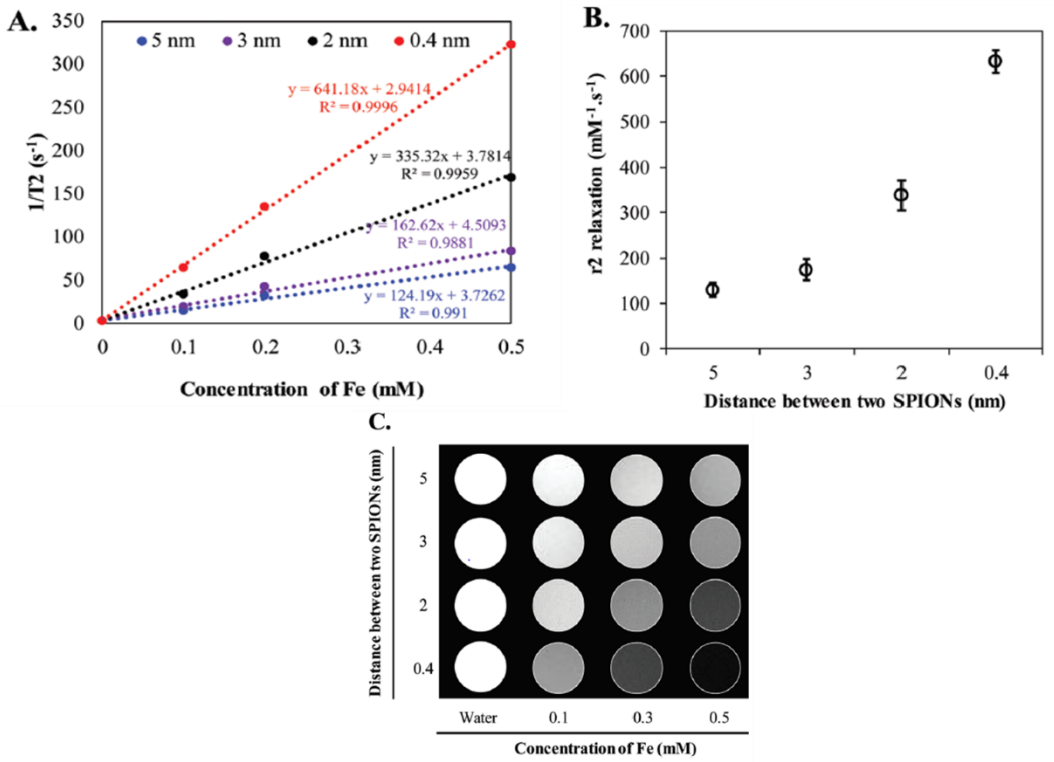

***Figure 9**: "Confinement driven relaxivity enhancement. (A) Plot of $1/T_2$ vs. the concentration of iron in aqueous solution measured in a 14.1 T MRI system at 25 °C. (B) r_2 relaxation rate (s^{-1} mM^{-1}) with different SPION packing density. (C) T_2-Weighted MR images of HNC aqueous suspensions with different concentrations and packing densities. The T_2- weighted MRI phantoms were taken at TR = 1500 ms, TE = 15 ms, and slice thickness = 1 mm" [Nguyen et al., 2018].*

Electrochemical biosensors can be also applied to identify bone cancer utilizing body fluids. Nanomaterials have greatly improve the sensitivity and selectivity of these devices in the last decades *[Zhong et al., 2012]*. Metal nanoparticles such as gold and silver are extensively used in cell imaging and proteins interaction owing to their exceptionally strong absorption and light scattering in the plasmon resonance. The research interest in using these materials in cancer diagnosis becomes from their unique optical properties, facile surface chemistry, and suitable size and shape which

can be controlled depending on the application. Furthermore, they can be conjugated with specific ligands or biomarkers which are overexpressed by cancer cells *[Ficai et al., 2015]*. Zhong et al., *[Zhong et al., 2012]* produced a sensitive chronocoulometric DNA biosensor based on a nanostructure gold electrode in order to detect femtomolar level survivin gene which is connected with osteosarcoma utilizing hexaamine-ruthenium III complexes, $[Ru(NH_3)_6]^{3+}$, as the electrochemical indicator. The develop process was a simple, economical, and controllable. The device presented superior conductivity, activity, and biocompatibility due to the large active surface providing by the presence of gold nanoparticles. The biosensor was able to detect the target DNA at a concentration as low as 5.6 fM.

Yue et al., *[Yue et al., 2016]* developed a novel ultrasensitive, biocompatible and stable SERS nanotag, based on BSA (Bull Serum Albumin) coated gold–silver core–shell nanorods modified with Raman reporter 5,5-dithiobis 2-nitrobenzoic acid (DTNB) (Au@AgNRs@BSA@Anti-MICA), for *in vitro* detection of Osteosarcoma cells (Fig. 10). They tested the SERS performance of the nanomaterial in the presence and absence of the silver shell and the results revealed that the presence of the silver coating strongly enhances the SERS performance due to the strong electromagnetic field around the two ends of the bimetallic nanoparticles. This phenomenon is ascribed to the lightening-rod effect reported by Nikoobakht et al. *[Nikoobakht et al., 2002].* They also examined the cytotoxicity of the prepared nanopraticles via the MTT assay method. Concentrations up to 2nM of Au@AgNRs@BSA were examined and it was found that the nanorods did not present toxicity over 24h, revealing the good biocompatibility and the potential to be suitable for *in vitro* and *in vivo* studies. Finally they examined the SERS performance of the nanotags over MICA positive U-2 OS cells and MICA negative Saos-2 cells via Raman microscopy. The results revealed that the antibody conjugated with nanoparticles was specifically bonded with MICA receptor over-expressing cells as high SERS signals were detected only from the over-expressing MICA receptors of U-2 OS cells, while negligible signals were observed in the case of nanoparticles cultured with MICA negative Saos-2.

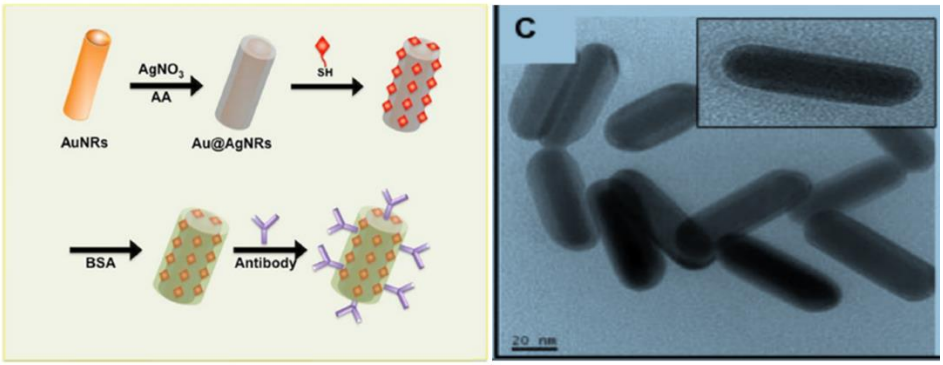

Figure 10: *Synthesis procedure of Au@AgNRs@BSA@Anti-MICA (left), TEM image of Au@AgNRs@BSA@Anti-MICA nanorods (right) [Yue et al., 2016].*

Conclusions and Future perspectives

In the past few years, important attention has been given in developing early cancer diagnosis tool using single or even more modalities. Nanomaterials offers many benefits in the field of diagnosis and imaging of bone cancer such as increased biocompatibility, tunable size, shape, and surface properties, high surface area which can be functionalized, long circulation times and quick and accurate detection and imaging. Nowadays, the ability to controllably synthesize nanomaterials as tumor detectors and imaging agents as well as the appetite for newer, better, and more tolerable nanoparticles to enhance disease diagnosis in living systems has great impact in orthopaedic oncology. The future for further development of diagnostic imaging materials is bright and the drive to diagnose disease earlier in its progression only strengthens the necessity of better and more effective diagnostic strategies.

Nanotechnology in bone cancer treatment

Compared to other types of cancer, bone tumor therapy, due to the particularities of the bony tissue, involves different approaches. More precisely, the low diffusion rate of the anticancer agents inside the tumor as well as the low penetration ability of different radiations into the bony tissue are the most important factors, which affect tumor therapy *[Fucai et al., 2015]*. Additional limitations are the wide excisions of tissue, low targeting efficiency and adverse effects on other organs and tissues when chemotherapy and radiotherapy are used and insufficient bone graft sources as well as risk of infection in cases of using bone grafts.

One reason why antitumor drugs fail to eradicate cancer cells is that they are administered systemically which leads to differences in drug bio-distribution, absorption and metabolism. Tumors are often located in areas that are hard to be penetrated by chemotherapeutic agents and they are shielded by the local microenvironment owing to enhanced hydrostatic pressure and the modified tumor vasculature. There are two ways in which nanomaterials can accumulate at the tumor target, the passive and the active targeting (Fig. 11) *[Susa et al., 2011]*.

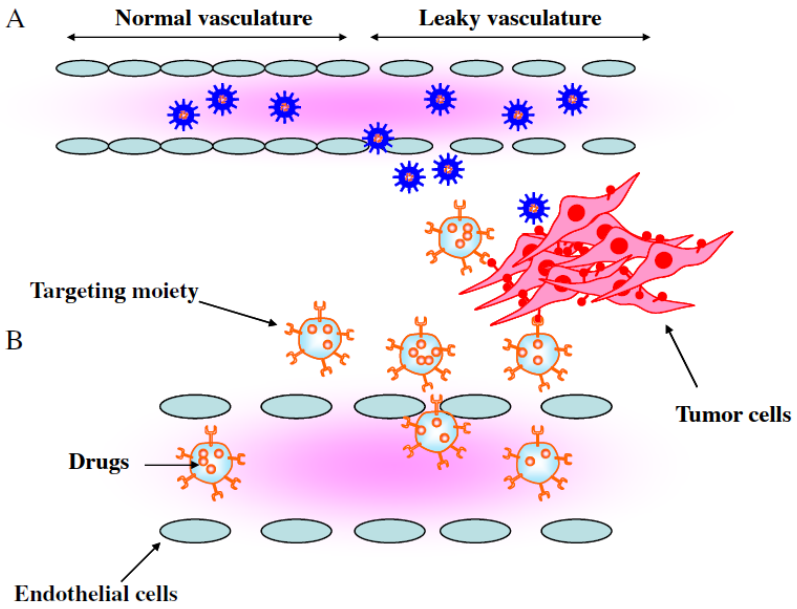

Figure 11: *"Passive targeting and active targeting. A. Pictorial representation of passive targeting. Tumor tissue vasculature is hyperpermeable compared to the normal vasculature, and nanoparticles are able to accumulate preferentially in the tumor environment due to the enhanced permeability and retention effect. B. Targeting ligand or antibody is conjugated to*

the nanoparticle, thereby allowing increased accumulation of the chemotherapeutic drugs or genes to the tumor site" [Susa et al., 2011].

Passive targeting refers to the ability of nanoparticles to enter tumors via the localized leaky vasculature of tumor and to remain in the tumor microenvironment due to poor lymphatic drainage. This passive targeting is also called the "Enhanced Permeation and Retention" (EPR) effect *[Pelaz et al., 2016]*. Active targeting refers to conjugation of NPs by antibodies or ligands specific to the tumor cells *[Susa et al., 2011]*.

The anticancer drug delivery to a solid tumor consists of five critical steps, termed the "CAPIR cascade": "circulation in blood, accumulation and penetration into the tumor, cellular internalization, and intracellular drug release". Therefore, the therapeutic efficiency of a nanomedicine is determined by its efficiency in each step (Fig. 12). Furthermore, in order to a nanocarrier accomplish the CAPIR cascade it should have 2R2SP properties, the termed of "drug retention vs release (2R)", "surface stealthy vs sticky (2S)" and "tumor penetration (P)"; thus to deliver active drugs at the right time and place and to provide a high therapeutic efficacy. Consequently, researchers have to tailoring the physicochemical properties of NPs such as size, surface properties to achieve 2R2SP properties and finally to accomplish the CAPIR cascade *[Pelaz et al., 2016]*.

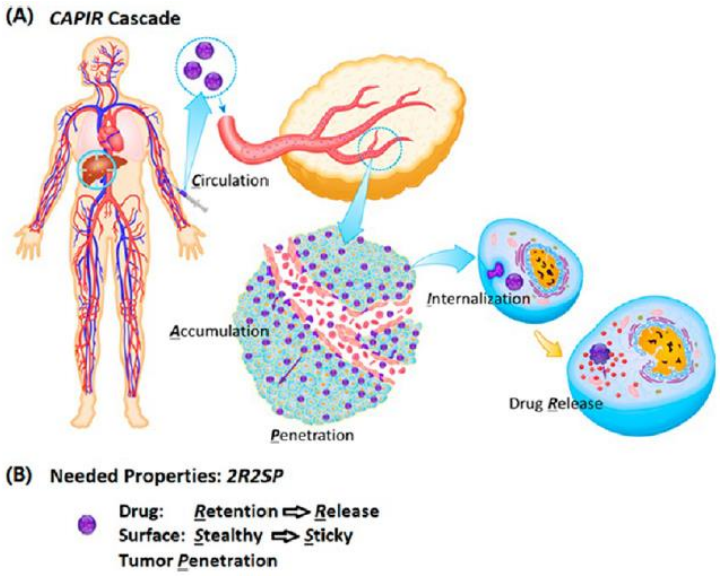

Figure 12: *"Five-step CAPIR cascade in targeted cancer drug delivery (A), Needed properties of a nanomedicine capable of accomplishing the cascade (B)" [Pelaz et al., 2016].*

In the last decades, nanotechnology have revolutionized traditional therapies of bone cancer, such as radio- and chemotherapy, by enhancing the effectiveness of drug delivery strategies. Nanomaterials, due their small size are able to traverse biological barriers resulting in more efficient delivery. Further advantages that NMs present are: (i) high drug-loading capacity due to the large surface area:volume ratios; (ii) increased drug solubility; (iii) increased drug stability; (iv) targeted drug delivery and controlled release via stimuli-responsive functional groups (v) reduced side effects on other tissues or organs; and (vi) enhanced penetration in cell membranes, allowing intracellular drug delivery or delivery to specific organelles. In addition, nanocarriers, such as calcium phosphate, can motivate new bone growth by stimulating mineralization or promoting bone cell growth *[Cheng 2017]*.

Although countless experiments have been conducted using different nanoparticles, each with its own benefits and drawbacks, the researchers have to share a common aim: nanomaterials must be able achieve the tumor by overcoming different difficulties without losing their drug cargo, and they have to effectively release the highest amount of the antitumor drug. Ideally, NPs should be inert, free from leachable impurities, and biodegradable. Nanoparticles' potential to cause systemic side effects has been well established, but most of the toxicity is due to the use of products which are not intended for *in vivo* use. As it has already mentioned, the small size of nanoparticles is advantageous because it is controllable and their surface can be functionalized by hydrophilic polymers allowing to avoid opsonization by macrophages *[Susa et al., 2011]*.

Several therapies using photodynamics and hyperthermia that increase the efficiency of the nanomaterials have been reported. Furthermore, cancer cells have specific characteristics that allow their targeting from nano-carriers, allowing them to bind and release the drugs into the cells. Several stimulants such as pH, temperature, tumor environment, redox condition, enzyme activity and ultrasound waves can trigger the nanoparticles to release the drug they carry. For example, pH value at cancerous environment is lower due to glycolysis *[Moradi et al., 2019]*. Encapsulation of a histidine-lysine peptide in a polymeric matrix has been reported to enhance endosomal release resulting in increased effect *[Cheng et al., 2002]*. Several types of nanoparticles have been utilized for enhancing drug delivery to bone malignancies,

such as liposomes, micelles, dendrimers, polymeric nanoparticles and solid lipids. *[Moradi et al., 2019]*. Except from the organic materials mentioned above, inorganic materials such as metals and ceramics, are also used in bone cancer treatment. In Table 3 several NPs that have been utilized in bone malignancy models are given, whereas FDA- and EMA-approved nanomedicine products are given in Table 4.

Table 3: *Examples of NPs utilizing in bone tumor therapy*

Study	Nanoparticles	Results	Reference
Halloysite nanotube-based drug delivery system for treating Osteosarcoma	Halloysite nanotube (HNTs)	A high potential anti-cancer drug delivery system	*Sun L and Mills DK, 2014*
Poloxamer surface modified trimethyl chitosan nanoparticle for the effective delivery of methotrexate in Osteosarcoma	Poloxamer surface modified trimethyl chitosan NP (TMCN)	Improved Efficacy of treatment and minimizing toxicity with distribution of drugs and increased apoptosis bone cancer cells	*Li S, et al., 2017*
The effective combination therapy against human Osteosarcoma: Doxorubicin plus curcumin co-encapsulated lipid-coated polymeric nanoparticulate drug delivery system	Doxorubicin plus curcumin co-encapsulated lipid-coated polymeric NPs (DOX+CUR LPNs)	Reduced restricting side effects of traditional chemotherapy drug dose, the DOX+CUR LPNs increased cell delivery and drug distribution to cancer cells.	*Wang L, et al., 2016*
Inhibition of ABCB1 (MDR1) expression by an siRNA nanoparticulate delivery system to overcome drug resistance in Osteosarcoma	Lipid-modified dextran-based polymeric nanoparticle for MDR1 siRNA delivery	Increased Doxorubicin concentration and improved delivery on MDR reduced resistance of bone cancer cell lines	*Susa M, et al., 2010*
Ceramic core with polymer corona hybrid nanocarrier for the treatment of Osteosarcoma with co-delivery of protein and anti-cancer Drug	Ceramic core with polymer corona hybrid nanocarrier	Increased protein and drug delivery to OMG-63 cell line.	*Prasad, et al., 2017*

Study	Nanoparticles	Results	Reference
Alendronate-modified polydopamine-coated paclitaxel nanoparticles for osteosarcoma-targeted therapy	PTX-PDA-ALN-NP: Polymeric NPs coated with polydopamine and grafted by alendronate as a targeting paclitaxel carrier	Superior cell proliferative inhibitory efficacy and high targeting therapeutic effects decreasing the side effects of PTX and achieved better therapeutic efficacy than PTX injection	*Lei et al., 2019*
Cancer cell membrane coated silica nanoparticles loaded with ICG for tumour specific photothermal therapy of osteosarcoma	CM/SLN/ICG: Encapsulated indocyanine green (ICG) as a photothermal into SGC7901 cell membrane modified silica nanoparticles.	Superior anticancer efficacy when compared with either SLN/ICG or free ICG	*Zhang et al., 2019*
Polydopamine-based surface modification of paclitaxel nanoparticles for osteosarcoma targeted therapy	PTX-PDA-ALN-NPs: Polydopamine coated paclitaxel NPs functionalized with alendronate as ligand.	Remarkably inhibit cell proliferation in vitro, higher tumor inhibition rate and lower toxicity compared to pure PTX in vivo.	*Zhao et al., 2019*
Nanodiamond as a Vector for siRNA Delivery to Ewing Sarcoma Cells	Polyethylene-imine (PEI) or polyallylamine hydrochloride (PAH)-coated NDs-siRNA	Sufficiently strong adsorption of the biomolecule onto the particle to go through the cell membrane without loss of material, the dissociation of the complex on the timescale of a cell division cycle, and low cellular toxicity. High efficiency in inhibiting the EWS-Fli1 Expression.	*Alhaddad et al., 2011*

Table 4: *FDA- and EMA-approved nanomedicine products.*

Trade name	Generic name	Nanoplatform (active agent)	Indications	Approval (date)	Manufacturer	Benefit of nanomaterials
Mepact	Liposomal mifamurtide	Liposome (Mifamurtide)	Osteosarco-ma	EMA (2009)	Takeda	Increase efficacy and decrease systemic toxicity
EquivaBone	Hydroxyapatite	Hydroxyapatite	Bone substitute	FDA (2009)	Zimmer Biomet	Mimics bone structure
Ostim	Hydroxyapatite	Hydroxyapatite	Bone substitute	FDA (2004)	Heraseus Kulzer	Mimics bone structure
OsSatura	Hydroxyapatite	Hydroxyapatite	Bone substitute	FDA (2005)	Rti Surgical	Mimics bone structure
Vitoss	Calcium phosphate	Calcium phosphate	Bone substitute	FDA (2003)	Stryker	Mimics bone structure

Chemotherapy and gene therapy

Since the current chemotherapy treatments do not differentiate between cancer and healthy tissue, it is essential to design nanoparticles that can differentiate this dissimilarity. As the rate of drug resistance in cancer patients is increased, incorporation of nanoparticles have been proposed in order to improve the drug delivery process. Healthy tissue toxicity and inactivation of drugs can be prevented by encapsulated pharmaceutical agents into nanoparticles *[Zare-Zardini et al., 2015]*.

Organic materials, such as polymers, are widely used as nanocarriers of drug and gene delivery systems (Table 5). Ding's group *[Ding et al., 2015]* produced a three poly(ethylene glycol)-polyleucine (PEG-PLeu) di- or triblock copolymers through ring-opening polymerization (ROP) of leucine N-carboxyanhydride (Leu NCA) with amino-terminated PEG as a macroinitiator. Doxorubicin (DOX), a common chemotherapeutic agent frequently used in cancer therapy and mainly for spinal tumors, was encapsulated into micelles through a nanoprecipitation method. The nanopharmaceutical compound showed effective drug release in both MG63 and Saos-2 cells, two types of human osteosarcoma cell lines improving the antiosteosarcoma efficiency.

Table 5: *NPs used in bone diseases for drug and gene delivery [Gu et al., 2013]*

Bone diseases	NanoParticles	Drugs delivered	Drug efficiency
Cancer bone metastasis	PLL-CD	BPs (RIS)	Increased
	PLGA	BPs (ZOL)	Increased
	PLGA	Doxorubicin	Increased
	PLGA	Alendronate	Not detected
	PTX–PEG–ALN	Aminobisphosphonate	Increased
Osteosarcoma and Ewing's sarcoma	MSN	siRNA	Cell model only
	Polymer	Camptothecin	Increased
	LDH	Methotrexate	Cell model only
	Chitosan NP	DNA enzyme	Increased
	Chitosan NP	DNA plasmid	Increased
	Polymerized liposomal NP	Doxorubicin	Increased
	Magnetic arsenic trioxide NP	Arsenic trioxide	Increased
	Calcium phosphate NP	Cisplatin	Increased
	Lipid-modified dextran-based	Doxorubicin	Increased
	Polymer NP		
	Dextran-PEI NP	Doxorubicin	Increased

Self-assembled polymeric nanoparticles have gained researchers' attention as effectively drug delivery carriers for cancer therapy. These amphiphilic nanoparticles commonly have a hydrophobic core protected by a hydrophilic shell when they exposed to aqueous means. The drug is encapsulated in the hydrophobic core whereas the hydrophilic shell prevents the drug delivery system alongside the reticuloendothelial system (RES). Additional advantages that these nanostructures offer are the core-shell structure, the increased loading capacity, the targeted drug delivery as well as the minimization of the adverse side-effects of the administered drug. Furthermore, high stability and consequently prolonged circulation in blood resulting in enhanced EPR based passive targeting. In this respect, Chen and coworkers *[Chen et al., 2015]* produced an acid-sensitive IFS-loaded poly(lactic-co-glycolic acid-) (PLGA-) dextran polymer nanomaterial (PD/IFS) for the inhibition of MG63 and SaOS-2 cancer cells. PLGA-dextran polymeric micelles with a mean particle size at 124 nm and an excellent dispersity index of 0.124 (PDI) were formed in aqueous media (Fig. 13 a). IFS was encapsulated into the nanoparticles with a loading and encapsulation efficiency of (20.15 ± 3.5)% and (89 ± 1.95)%, respectively. The release studies were

shown promoted sustained drug release at pH 7.4 and induced accelerated release at pH 5.0. The in vitro studies against MG63 and Saos-2 cancer cells indicated higher antitumor activity and greater induction of apoptosis compared to free IFS (Fig. 13 b-c, d-e). Overall, their study reveals that the encapsulation of the anticancer agent into polymeric micelles enhances the therapeutic efficacy and may be a capable method for the therapy of malignant spinal tumors.

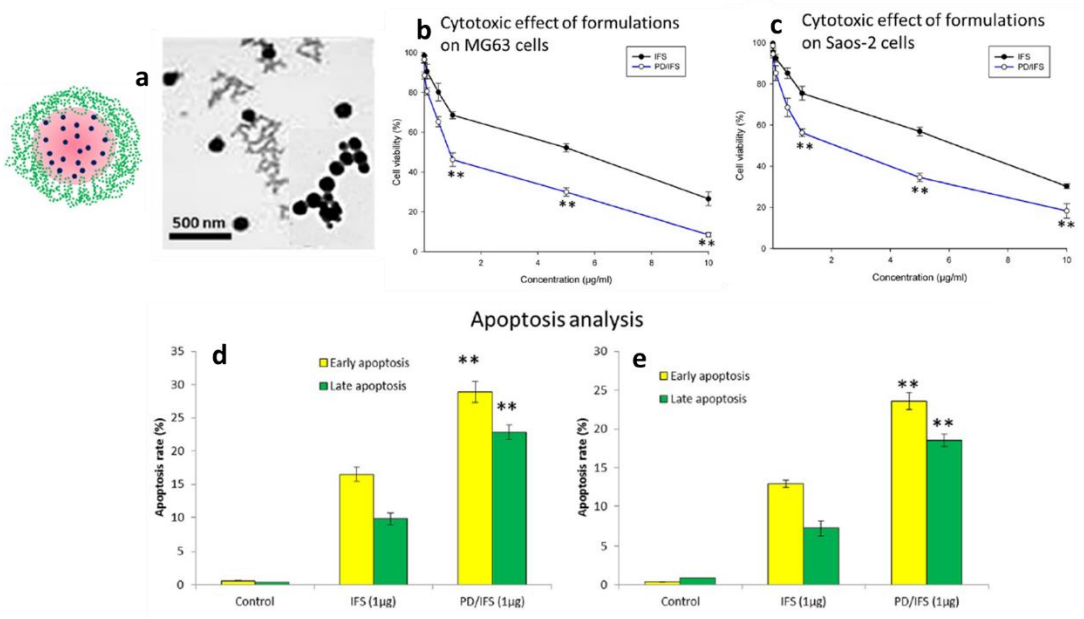

Figure 13: *"Schematic illustration and TEM image of conjugation of PLGA polymer with the dextran block. The ifosfamide and block copolymer self-assembled to form the polymeric nanoparticles (a), Cytotoxicity of free IFS and PD/IFS (with equivalent IFS concentration) on (b) MG63 (c) Saos-2 osteosarcoma cancer cells, Apoptosis of (d) MG63 (e) Saos-2 cancer cells". [Chen et al., 2015]*

Based on the need for a cancer therapy that maximizes drug exposure to diseased tissues while minimizing adverse side effects, Morton et al. *[Morton et al., 2014]*, developed DOX-loaded liposomes where a polyelectrolyte, poly(acrylic acid) (PAA), was modified with a bisphosphonate, alendronate, and subsequently electrostatically assembled in a nanoparticle coating for treating primary osteosarcoma. The results showed that the nanoparticles accumulated in subcutaneous 143B osteosarcoma xenografts, releasing DOX, resulting in attenuation of tumor burden and extended animal survival, whereas in some cases even completely eliminating tumors.

Several inorganic materials such as CNTs, CaP, SiO_2, TiO_2 as well as GNPs have received increased attention in drug delivery for treating bone cancer (Table 3). CNTs composed of a layer of graphene and have widely been applied to carry numerous biological molecules, ranging from small drug molecules to biomacromolecules such as proteins, DNA and RNA into different types of cells via endocytosis with very promising results. CNTs are able to penetrate the cell membrane and release the biological cargo in the cell. Therefore, their high surface area and hydrophobicity are the main obstacles for their application in biological systems as they cause intrinsic toxicity. One common strategy to overcome this limitation is the functionalization of their surface with organic materials such as polymers. However, the cellular uptake mechanism may vary depending on the size and functionalization of CNTs, hence it is difficult to control the release profile in a specified manner *[De la Zerda et al., 20018; Liu et al., 2009; Cheng et al., 2017]*. Cheng et al., *[Cheng et al., 2013]* developed a novel PLGA coated-CNT drug delivery system for intracellular delivery of caspase-3 (proapoptotic protein) into osteosarcoma cells. PLGA is a biodegradable polymer which allows the release of the cargo in a controlled manner and tune cell behavior. The proposed novel system is advantageous due to its ability to efficiently transfect cells with the unique needle-like shape of CNTs, decreased cytotoxicity compared to pure CNTs via a biocompatible PLGA coating, and program the protein release times by controlling the molecular weight and ratio of PLGA and consequently the degradation profile of it. The research team tested the capacity of the novel nanostructure bearing caspase-3 as antitumoral agent to induce apoptosis of MG-63 cancer cells *in vitro*. The results showed high efficiency compared to common anticancer agents revealing a promising nanostructure for drug delivery as well as for developing scaffolds for bone tissue engineering.

Research has shown that the combination of chemotherapeutic drugs and bisphosphonates (BPs) enhance the therapeutic efficiency of treating bone cancer. Nevertheless, common chemotherapy is unsuccessful due to the low permeability in the skeleton tumor tissues and decreased selectivity to the multiple bone metastatic nodules. Thus, side effects due to the non targeted drug release is still an obstacle in cancer therapy. In addition, the efficiency of BPs for inhibiting the viability of cancer cells is limited, whereas clinical trials have shown that high doses usually cause

osteonecrosis of the jaw *[Coxon et al., 2006; Saad et al., 2012; Rebucci et al., 2013; Shukla et al., 2014; Ye et al., 2015;]*. Consequently, it is essential to develop new strategies for treating cancer metastasis in bone. In order to overcome these problems, Sun et al. *[Sun et al., 2016]* developed pH-sensitive mesoporous silica nanoparticles loaded with DOX and linked to zoledronate (ZOL). The pH-sensitive nanocarriers, showed an enhanced cumulative drug release of 38% at pH 5.0, compared to 10% at pH 7.4, due to the protonation of the amino groups of DOX at acidic pH values. The antitumor tests against A549 cells showed high cytotoxicity and significantly decreased cancer cell migration in vitro (Fig. 14).

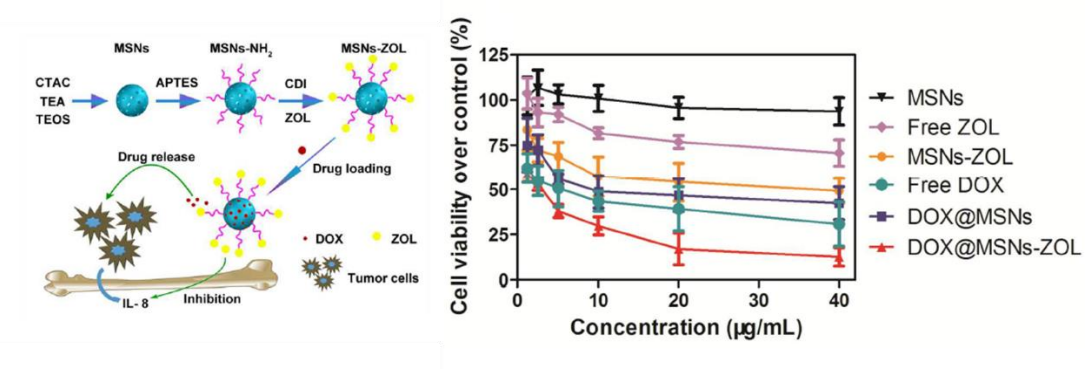

Figure 14: *"Cell viability of A549 cells incubated with free DOX, free ZOL, MSNs, MSNs-ZOL, DOX@MSNs, and DOX@MSNs-ZOL for 24 h" [Sun et al., 2016].*

A major problem in chemotherapeutic treatment of cancer is that many patients after prolonged therapy present multi-drug resistance (MDR), thus the effectiveness of the therapy is diminished. Tumor cells presenting drug-resistance overexpress P-glycoprotein 1 which transports the chemotherapeutic agent out of the cell resulting in low intracellular concentration of it *[Susa et al., 2010; Savvidou et al., 2016]*. Consequently, the development of agents that effectively reverse MDR with low toxicity is on demand. Susa et al. *[Susa et al., 2010]*, in order to overcome the drug resistance as well as the adverse effects of conventional chemotherapeutic agents designed and evaluated a novel biocompatible and stable lipid-modified dextran-based polymeric carrier for MDR1 siRNA delivery against multi-drug resistant osteosarcoma cell lines ($KHOS_{R2}$ and $U\text{-}2OS_{R2}$). The liposomes were PEGylated in order to become stable and stealthy. The results showed suppressed P-gp expression in both $KHOS_{R2}$ and $U\text{-}2OS_{R2}$ cells after treating with MDR1 siRNA NPs at concentrations of 30

nM or higher. Furthermore, the novel nanoplatforms showed a longer period of suppression in comparison with commercially available agents (96 hrs. vs 48 hrs.).

In another study, Cebrián et al., *[Cebrián et al., 2011]* developed poly(ethylenimine)-coated gold nanoparticles (Au-PEI NPs) as promising non-viral vectors for transfecting a variety of plasmids into human osteosarcoma cells. Au NPs are advantageous due to their low cytotoxicity, low immunogenicity and biocompatibility as well as due to their easy production synthesis and functionalization of their surface. The research team tested the effect of particle size on cell transfection, and the results revealed that cells were transfected with complexes derived from <10 nm Au-PEI NPs, but not with the <100 nm Au-PEI NPs due to the big aggregates (Fig. 15). In addition, they tested uncoated Au/DNA complexes and the results showed that the bare Au/DNA complexes were unable to transfect target cells regardless the DNA:Au NPs ratio tested, indicating that PEI is coupled to the surface of the Au NPs as well as that PEI is particularly effective firstly at condensing DNA and facilitating cell entry, and then at engineering endosomal escape, through weakening of the endosomal membrane via osmotic swelling.

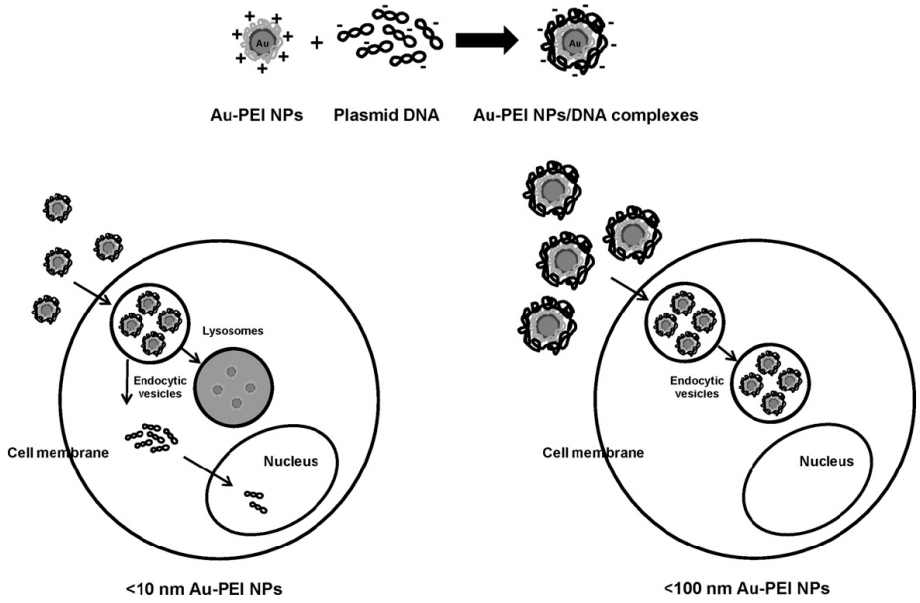

Figure 15: *"Schematic illustration of the structure of Au-PEI NPs/DNA complexes and the relationship between transfection ability of the Au-PEI NPs and cell trafficking" [Cebrián et al., 2011].*

Radiotherapy

Radiotherapy has a primary role in bone metastases treatment *[Scoccianti and Capana 2018]*. It is used external-beam radiotherapy or radiopharmaceuticals to induce damage of DNA and hence apoptosis of tumor cells. Strontium-89 (^{89}Sr), phosphorous-32 (^{32}P) and rhenium-186 (^{186}Re) are bone-seeking radiopharmaceuticals which accumulate favorably in osteoblastic bone metastases because of the increased rate of bone formation. However, when they used alone, they present side effects such as myelotoxicity, which causes low blood-cell counts. The implementation of nanomaterials can decrease the toxicity of radiopharmaceutical-mediated therapy, enhance the radioisotope localization into tumors and decrease the accumulation of radioisotopes in healthy tissues *[Adjei et al., 2018]*. One common treatment for bone metastasis via radiotherapy is the use of ethylenediamine-tetramethylenephosphonic acid (EDTMP) labeled with the radioisotope ^{153}Sm. The development of nanoparticles consisting of polylactic acid (PLA), polyvinyl alcohol (PVA) and EDTMP have been investigated in an attempt to overcome the drawbacks of ^{153}Sm-EDTMP such as myelosuppression. Indeed, biodistribution analyses conducted, after their radiolabeling with ^{99m}Tc, showed an increase in accumulation of these ^{99m}Tc-PLA/PVA/EDTMP nanocomplexes in osseous tissues [Patricio et al., 2014]. Furthermore, nanoparticles in radiotherapy give the advantage of having multimodality treatments to improve tumor response. For instance, ^{64}CuS NPs can combine the ionization radiation from ^{64}Cu and the plasmonic properties of the CuS NPs to enable both radiotherapy and photothermal therapy *[Zhou et al., 2015]*. Nanomedicines can also act as radiosensitizers, increasing the efficacy of external-beam radiotherapy. Nqaw et al., *[Nqwa et al., 2013]* investigated the use of GNPs as radiosensitizers during continuous low-dose-rate irradiation with brachytherapy sources and the results revealed the effectiveness of utilizing GNPs, as radiosensitization was achievable with lower kV energy brachytherapy sources (I-125), enhancing the apoptosis of radiation-resistant cancer cells and improving the survival of tumor-bearing mice.

Hyperthermia

Hyperthermia is a type of cancer therapy in which body tissue is exposed to high temperatures (41-48 ^{o}C). Research has shown that high temperatures can damage and kill cancer cells, usually with minimal injury to normal tissues. By killing cancer cells and damaging proteins and structures within cells, hyperthermia may shrink tumors. The hyperthermia treatment can be conducted with: radiofrequency, microwave, ultrasounds and laser which are less invasive treatments. Nowadays, this method is usually applied in combination with other cancer treatments, such as radiotherapy or chemotherapy that are more effectively when applied after a hyperthermia cycle. It is well known that in the 41–48 ^{o}C temperature range, several processes of relevance at the cellular level are simultaneously activated. Hyperthermia is connected with cell death via three mechanisms: cell apoptosis, cell necrosis and with necroptosis which is a type of programmed necrosis *[Blanco-Andujar et al., 2014; Jaque et al., 2014]*. Magnetic nanoparticles are the most common nanoparticles used in hyperthermia due to their exceptional magnetic properties which make possible their manipulation by applying an external magnetic field. The application of high frequency alternating magnetic field with a frequency of 50 kHz–1.2 MHz causes losses at reversal magnetizing of MNPs resulting in local heating followed by thermal destruction of the tumor *[Kubovcikova et al., 2019]*. An *in vivo* model of osteosarcoma was presented by Shido et al. *[Shido et al., 2009]* in which magnetite cationic liposomes (MCLs) manipulated by a magnetic field were able to reduce both local tumors and lung metastasis via necrosis of tumor cells via effectively heated the targeted tumor tissues at 45°C. Wu et al. *[Wu et al., 2019]* produced a ferrofluid consisting of superparamagnetic IO, SiO_2 and CNPs and tested its efficacy in hyperthermia treatment against osteosarcoma. The double coating on SPIONs enhanced the colloidal stability and cancer targeting efficacy. The prepared NPs decreased the viability of osteosarcoma cells and tumors compared to their primary and non-transformed analogues. In addition, they showed a higher preference for cancer cells because of a higher rate of uptake by these cells and a pronounced adherence to cancer cell membrane reducing the viability of cancerous cells up to 60%. In another study, Mondal et al., *[Mondal et al., 2017]* synthesized HAp-coated

magnetic nanoparticles via a facile 2 step synthetic process (Fig. 16a) which enhances the reproducibility. Their efficacy in bone cancer treatment using HT was tested *in vitro*. The produced nanomaterial presented high biocompatibility and enhanced heating efficiency compared to conventional HAp coated iron oxide. In addition, HAp coating acts as insulator causing less increase of temperature compared to pure IO NPs (Fig. 16b). As it was mentioned above, for efficient HT treatment and for the protection of the surrounding tissue the ideal temperature is between 41 and 48°C. The cytotoxicity assay test indicated that the HT (~45°C) mediated cell death to the cancer cells, via cell degradation and development of blebs, which causes deformities or lysis of the cells (Fig. c). Consequently, the use of IO-HAp NPs as heaters in magnetic HT can be a nontoxic and effective therapeutic strategy for several types of cancer therapy.

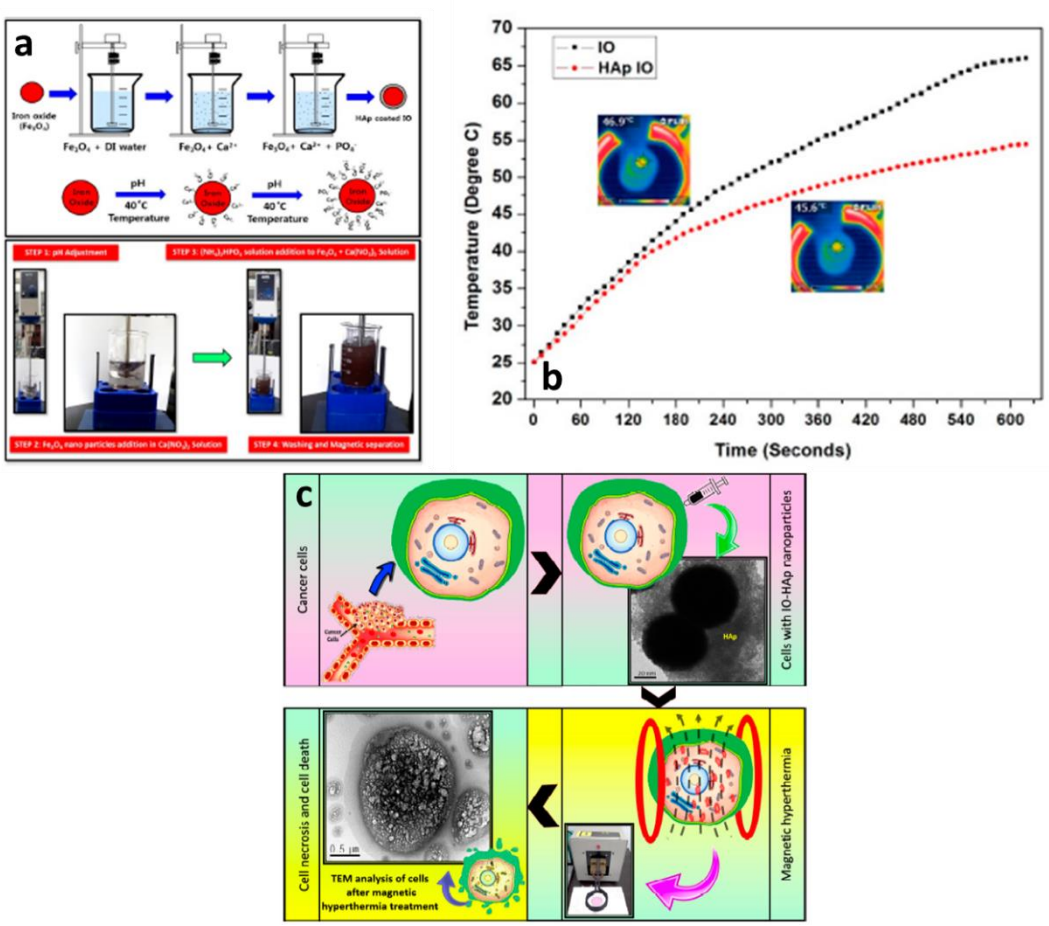

Figure 16: *"Synthesis of hydroxyapatite coated iron oxide (IO-HAp) nanoparticles (a), magnetic hyperthermia study of IO and HAp coated IO (IO-HAp) (b), magnetic hydroxyapatite (IO-HAp) mediated hyperthermia study to treat cancer (c)" [Mondal et al., 2017].*

Photothermal therapy (PTT)

Photothermal therapy is a noninvasive cancer therapy based on the local light application after systemic photosensitizer injection which is able to transfer NIR light into heat to cause cytotoxic on cells *[Zhang et al., 2019]*. A major advantage of this method is that the energy source can be adjusted and shaped in order to provide relatively uniform distribution of heat depending on the tumor volume. Thus, PTT offers better photothermal ablation of tumor, resulting in a more effective and minimally invasive therapy. Nanomaterials, such as Au nanoshells, *[Bardhan et al., 2011]* CuS NPs, *[Tian et al., 2011]* CNTs, *[Antaris et al., 2013]* have been developed as photothermal agents.

Multiwall carbon nanotubes (MWCNTs) have been suggested as promising photothermal agents for PTT of cancer, as, due to their high electrons capacity and high surface area they can absorb more NIR irradiation, thus reducing the amount of NIR radiation and subsequently the risk of skin damage *[Burke et al., 2009; Fisher et al., 2010; Mocan et al., 2014]*. Lin et al., *[Lin et al., 2015]* produced PEGylated MWCNTs and they investigated the therapeutic efficacy of PTT in combination to the PEGylated-MWCNTs in an orthotropic xenograft model of bone metastasis. PEG coating assures high biocompatibility, lower agglomeration tendency, and protecting of triggering the immune system. Their results showed that PEGylated-MWCNTs presented low toxicity whereas their application in PPT process dramatically increase cell apoptosis. PEGylated MWCNTs mediated photothermal effect generates significantly temperature raise both *in vitro* and *in vivo* studies. In addition, PEGylated-MWCNTs in combination to NIR laser irradiation remarkably suppressed the tumor growth compared with treatment with either MWNTs injection or NIR irradiation alone, as well as they significantly reducing the amount of tumor-induced bone destruction.

In another study, Wang and co-workers *[Wang et al., 2015]* developed via a facile and green method trifolium-like Pt nanoparticles (TPNs) as photosensitizers and tested for the first time their efficacy for PTT therapy of bone metastasis *in vivo*. The prepared material showed minimal cytotoxicity *in vitro* and low systemic toxicity *in vivo*. The photothermal conversion efficacy both in vitro and in vivo was high with the optimum quantum of TPA NPs found to be 48.8 ppm. Animals treated with TPNs

mediated PTT showed significantly reduced tumor growth (Fig. 17), revealing the effectiveness of this method in the bone metastasis model. They also tested the inhibition of osteolysis via X-ray imaging before and after the therapy and the results showed negligible changes.

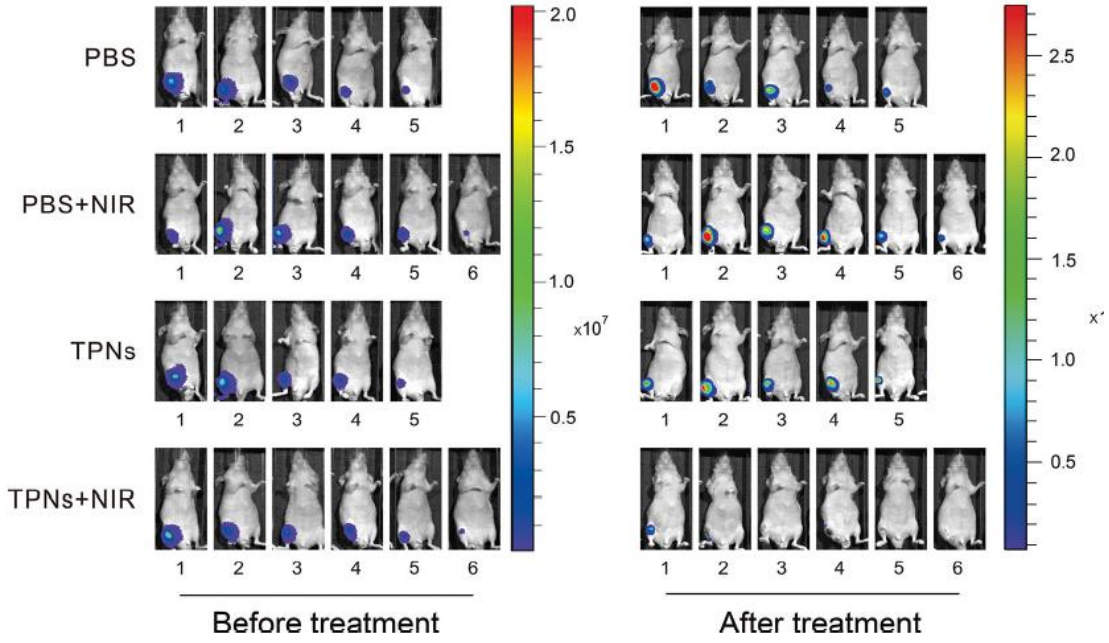

***Figure 17**: "In vivo luminescence images of animals bearing bone metastasis before and after PTT treatment. The NIR irradiation experiments were performed using an 808 nm NIR laser at a power density of 2.5 W cm −2 for 10 min immediately after the injection of PBS or TPNs. The luminescence images were recorded before the first treatment and 2 d after the treatments" [Wang et al., 2015].*

Indocyanine green (ICG) is a well-known PTT agent both in experimental and clinical applications. Though, ICG has some limitations, such as irreversible degradation, short blood half-life as well as rapid photobleaching. Thus, Zhang et al., *[Zhang et al., 2019]* developed a monodispersed core-shell structure consisting of ICG-loaded silica nanoparticles as a core and cell membrane (CM) derived from 143B cells as a shell, capable of tumour-targeted PTT of osteosarcoma. Due to the modification of CM, the new nanoplatform could specifically target the 143B cancer cells both *in vitro* and *in vivo*, which proved superior anticancer efficacy in comparison to either SLN/ICG or pure ICG. The photothermal conversion efficiency of CM/SLN/ICG was similar to free ICG 57.93 and 57.21 ^{o}C, respectively, indicating that the ICG molecule is well protected to exert comparable photothermal conversion capability to free ICG.

In addition, drug release studies showed a pH dependent release of ICG where after 120 h of incubation, the accumulated drug release was 32.96% at pH 7.4 and 74.61% at pH 5.5, which is a characteristic pH value of a cancerous area.

Nanotechnology in therapy and regeneration of cancelous bones

Up to now, treatment of bone tumors includes surgical removal of diseased bone and chemo- or radiotherapy. However, cancer cells are always survived around the bone tissues due to the difficulty to eliminate bone-tumor cells completely from patients during the surgery intervention. These residual cancer cells traditionally killed via chemo/radiotherapy, but the chemo/radiation-resistance and severe side-effects result in endless suffering to patients. In addition, the tumoral bone tissue that is surgically removed leads to large bone defects, which is difficult to be cured by themselves; thus repairing these large bone defects still remains a major challenge Therefore, it is of great importance to design and develop novel biomaterials with antitumor and regenerative properties *[Meijer et al., 2013; Thakor et al., 2013; Luetke et al., 2014]*. In this respect, several researchers have developed nanomaterials which combine the regeneration of the bone defect with an anticancer therapy such as chemotherapy, hyperthermia and photothermia by incorporating the suitable nanomaterial into the implant. Andronescu and co-workers *[Andronescu et al., 2010]* produced a collagen/hydroxyapatite composite material enriched with magnetite, with multifunctional role: as a bone graft material and as a hyperthermia generator for bone cancer therapy (Fig. 18). Magnetites' role is to induce hyperthermia causing cell apoptosis by an applied electromagnetic field, any time after implantation. The optimum content of Fe_3O_4 was found to be 5% where the temperature reached the value of 45^{o}C at around 20-30 min.

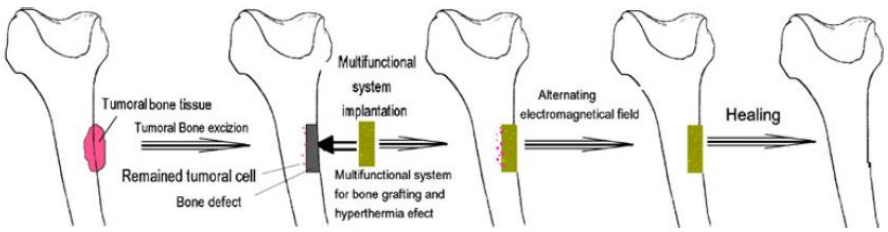

***Figure 18**: Bone cancer treatment of osteosarcoma [Andronescu et al., 2010].*

A bifunctional graphene oxide (GO)-modified β-tricalcium phosphate (GO-TCP) composite scaffold with high photothermal effect and improved bone-regeneration capability was prepared via 3D-printing and surface-modification strategies by Ma et al. (Fig. 19) *[Ma et al., 2016]*. The novel scaffolds showed excellent photothermal effect to eradicate bone-tumor cells *in vitro*, inducing more than 90% of cell death and to inhibit tumors *in vivo*, due to the significant advantages of GO such as strong NIR absorbance, high-photothermal-conversion efficiency and excellent thermal conductivity. They showed that the photothermal temperature can be controlled in the range of 40–90 °C by altering GO concentration, surface-modification times, and power densities of NIR. In addition, the scaffolds exhibited significantly enhanced bone regeneration activity by stimulating osteogenic differentiation of rBMSCs, comparing to pure β-TCP scaffolds.

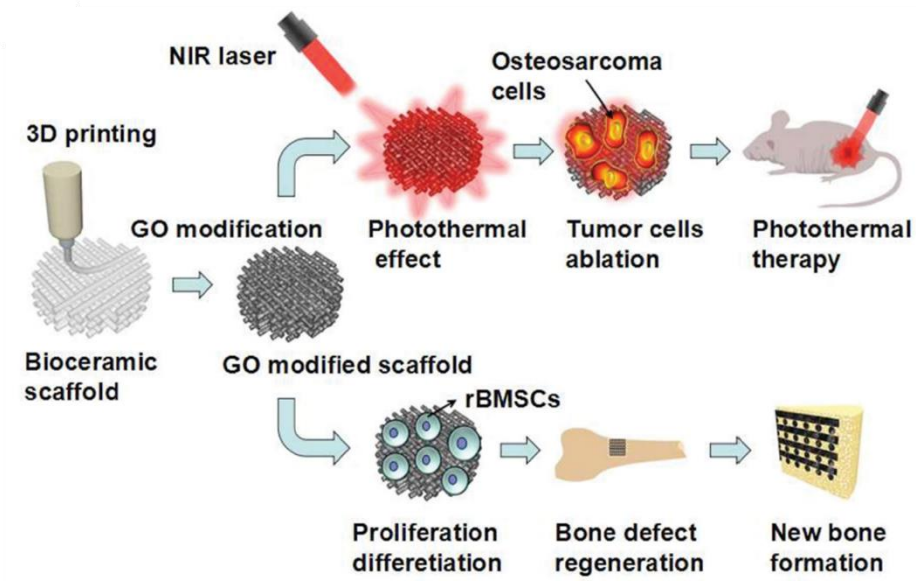

Figure 19: *"Schematic illustration for the formation of bifuctional GO-TCP scaffolds and bioapplication" [Ma et al., 2016].*

Selenium (Se) is a metalloid, which is naturally found in humans and animals and it is well-known for its chemopreventive and chemotherapeutic properties. Animal tests have revealed that selenium intake in excess of the nutritional requirement can inhibit and/or retard carcinogenesis. High concentration of selenium in blood (154µg/mL), have been associated to low cancer rates including pancreatic, gastric, lung, nasopharyngeal, breast, uterine, respiratory, digestive, hematological and gynecological. Perla and Webster *[Perla & Webster 2005]* showed that selenium

implants with nano-surface roughness promotes healthy osteoblast. However, selenium, as a metalloid, does not have the appropriate mechanical strength needed for implant. Hence, Tran et al. *[Tran et al., 2009]* developed a new nanoselenium coated titanium implant which is able to restore a bone defect and simultaneously prevent cancer growth at the implant-tissue interface. They studied 3 different concentrations of Se NPs as coatings and they compared them with conventional untreated titanium implants. The results showed that healthy osteoblast growth increased after 1 day of cell culture on medium and high concentrations of Se NPs revealing the good osteointegration whereas after 3 days of culture, cancerous cells density on the high concentration of Se titanium implant was significantly reduced compared to all other substrates (Fig. 20).

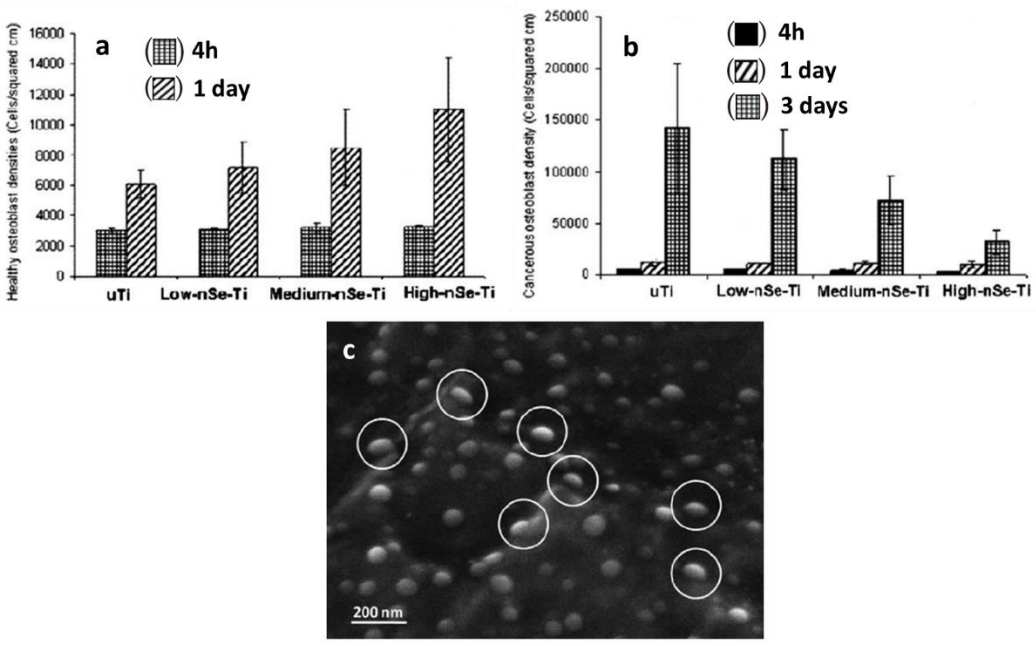

Figure 20: *"Healthy osteoblast densities after 4 h and 1 day (a), Cancerous osteoblast densities after 4 h, 1 and 3 days (b), SEM image, of High-nSe-Ti showing the hemispherical shape of the selenium nanoclusters on the titanium surface (c)" [Tran et al., 2009].*

Other nanoparticles such as Fe_3O_4, *[Zanganeh et al., 2016]* Mn_3O_4, *[Khan et al., 2016]* ZnO, *[Zhu et al., 2016]* and VO_2 *[Li et al., 2019]* have been also examined for their anticancer properties as coatings on biomedical implants with very promising results.

Nanotechnology in imaging and therapy of bone cancer-Theranostics.

Nowadays, theranostic nanomedicine has attracted much interest because it can combine diagnosis and therapy within a single multifunctional nanomaterial. Incorporating detecting, imaging and treatment functions into a single nanomaterial offers the opportunity of combining diagnosis of disease, tissue imaging and real-time drug delivery monitoring. For this purpose, several types of NPs have been proposed i.e magnetic nanoparticles, metal nanoparticles, liposome etc. The theranostic nanomaterials should have the ability to selectively accumulate to the diseased tissue, the capability to deliver selectively and safely the drug and finally to be able to undergo biodegradation into nontoxic by-products *[Fan et al., 2014; Han et al., 2016]*.

Bionanomaterials i.e hydroxyapatite (HAp) nanorods carrying magnetic-radioisotopes, have become promising theranostic agents for cancer due to their significant features such as nonimmunogenicity, biocompatibility, bioactivity and high osteoinductivity. HAp nanoparticles can easily be adhered on osteosarcoma and osteoblast cells, promoting osteoblast growth and osteosarcoma cells uptake; thus, HAp NPs bearing magnetic-radioisotopes can have a triple role, imaging, treatment and bone regeneration. In the same direction gadolinium doped HAp nanorods functionalized with folic acid (HAp-^{159}Gd-^{32}P) nanorods were fabricated and characterized in order to investigate their potential application as theranostic system for osteosarcomas by Cipreste et al., *[Cipreste et al., 2016]*. The presence of folic acid enables active targeting to osteosarcomas. The physicochemical characterization showed that Gd^{3+} ions were trapped in the HAp nanorods crystal net resulting in great stability of the final material. The incorporation of Gd in the composite material change the diamagnetic nature of pure HAp to paramagnetic indicating the potential efficacy of the new structure as contrast agent for MRI diagnosis. In addition, they showed that phosphorous and gadolinium can be activated to induced gamma and beta activity, making possible to obtain a stable theranostic system.

A new type of biocompatible mesoporous silica–coated bismuth sulfide nanoparticles (Bi_2S_3@MSN NPs) was for the first time developed by Lu et al. *[Lu et al., 2018]* for CT imaging and synergistic photothermal therapy and chemotherapy of osteosarcoma both *in vitro* and *in vivo* (Fig 21a.). An efficient encapsulation (99.85%)

and protection of DOX was achieved due to the large surface area and the well distributed mesoporous pores. In addition, the incorporation of Bi_2S_3 resulted in high photo-thermal efficiency (Fig. 21d) thus, offering a great possibility for cancer synergistic treatment and highly near-infrared-triggered DOX release (Fig. 21b). In order to enhance the accumulation in the tumor, for computed tomography (CT) imaging and tumor ablation, the nanoparticles were covalently conjugated to arginine-glycine-aspartic acid (RGD) peptide [c(RGDyC)]. The prepared nanocomplex were compared to a clinically used agent (iobitridol) and the results revealed that the RGD–Bi_2S_3@MSN could be utilized as an ideal CT contrast agent (32.83 HU L g^{-1}) at a lower dose (iobitridol, 25.63 HU L g^{-1}) showing distinct CT contrast imaging at the tumor site (Fig. 21c). Synergistic application of photothermal therapy and chemotherapy significantly ablates the highly malignant OS (Fig. 21e) via mitochondrial apoptosis pathway, thus preventing cancer recurrence. Consequently, the novel nanocomplex could be a promising tool for malignant tumor diagnosis combined with photothermal therapy–chemotherapy.

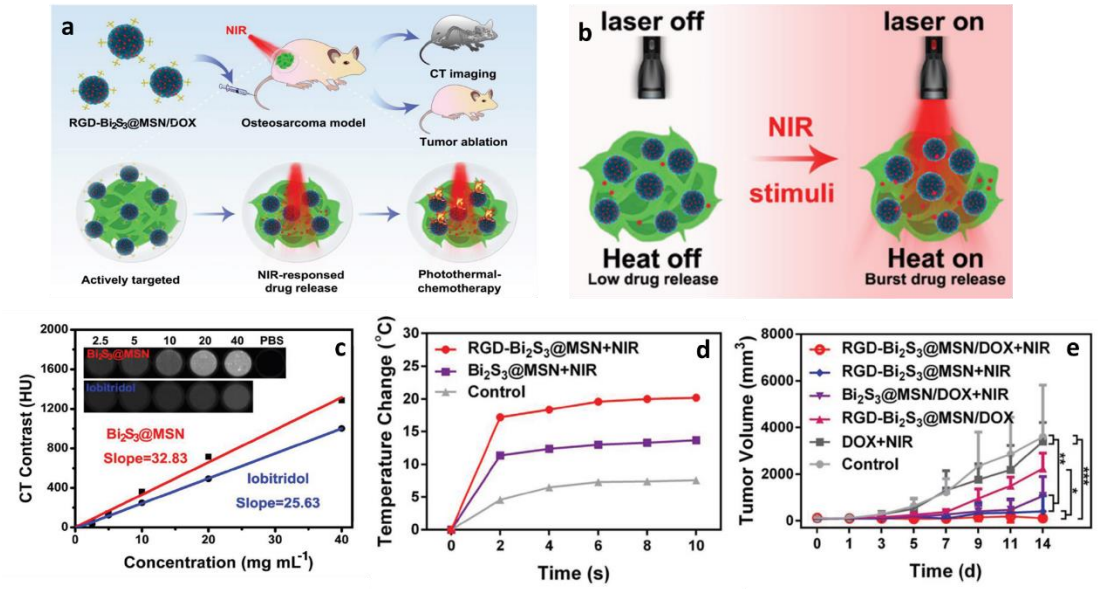

Figure 21: *"The smart RGD–Bi_2S_3@MSN/DOX nanoplatform for OS real-time X-ray CT imaging and NIR-responsive photothermal therapy–chemotherapy (a), Illustration of drug release behavior with or without NIR laser irradiation. Burst drug release occurred after applying NIR laser irradiation (b), in vitro CT value (HU) of Bi_2S_3@MSN and iobitridol. Inset: CT images of the Bi_2S_3@MSN and iobitridol suspensions with different concentrations (c), quantitative temperature change of the tumor site from (d) and tumor volume growth curves of different treatment groups (e)" [Lu et al., 2018].*

Conclusion and future perspectives

The impact of nanotechnology in bone cancer treatment has shown a bright future, as it is proved by several promising results both in *in vitro* and *in vivo* studies. Due to NPs' exceptional characteristics such as tiny size, controllable shape, exceptional optical and thermal properties, versatility for conjugating secondary functional groups, ability to traverse to the diseased site in bone etc, NMs are a very promising tool in therapy of bone cancer. These promising nano-systems can be constructed of organic and inorganic materials, via a plethora of production methods. Thus, several choices of delivery vehicles and administration strategies are emerging in order to treat bone cancer, giving many possibilities for future personalized medication. At the same time, it is also possible to expect a greater therapeutic index because functionalized NMs are capable of targeting and delivering drugs exactly to subcellular areas.

Regardless the nanotechnology's remarkable progress in treating bone cancer, most of the researches are in the early stages of studies. Critical difficulties, such as lack of understanding the NPs' nanotoxicity, inadequate drug-loading capacity, low delivery efficacy, and inflexibility of drug release kinetics, make their clinical application difficult.

Some nanomaterials like LDH, with the same composition of an already FDA-approved alum adjuvant will be ready to use in humans. Polymer-based NPs such as CS and PLGA present low cytotoxicity and are be expected to be applied in the near future to humans as well. Also CaP-based NPs have already been used in drug delivery for bone diseases with no cytotoxic effects. Thus, future research and clinical applications will definitely concentrate on these nanomaterials. Nanotechnology is anticipated to play more significant roles in future for bone cancer treatment and bone regeneration. In the near future, targeted-delivery nano-systems and multifunctional NPs will be emerged at the frontier of cancer treatment with better and controlled drug-release profile. Improvement of the current bone tumor therapies as well as novel and more effective treatments will be arisen with the advancement of the technology in the near future.

Local delivery systems and multifunctional NPs which can delivery agents specific to bone tissues or cells will quickly be seen, with improved controlled release profiles and ability to escape from endosomes, when drug delivery needs to occur in the cytoplasm (such as siRNA). More active therapies, including the improvement of the already existed, for bone diseases will be seen with the advancement of the technology in years to come.

Nanotoxicology-Are nanomaterials safe?

In the last few decades, nanotechnology has provided a wide range of advanced alternatives that allow medical procedures at the molecular scale for disease prevention, diagnosis and therapy as well as tissue repair by utilizing nanomaterials with distinctive optical, thermal, magnetic or redox properties *[Yan et al., 2019]*. Even though the application of nanotechnology in medicine has produced an offspring able of making significant progress in the therapy of several diseases, including bone cancer, the extensive use of nanomaterials in medicine has as a result the increasing of worries about their possible adverse effects on human health *[Mukhopadhyay et al., 2017]*. These adverse effects are not only about the patients receiving the treatment but they are directly related to all those who come into contact with the product, from those who produce it to those who use it. Adverse effects are also caused on the environment via the nanopollution created by toxic wastes from nanomaterial manufacturing.

Although drug delivery nano-systems have been designed with the aim to reduce toxicity of drugs and to increase biocompatibility, there might be some risks involved because of their unique characteristics *[Vlasceanu et al., 2017]*. NPs used in drug delivery, are needed to cross the cell membranes in order to interact with particular components. Therefore, the drug delivery success rate is based on NPs' biocompatibility *[González-Muñoz et al., 2015]*. Research has shown that several physicochemical properties of the utilized NPs play an important role in their toxicity such as solubility, degree of agglomeration, size, shape, charge, surface chemistry, hydrophobicity and hydrophilicity, chirality, degradability, and catalytic ability. All these factors may influence the toxicity of the particles. Thus, characterization of the

various properties of the NPs has been established as a crucial task for the evaluation of the potential effects on health and environmental safety *[Aguilar et al., 2012; Pelaz et al., 2017]*. A new area called "nanotoxicology" have been adopted and defined as the science dealing with the effects of nanostructures and nanodevices in living organisms *[Vlasceanu et al., 2017]*.

Nanoparticles can present toxicity either due to its chemical composition or due to their tiny size. Regarding the chemical composition, the toxic effect could be arise by several chemical interactions i.e at the bio-nano interface between their surface and the local physiological environment, or by the release of toxic components such as Cd^{2+} ions from Cd-based QDs to the surrounding area. In addition, the products generated by the biodegradation of polymeric NPs may exhibit toxicity *[Vlasceanu et al., 2017; Yan et al., 2019]*.

The physical and surface properties of NPs play also a major role in producing toxicity. Due to the high surface-to-volume ratio that NPs have, surface-initiated processes are significantly enhanced. Also, NPs' toxicity profile differs from that of larger particles, due to their extremely small size and high surface area. The small size and high surface energy enable NPs to interact with cellular components and biological systems, possibly resulting in unwanted chemical, biological and toxicological reactivity within normal tissues. Its high surface area results in increased formation of free radicals, such as superoxide anion or hydroxyl radical. The charge of the NPs' surface has a pronounced impact on the produced toxicity *[Pittella et al., 2011; Engin et al., 2017; Vlasceanu, et al., 2017]*.

Absorption, metabolism, distribution, accumulation, and elimination of NPs are important factors for health risk evaluation and have to be studied both in vitro and in vivo. Inert nanoparticles like gold and silver particles, carbon nanotubes and fullerenes may not be able to undergo efficient enzymatic metabolism. It has been reported that NPs with modified groups may be metabolized *[Aguilar et al., 2012; Vlasceanu et al., 2017]*. However, surfactants, such as CTAB, which has been extensively used to prepare and stabilize gold nanorods, exhibit considerable toxicity. Other substances such as PEG, citric acid, and transferrin are considered to reduce gold nanorods toxicity *[Niidome et al., 2006; Wang et al., 2008]*. NPs in the systemic

circulation can interact with plasma proteins, coagulation factors, platelets, and red or white blood cells resulting in the formation of a biocorona. Binding to plasma elements can have a significant impact on nanoparticles' metabolism, distribution, and excretion. Change in biokinetics, may cause undesirably accumulation of the NPs to some specific tissues, thus leading to high local concentrations at these parts of the body. These changes in distribution and metabolism may cause new and unpredictable impacts that need to be taken into account *[Qiu et al., 2010; Zhang et al., 2014]*. The formation of a biocorona may enhance the nanotoxicity, if during adsorption to NPs' surface the endogenous proteins experience misfolding. For example, conformation modifications in fibrinogen induced by nanoparticles have been shown to activate inflammatory signaling pathways *[Deng et al. 2011]*.

Another critical issue and significant problem for nanomedicine is the rapid uptake by cells typically in the reticuloendothelial system (RES), resulting in their raised hepatic and spleen accumulation. Toxicity in these cells and tissues should be thoroughly tested *[Pelaz et al., 2017]*. Oxidative stress can play a significant role in the toxicity of nanoparticles, particularly for NPs based on metals. Overproduction of reactive oxygen species (ROS) by NMs can cause oxidative stress, and consequently mitochondrial damage, lipid peroxidation, and DNA damage. For instance, inflammatory responses to NPs can be attributed to free radical formation *[Vlasceanu et al., 2017; Yan et al., 2019]*.

Table 6: *Physiological effects of NPs on different Cells depending upon their dose, surface area and route of administration in the human body.*

Type of NPs	Cell	Physiological effects
Gold	Renal	Cytotoxicity
	Myocardium	Cytotoxicity
	Hepatocytes	Atrophy/nacrosis
	Lungs fibroblast	Genotoxicity/autophagy
	Bone marrow	Cytotoxicity
Silver	Lungs fibroblast	Cytotoxicity/DNA damage
	Bone marrow	Genotoxicity/cytotoxicity/ carcinogenicity
	Hepatocytes	Cytotoxicity/induction of oxidative stress
	Osteoblast	Cytotoxicity/generation of ROS

Type of NPs	Cell	Physiological effects
Superparamagnetic	Renal	Genotoxicity/cytotoxicity
	Fibrolast	Cytotoxicity / loss of adhesion skill
Quantum dots	Lymphocyte	Induction of DNA damage/formation of micronuclei/generation of DNA adduct
Polymeric	Lungs fibroblast/epithelial	Cytotoxicity/Inflammatory
Carbon nanotubes	Renal	Cytotoxicity
	Neurons	Neurotoxic effect
	Hepatocytes	Inflammatory/genotoxic effect
	Lungs epithelial	Cytotoxicity
Silica	Hepatocytes	Hepatotoxicity
	Neurons	Dopaminergic neurons damage pathway
	Lungs epithelial	Inflammatory response/toxicity
	Lymphocyte	Cytotoxicity/genotoxicity
	Fibroblast	Cytotoxicity
Titanium dioxide	Renal	Induction of oxidative stress/cytotoxicity
	Neurons	Neurotoxicity
	Hepatocytes	Genotoxicity/carcinogenicity/ inflammation
	Lungs epithelial	Genotoxicity/mutagenicity/ carcinogenicity
	Lymphocyte	Induction of oxidative stress/genotoxicity

In vitro studies of using CdSe QDs as bioimaging agents, showed apparent hepatocyte toxicity due to the degradation of their structure and the subsequent leakage of cadmium ions *[Derfus et al., 2004]*. Furthermore, several reports have shown that CNTs could induce important cellular responses like inflammation and gene damage in several cell types, although the results on this issue have been conflicting *[Liu et al., 2013; Wang et al., 2016; Luo et al., 2016]*. Jia et al., *[Jia et al., 2019]* prepared graphene (G) and graphene oxide (GO) NPs and tested their toxicity both *in vitro* and *in vivo* regarding the impact of the NPs' size (S-small, M-medium, and L-large). *In vitro* studies indicated that the small and large G and GO NPs considerably decreased cell viability and induced DNA damage, accompanied with generation of (ROS) and caused different expressions of related critical genetic markers. G showed

higher effects than GO which were size dependent (L>M>S) whereas the medium size of GO encouraged mild genetic toxicity on RecA bacteria. *In vivo* tests revealed that both G and GO in concentration of 100 mg/L caused toxicity, induced ROS generation, and activated related pathways in zebrafish. The research team concluded that the two carbon compounds were induced toxicity in a different way, based on their physical characteristics, particularly size and oxidation state, as well as by exposure concentrations.

Iron oxides (IOs) have drawn considerable attention not only due to their superparamagnetic characteristics, which make them appropriate for interesting biomedical applications, but also because they are linked to low toxicity in the human body. Therefore, IOs are categorized as biocompatible materials. For instance, an in vitro research comparing several metal oxides showed that IOs were non-cytotoxic at concentrations below 100mg/ml. Though, the lack of cytotoxicity does not ensure that IOs are completely safe to be used in medical applications. Several studies report various damaging cellular impacts, including DNA damage, oxidative stress, mitochondrial membrane dysfunction and alterations in gene expression due to IOs exposure in the absence of cytotoxicity. The main mechanism causes these adverse side effects is the overproduction of ROS. Magnetite/maghemite nanostructures have already been approved for clinical use as MRI contrast agents. However, there are some contradictions in the literature about the cytotoxicological evaluation in different cells and the explanation of these results. Based on several studies, the main factors affecting the results are dose, exposure time and the type of the cell *[Valdiglesias et al., 2015; Gokduman et al., 2018]*. For instance, IO (II,III) NPs induced moderate time- and concentration-dependent cytotoxicity in Vero cells after 24 h whereas they were non-mutagenic and did not cause histopathological modifications in rats following a single intratracheal instillation *[Szalay et al., 2012]*.

Recent *in vivo* studies have shown that silver NPs (Ag NPs) cause toxicity on Caenorhabditis elegans. In mice models orally treated with several sizes of Ag NPs (1 mg/kg of body weight for 14 days), small particles in brain, lung, liver, kidneys, and testis were found. In addition, in the same groups, the levels of transforming growth factor beta (TGF-β) in serum were significantly increased. In a mice model treated for 28 days with several doses of AgNPs, high levels of IL-1, IL-6, IL-4, IL-10, IL-12, and TGF-

β in a dose-dependent manner were detected, indicating that frequent administration may cause organ toxicity and inflammation *[Park et al., 2010]*. Several studies have shown that AgNPs can induce cytotoxicity in phagocytic cells, macrophages, and monocytic cells via generation of ROS and induction of apoptosis *[Clichici & Filip 2015]*. Furthermore, AgNPs can be captured by central nervous system through microglia and astrocytes cells, being a threat to neuronal cells. *In vivo* studies have revealed that AgNPs can accumulate on the developing brain, resulting in developmental dysmorphologies. Neurotoxicity of AgNPs is also attributed to generation of ROS induced by NPs *[Souza et al., 2018]*. A study of Hyun et al. *[Hyun et al., 2008]* showed that chronic and repeated exposure to AgNPs improved the development of mucins in nasal respiratory mucosa revealing the importance of mucus in defense against air pollutants. The multiple outcomes obtained can be attributed to the variation of the synthesis techniques, sizes, concentrations and time exposures and the presence or lack of capping agents. Thus, NPs' risks have to be assessed on a case-by-case basis and require more extensive investigations *[Clichici & Filip 2015]*. Nevertheless, studies evaluating the implications and applications of AgNPs in bio-logical systems are still recent and how this NPs influence people health remains unanswered *[Souza et al., 2018]*.

Although gold nanoparticles (GNPs) are very useful in the diagnosis and therapeutics a much discussed issue is the correlation between the size and the shape of nanoparticles and their toxicity. Small GNPs are widely used in medical applications because they can penetrate into cells and transport various drugs without causing cell injury. Exposure to GNPs, results in an induction in pro-inflammatory cytokines (e.g. TNF-α, IL-β and IL-2) leading to brain toxicity of rats. GNPs at smaller sizes (3 nm), showed lower toxicity compared to the larger ones (5 nm). The toxicity of gold nanomaterials has to do with their shape too *[Clichici S., Filip 2015; Jamil et al., 2018]*. Researches have shown that gold nanorods are more toxic than nanospheres, both on human keratinocyte (HaCaT) cells and on human breast adenocarcinoma cell line (MCF-7) *[Chithrani et al., 2006; Wang et al., 2008]*. Several researches have shown that GNPs cause nephrotoxicity, hemolysis, cytotoxicity and genotoxicity. Dobrovolskaia et al. *[Dobrovolskaia et al. 2008]* reported potential hemolysis and immunotoxicity of GNPs. Similarly, Chen et al. *[Chen et al., 2009]* reported the

accumulation and consequent toxicity (inflammation and cellular damage) of GNPs in the liver and spleen. Nevertheless, the results from the literature about GNPs' toxicity are quite conflicting and inadequately documented. Thus, it is essential to investigate toxicity mechanisms because GNPs have been widely used in several medical applications, including drug and protein delivery, gene therapy, in vivo delivery and targeting, etc *[Clichici S., Filip 2015; Souza et al., 2018]*.

Polymers have different chain length and molecular weight, different chemical structures, and consequently different *in vivo* behavior (biodistribution, pharmacokinetics, stability, and toxicity). Despite the fact that polymers have many advantages such as low viscosity, narrow polydispersity, and high density, their toxicity has not been systematically examined. Positively charged dendrimers and cationic macromolecules can interact with blood constituents leading to hemolysis, which may cause nephrotoxicity and hepatotoxicity. Dendrimer toxicity depends on dose and generation and is connected to surface charge, since cationic dendrimers are more toxic than anionic compounds *[Clichici & Filip 2015]*.

The majority of nanomedicines that are already used for therapy and diagnosis are lipid-based. From almost 40 years ago, the intravenous infusion of certain micellar formulations of paclitaxel Taxol®, docetaxel (Taxotere), cyclosporine (Neoral, Sandimmune), etoposide (Etopophos) and different iodinated contrast media, are known to induce idiopathic hypersensitivity (HSR). HSR responses occur at first exposure without previous sensitization. They decrease, resolve, or disappear on later treatment. Some people have a delayed onset of symptoms. Common symptoms of HSR are facial swelling, fever, rash, cough, chill, and shortness of breath, hypertension, tachypnea, hypotension, back pain and chest pain. Fatal reactions during the infusion of Taxol ® have been reported and attributed to cardiac arrest. HSRs' highest rate (>10%) arise with certain monoclonal antibodies (mAbs; rituximab, infliximab), liposomal drugs [Doxil (Caelyx), AmB (AmBisome)] and anticancer agents (taxanes: (paclitaxel, docetaxel) which are delivered in micellar solvents, such as Cremophor EL (CrEL). Liposome reactions were reported in 1986, the first human study when high doses of liposomes were infused in patients suffering from cancer. Since then, all kinds of liposomal drugs (Doxil® (or Caeylx®, Lipodox® and Ambisome®-liposomal-based

formulations of doxorubicin and amphotericin B, respectively), were presented to cause HSRs *[Morilla & Romero 2018]*.

In order to increase preclinical and clinical use of novel nanomaterials in medicine, it is essential that these nanomaterials are sufficiently secure while at the same time preserving their parental nano-medical functions throughout their entire life cycles *[Yan et al., 2019]*. Dose, dose rate, dose metric, and biokinetics are very crucial factors for the safety assessment of newly engineered nanoparticles *[Vlasceanu et al., 2017].*

Several approaches have been developed for the development of safe-by-design nanomedicines such as coating, doping, loading and grafting. Coating toxic nanostructures with biocompatible materials, is one of the most extensively used method to reduce their potential adverse effects (Fig. 22a). Loading is the physical attachment of organic molecules, polymers, or biomolecules to the surface of nanomaterials via van der Waals forces, hydrophobic and electrostatic interaction, π–π stacking, or hydrogen bonding (Fig. 22b). The loaded molecules are capable of further loading of drugs and various targeting ligands. Grafting is a method by which functional moieties such as -NH_2 and -COOH are using for the covalently attachment of probe molecules and targeting ligands to nanomaterials (Fig. 22c). Doping of NMs refers to the introduction of a low percentage of different atoms into a pure material to alter their electrical, optical, or magnetic properties (Fig. 22d) *[Yan et al., 2019]*.

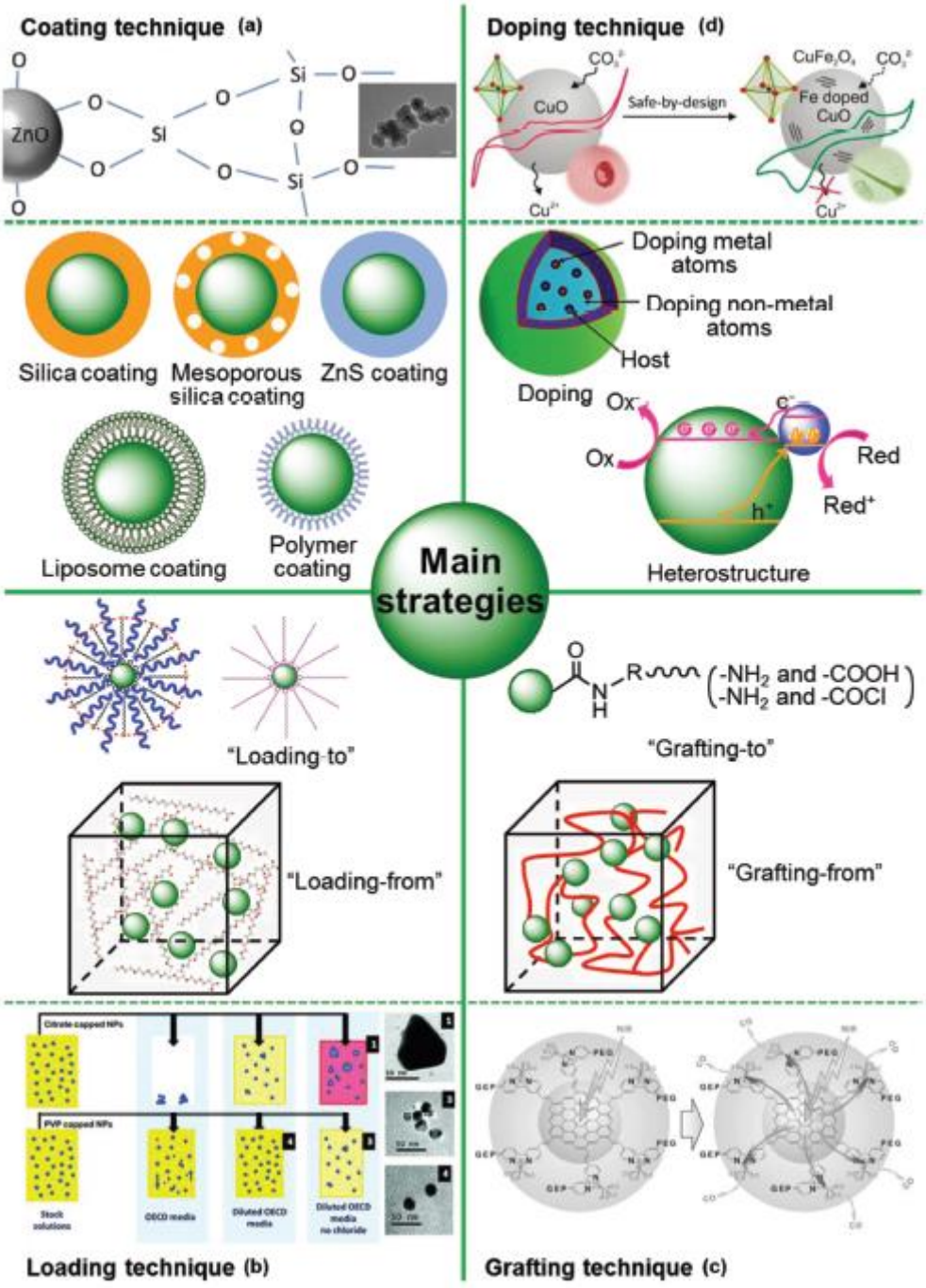

Figure 22: *"Common safe-by-design strategies available for the fabrication of safe nanomedicines" [Yan et al., 2019]*

Principles for safer design of nanomedicines, include optimization of the size and structure of NPs, regulation of ROS generation, prevention of the leakage of toxic components, passivation of defect sites, reducing the interaction between NPs and biomolecules, controlling the biopersistence of NPs, as well as introduction of stimuli responsiveness (Fig 23a-f).

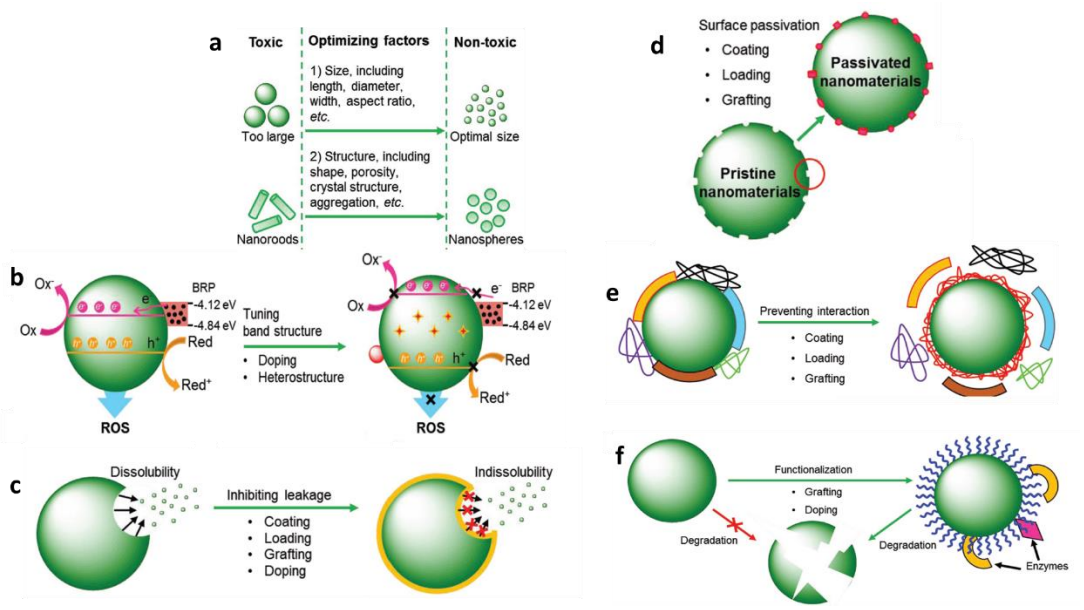

Figure 23: *"Optimization of the size and structure of nanomaterials in nanomedicines (a), Nanomaterial-related perturbation of cellular redox equilibrium and its regulation (b), Approaches to inhibit the leakage of toxic components from medical NPs (c), Surface defects of nanomaterials and their passivation (d), Approaches to reduce nanomaterial interactions with biomolecules in vivo (e), Strategies to control the biopersistence of nanomaterials (f)" [Yan et al., 2019].*

Conclusions and future perspectives

Although important progress has been made in the growth of safe-by-design nanomedicines, this area is still in its infancy and many difficulties remain to be addressed. While growing evidence has shown that nanomaterials' toxicity is directly associated to their physicochemical features, the main factors affecting the toxicity and the connection between nanomaterials' properties and biological impacts are still far from fully identified. This problem is further complexed by the reality that tuning one property through nanomaterial engineering often results in many other significant characteristics being changed. This concern is further complicated due to the easy change in the properties of a material resulting from the change of a single property during the synthesis procedure. For instance, silica coating avoids the release of toxic ions from nanomaterials, but can also change entirely their biological fate. Cancer treatment is a major challenge for the scientific community who is trying to develop new materials, new particles and new drugs and combine them to achieve a high-performance system that can deliver the drug in a timely manner and specifically

response to the damaged tissue. However, there is still a need for comprehensive toxicology research to effectively use antitumor nanoparticles as the up to now available data are still insufficient.

General conclusions

In the last decades, the interest in the use of nanomaterials in orthopaedic oncology has increased due to their potential to improve the way that cancer is diagnosed and treated. Significant progress has been made in production methods so that to develop NMs with controlled size, shape, surface charge and physicochemical properties. In addition functionalization of NMs' surface can be also conducted in order to enhance biocompatibility and attain effective targeting. NMs present a plethora of advantages such as high surface area:volume ratio, exceptional optical, electrical and thermal properties, high drug loading capacity, reduced toxicity, ideally favorable for endocytic intracellular uptake, specific targeting to the tumor area etc. However, lack of reproducibility in batch to batch production is a major issue, as the properties of the NMs can be easily changed due to their nano size.

Several nanostructures have been produced which are capable of detecting, imaging and treating bone tumors by different therapeutic techniques. Since NMs can carry and deliver several diagnostic agents and drugs to the target cells, it is possible the rate of patient survival to be increased as well as the quality of life to be improved. Magnetic nanoparticles, quantum dots, and fluorescent nanoparticles are very useful for tumor imaging. Lipid NPs, liposomes, micelles, dendrimers, nanocrystals, ceramics, metallic NPs and carbon NMs could be used for passive or targeted drug delivery to the tumor cells *[Moradi et al., 2019]*. Selinium nanoparticles can cause apoptosis of cancerous cells *[Tran et al., 2009]*, whereas various nano-structure scaffolds could be helpful for bone regeneration with simultaneous treating properties for the bone defects left by their surgical removal *[Andronescu et al., 2010; Ma et al., 2016]*. Ongoing research is conducted in the field of orthopaedic oncology in order to develop multifunctional theranostic NMs that can combine imaging, treating and regenerative properties. Considerable attention has been given in the development of stimuli-responsive nanostructures which are considered to enhance the localization and efficacy of therapeutic cargos, whereas new strategies for controlled drug release are frequently being proposed. Undoubtedly, future research will reveal new types of NMs.

Despite important improvements in NMs-based cancer diagnostics and therapy tools, our general knowledge on NPs' pharmacokinetics (adsorption, uptake, distribution, metabolism, and excretion) is currently quite limited *[Yan et al., 2019]*. In terms of structure, composition and state of aggregation, nanomedicines are much more complicated compared to conventional medicines, making almost all standard methods insufficient for quantitative nanomedical analyses. Advanced analytical methodologies are therefore needed to build a reliably extensive spectrum of nanomedicines' in vivo. In addition, thorough studies on the safety profile of these NMs need to be carried out before they applied in clinic studies. Understanding the biophysicochemical interactions occurred when NMs are exposed to physiological environments is of fundamental importance. It should be pointed out that drug delivery and nanotoxicity are strongly correlated. To induce toxicity to cancer cells in a selective manner resulting in elimination of the targeted tumors, but unfavourable toxicity of NMs could also presented causing side-effects and dysfunctions. Thus, novel reliable techniques for characterizing the real risks of NMs need to be established and implemented.

Overall, the application of nanotechnology in orthopaedic oncology in terms of diagnosis and therapy seems to be very promising for the years to come, however, there is still much room for improvement.

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