In situ forming implants for local chemotherapy and hyperthermia of bone tumors

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In situ forming implants for locally combined chemotherapy and hyperthermia of bone tumors

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Abstract

Hyperthermia, an established adjuvant in cancer treatment, potentiates the effects of anticancer drugs and synergetic combination can be obtained improving the therapeutic outcome. Bone metastases may benefit from this combination when treated through *in situ* forming implants. Once loaded with magnetizable (nano-) particles and antineoplastic agents, these implants allow for the local application of magnetically-induced hyperthermia and the release of an anticancer drug. The implant can be heated applying an external magnetic field, sensitizing the surrounding tumoral tissues, while releasing the chemotherapeutic agent. Moreover, bone implants provide mechanical support to the weakened bone and consequently relieve pain.

This review gives an overview of the current knowledge as well as the rationale of combining locally both magnetic hyperthermia and chemotherapy using *in situ* forming implants in the field of bone metastases treatment.

Keywords

Induced hyperthermia, chemotherapy, drug delivery systems, *in situ* forming implants, iron oxide nanoparticles, bone metastases, cementoplasty, vertebroplasty

1. Introduction

Cancer remains a leading cause of mortality and consequently an important public health issue. For the year 2008, it is estimated that over 7 million deaths due to various cancers occurred worldwide, representing around 13% of all casualties. Increase of these numbers is driven by an aging society, increase in the world population and augmented standard of living in developing countries [1, 2]. Usual curative treatments of solid tumors consist of systemic chemotherapy or radiotherapy combined with surgery, depending on the type, stage and localization of the tumor. Other treatment strategies, such as immunotherapy, hormonotherapy or photodynamic therapy are also used today to treat cancer through a systemic or local approach [3-6]. In spite of the progress that has been made in the field of cancer therapy, these current approaches are not yet satisfactory for many reasons. Although new minimally invasive surgery techniques are available, e.g., laparoscopic tumor resection, not all solid tumors are eligible for surgical resection. Oncological surgery is mainly limited by the size of the tumors, its localization and by the presence of metastases. Systemic administration of an anticancer agent at high resulting plasma concentrations is mainly responsible for the observed side effects, while drug concentration in the tumor itself is usually low. This dose-limiting toxicity is an important obstacle to chemotherapy, as most anticancer agents would be more effective at sufficiently high doses. Thus, systemic chemotherapy of solid tumors still suffers from limited effectiveness. To improve drug therapeutic effects, cancer cell killing should be increased, while preserving healthy cells. In this view, the use of a local drug delivery system appears attractive as it will directly target the tumor cells and decrease the circulating drug concentration, which would result in fewer side effects. An enhanced delivery of effective therapeutic agents to their target site would improve clinical outcome. Intratumoral therapies were investigated with different systems ranging from simple intratumoral injections to a polymeric matrix forming an implant. The latter can be divided

into two distinct groups: i) preformed implants, which need a surgical intervention in order to be placed close to the tumor and ii) *in situ* forming implants. *In situ* forming implants (ISFIs), once loaded with an antiblastic drug, offer an interesting alternative to overcome dose-limiting toxicity. Injected as a liquid mixture, these polymeric formulations solidify within the injection site generating locally a (semi-)solid implant. The physicochemical processes that underlie the phase transition occur in response to an environmental stimulus such as temperature, pH and water or through an *in situ* polymerization or cross-linking reaction [7-10]. The polymeric matrix formed may be biodegradable or not depending on the therapeutic goal [11]. Consequently, the release of drugs will be mainly dictated either by diffusion process through the polymeric matrix, or by a potential drug-polymer interaction, or by the kinetics of formation/degradation of the implant itself, or most probably by a combination of all of these mechanisms. ISFIs are used for a localized treatment using different therapeutic drugs such as antibiotics, proteins, genes and antiblastic substances or in order to achieve tissue engineering [12-15]. These minimally invasive delivery systems are of great interest as they will create drug depots locally and may offer a sustained release of therapeutic agents over a long period of time.

1.1. Hyperthermia: techniques and limitations

Hyperthermia, i.e. heating of a part of or the whole body of the patient is being increasingly used worldwide as an adjuvant in cancer treatment [16]. By exposing the body tissue to high temperatures – using external or internal heating devices – it is possible to kill tumor cells or at least to sensitize them to radiotherapy or chemotherapy. While cancerous tissues show significant sensitivity, normal tissues are usually less injured by heat [17]. Depending on the range of temperature achieved within the tumor and the exposure time, hyperthermia might be used alone or in combination with established therapy modalities (i.e. surgery and/or

radiotherapy and/or chemotherapy). At high temperatures, above 47°C (and for few minutes of exposure), tumor coagulation necrosis occurs. This thermoablation is used as a therapy modality and had resulted in interesting *in vivo* outcomes [18-22]. However, risk of shock syndrome following thermoablation treatment due to a sudden release of necrotic tumor material is a serious limiting side effect [23]. At mild and moderate temperatures, ranging from 40-42°C and 42-47°C, respectively, hyperthermia is mainly used as an adjuvant therapy, has shown relevant outcomes in several preclinical and clinical trials and is now recognized as an established clinical modality [24-30]. Technical aspects of hyperthermia involve the use of microwaves (MW), radiofrequencies (RF) or ultrasound (US) as sources of energy [31]. The interactions between biological tissues and these sources of energy result in the transfer of energy as the deposition of heat. The rate of electromagnetic energy converted to heat energy is represented by the "specific absorption rate". The main issues are to achieve a targeted, deep-seated and homogenous heat deposition at the tumor site while preserving healthy tissue. Microwaves are not suitable for deep-seated neoplastic tumors because biological tissues are opaque to this radiation. The attenuation of the MW by the body hinders strongly their use for deep-seated tumors and thus MW-induced hyperthermia will preferably be used for superficial tumors. Due to the variability of impedance between different biological tissues, ultrasound waves are strongly reflected at each tissue surface and, as for MW, US reached with difficulty deep-seated tumors. Clinically, hyperthermia therapy methods may be divided into different techniques (Table I) depending on the area to treat [26, 31-34]. Among these techniques, magnetic fluid hyperthermia (MFH), which consists of heating magnetizable (nano-) particles through application of an external alternating magnetic field, is of great interest as it will selectively deposit heat at the tumor site. This technique combines a maximally targeted deposition of heat to the tumor at a maximal protection of surrounding healthy tissue. Moreover, MFH overcomes almost all shortcomings of the other techniques, as

its source of external energy (i.e. alternating magnetic field) crosses biological barriers without loss allowing its application from superficial to deep-located tumors. MFH has proven benefits *in vivo* using animal models [35-41], as well as in clinical trials, the first of which have been described in publications by Jordan and coworkers [27, 28, 42-44]. Magnetic particles used in MFH are mostly the magnetic iron oxides Fe_3O_4 (magnetite) and γ-Fe_2O_3 (maghemite), as they are recognized for their biocompatibility and safety profile [45]. Depending on the particle size and nature, iron oxides may be divided into two main groups: i) ferro- or ferrimagnetic particles (multi- or single-domains) and ii) superparamagnetic particles, which advantageously lack remanent magnetic field, precluding their potentially deleterious aggregation. Superparamagnetic iron oxide nanoparticles, so-called SPIONs, offer a much more efficient heat release mechanism than the iron oxide particles. This, in turn, allows the use of lower and clinically safer magnetic field amplitudes [46]. MFH efficiency is depending on the frequency and squared amplitude of the external magnetic field, the size of the iron oxide particles used and their concentration at the tumor site. The physical principles inducing heat involve both Brownian and Neel relaxation. Magnetic fluid hyperthermia is currently administered using three different approaches, i.e. through tumor arterial supply, the so-called arterial embolization hyperthermia, direct injection hyperthermia or intracellular hyperthermia [23], the latter being a particular form of MFH in which the magnetic particles are coated to target the cancer cells. Following the injection, the possibility of particles to migrate within the body represents a shortcoming of these approaches, as it may result in the risk of particle accumulation in sensitive organs (e.g., brain, lung, and heart) and compromise treatment repetition, which is of great interest to avoid tumor recurrence. Indeed it was reported that up to 17.5% of the iron oxide injected into the prostate was not detected within the targeted organ, and almost 40% of the disseminated iron oxide was found in distant organs including liver, lung and spleen [38]. The distant migration may be circumvented by trapping

the magnetic particles into an ISFI, thus decreasing the potential deleterious effect of spreading particles and maintaining them within the tumor site for successive heating. Such an approach was successfully investigated using organogel preparations (i.e. water-precipitating polymer dissolved in a water-miscible organic solvent) for treating nude mice carrying a subcutaneous human colon carcinoma [40, 41, 47].

1.2. Bone tumors

For many tumors, a high-risk incidence of bone metastases exists [48-50]. In particular, patients with breast, prostate, lung, kidney and thyroid cancer may develop bone metastases, and these account for more than 80% of cases of metastatic bone disease [51]. Bone metastases are mainly treated through radiotherapy, systemic chemotherapy, bisphosphonates and radioisotopes [52]. By attracting cancerous cells through a complex crosstalk, the skeleton represents a fatal “pre-metastatic niche” and is the third most common metastatic site after the lung and liver. In particular, the axial skeleton is the most common bone site affected by metastases [53-56]. The main complications are bone pain, pathologic bone fracture, impaired mobility, spinal cord and/or nerve root compression, bone marrow infiltration and hypercalcaemia. The patient’s quality of life is strongly degraded and healthcare costs increase as the disease progresses [48, 57-59]. Despite available treatments, once cells are engrafted in the skeleton, curative therapies are no longer possible and palliative treatment becomes the only option [60]. Current radiotherapy and radioisotopes (e.g., strontium89, samarium153) represent the first advance guard in palliative therapy mainly for pain relief. Systemic anticancer treatment is usually added depending on cancer pathology. Bisphosphonates, and here mostly the new generation of such compounds, are the treatment of choice to mitigate hypercalcaemia and its complications. Finally, orthopedic surgery treats spine fractures mainly through cementoplasty. This percutaneous image-guided interventional

radiology procedure involves the intra-osseous injection of an ISFI, which will lead to a solid implant allowing stabilization of a vertebral body and thus, result in pain relief [61]. Cementoplasty may be divided in two approaches: the vertebroplasty and the kyphoplasty. Vertebroplasty consists of the direct injection of bone cements while kyphoplasty implies the placement of an inflatable balloon, which will restore some of the vertebral height and deformity prior to cement injection. Cementoplasty has shown great results regarding pain relief, vertebrae stabilization, and patient mobility avoiding much of the co-morbidity of prolonged conservative treatment and bed rest [62].

The observation that, in some cancer patients undergoing vertebroplasty procedures, tumor shrunk after cement-induced exothermic heating [63] led to the idea of combining cementoplasty with controlled hyperthermia. In addition, local combination of drug release with heat exposure might be of interest to potentiate the antitumor effect. Bone metastases are particularly suitable to be treated through *in situ* forming implants delivering locally both heat and drugs.

The scope of this review is thus to give an overview of the current knowledge as well as the rationale of combining locally both magnetic fluid hyperthermia and chemotherapy using *in situ* forming implants in the field of bone metastases treatment.

2. Hyperthermia and its use in oncology

2.1. Biological rationale for hyperthermia in oncology

Hyperthermia induces plethoric effects including molecular, metabolic, cellular, tissular, pathophysiologic and immune aspects. These effects are related to the combination of the temperature achieved and time of exposure, which is characterized as the thermal dose (D). Experimental evidence had shown a threshold of 42-43°C reflecting a two-step process of cell killing with a linear growth arrest below this threshold followed by exponential cell death [64-

66]. The mechanism of hyperthermic cell killing depends on the thermal dose applied, but may be divided into two main pathways differing in their initiation mode, their morphological, biochemical as well as their genetic features. In fact, cell death occurs either through apoptosis or necrosis, defined as a cellular self-controlled destruction mechanism or a severe cellular injury accompanied by a loss of vital function, respectively. Thus, at moderate hyperthermia, i.e. above or equal to 42°C, apoptosis is the main response of cells to heat while it was observed that above 47°C, cell killing is induced through a necrotic pathway, as observed in thermoablation treatment [67]. Apoptosis is a highly complex and interconnected process including several modulators such as genes, proteins, receptors, extracellular factors, organelles and various ion species [68-73]. Apoptotic cell death above this temperature threshold of 42°C was correlated to gene expression. Actually, gene networks mainly associated with heat-induced apoptosis were identified as up-regulated in several *in vitro* studies [74-79]. Several papers studied the effects of heat on cellular membranes (i.e. bleb formation/apparition) and morphological aspects of different cell lines [80-83]. It was proposed that the process of blebbing may be caused by a significant decrease of both integrin and actin cell content [84]. The integrin surface expression was also decreased and lead to an altered adhesion mechanism, suggesting a hypothetic hyperthermic role in down-regulated metastatic spreading [85]. Heat shock was shown to induce multiple effects: i) to provoke cellular membrane disruption, improving the uptake of molecules and thus enhancing therapeutic effects [80, 86, 87]; ii) to increase membrane fluidity, which may contribute to cell death either through deregulation of both passive diffusion and active transport processes or through an enhanced activity of the tumor necrosis factors receptors (TNFR) [88-90], and iii) to modify transmembrane potential, which was correlated to a significant decrease in cell survival [91]. The morphological changes observed after heat-shock treatment are mostly due to the interaction between heat and cytoskeleton. Modifications in the cytoskeletal

organization were observed in several studies but few of them established a clear correlation between morphological aspects of cell and the rate of cell death *in vitro* [82, 92, 93]. It is interesting to note that different effects – for the same heating conditions and a given cell line – were observed depending on the cell cycle. The results suggested that the S-phase is the most thermosensitive cell cycle phase [92, 94]. Intracellular metabolism – including glycolysis, citrate cycle, lipid metabolism and oxidative phosphorylation – is highly influenced by heat treatment through modifications of extra- or intracellular parameters such as oxygen up-take, pH and/or nutrient supply [95-98]. In particular, metabolism disturbances after a heat-shock result in an increased production of reactive oxygen species (ROS), which is responsible for macromolecules damage and, at least for a part, of heat-induced anti-tumoral response [99-103]. Heat shock treatment also affects nuclei and cytoplasmic organelles such as lysosomes and mitochondria [81, 104]. Proteins of the nuclear matrix are among the most important intracellular macromolecules as they are involved in many vital functions (e.g., DNA packing, replication, transcription and repair, cell death). Surprisingly, they are also highly thermolabile proteins and, under heat treatment, they undergo denaturation leading to their intranuclear aggregation, which is closely associated to the nuclear matrix [105]. This aberrant protein binding to the nuclear matrix is time and temperature-dependent as well as dependent on the delay necessary to repair and recover a normal status. In addition, it was correlated to the surviving fraction of cells [106]. Macromolecules *de novo* synthesis, including RNA, DNA and proteins, is limited during or after a heat stress and the rate of synthesis recovery is depending on the thermal dose as well as the type of macromolecules - for instance protein synthesis recovery is more efficient compared to DNA. Noteworthy, the delay to recover protein synthesis corresponds to the onset of thermotolerance [107-109].

2.2. Thermotolerance and heat shock proteins

Thermotolerance is defined as "a rapid, short-acting molecular process associated with the synthesis of several families of heat shock proteins (HSPs) of different molecular weights elicited as a result of acute short sub-lethal heat injury" [110]. HSPs are grouped into five families (HSP100, HSP90, HSP70, HSP60 and HSP20) and are involved in intracellular hemostasis and preservation against various internal as well as external lethal stimuli [111-116]. As RNA synthesis is almost completely inhibited during a heat shock, *de novo* synthesis of HSPs will not take effect immediately, but after heat shock exposure. In order to prevent irreversible damages, "prompt HSPs" are rapidly mobilized as an early response to increased temperature. These "prompt HSPs" play a role in the acute stress, whereas other HSPs will confer a subsequent thermotolerance to a second heat exposure [117]. In the early 1980s, the correlation between HSPs expression and thermotolerance was published in several papers [118-120]. Thus on one hand, HSPs prevent cells from heat-induced damage by conferring a temperature-resistant phenotype. On the other hand, HSPs have been recently shown to be immunogenic since they can stimulate both innate and adaptive immune system [121-126]. Three probably interconnected mechanisms are proposed to explain the influence of HSPs on the immune response. First, HSPs released by tumor tissue injury (due to radiotherapy, chemotherapy or hyperthermia) stimulate the innate immune system to activate an inflammatory response. Secondly, several studies concluded that HSPs are able to present tumor antigens to the antigen-presenting cells (APC) and consecutively activate the tumor-specific T cells. Finally, HSPs seemed to enhance the ability of intact tumor cells to process and present endogenous tumor antigens directly to tumor-specific cells [127-129].

Besides the effects of heat on HSP synthesis and their subsequent implication as immunomodulators, hyperthermia has been reported to influence directly several aspects of both innate and adaptive immunity but the results obtained are still equivocal [130-134].

Despite all the data obtained concerning the probable positive effects of hyperthermia on the immune system, there is still a lack of knowledge regarding the thermal dose at which effects or damages on immune cells could occur *in vivo* [135]. This major gap has to be completed to ensure quality and safety of treatment.

2.3. Tumor metabolic microenvironment

The so-called metabolic microenvironment (MME) – defined as closely linked factors such as blood flow, oxygen and nutrient supply, bioenergetics or metabolic status and pH distribution – is of great importance in cancer treatment. In fact, it is now widely accepted that the MME can dramatically influence several factors such as proliferation rate, cell cycle position, growth rate, the development of apoptosis and/or necrosis, and the effects of anticancer treatments including hyperthermia, radiotherapy as well as chemotherapy. First, the tumor blood flow (TBF) is heterogeneous both spatially and temporally, which correlates to the chaotic microvascularization observed in a tumor and also to the critical lack of structural and functional normality [136-138]. The critical role of TBF in hyperthermia treatment is related to two components: i) its involvement in convective thermoregulation and ii) its influence on the other MME parameters. The former is correlated to the ability of tumor tissue to adapt to heat and the latter dictates the sensitivity of the tumor to heat [139]. In healthy tissue, the physiological reaction to heat is an active vasodilation followed by an increase in blood flow in order to improve heat dissipation by convection and in turn reduce tissue temperature down to normal values. By contrast, tumor microvasculature is limited in its response to heat as its convective dissipation is reduced. Thus, tumor vessels appeared to be more sensitive to heat and the consequence of this abnormality is a decrease of TBF at temperatures above 42-43°C [65, 138, 140]. Moreover, above this temperature threshold, hyperthermia injured tumor blood vessels and in particular endothelial cells, and provoked erythrocyte aggregation, leukocyte

adhesion and even angiogenesis inhibition [141-143]. It also induced a significant vascular permeability increase in rats, which may explain plasma fluid shift into the interstitium [65, 144]. Furthermore, more recently thermotolerance was correlated to a normalization of tumor vessels [145]. In contrast to these observations, and mostly below the threshold of 42-43°C, which corresponds more closely to the clinical temperature usually achieved, TBF seemed to be increased [65, 140], probably allowing to achieve a higher drug delivery within the tumor tissues. One has however to keep in mind the difficulty of promulgating a general principle. In fact, a recent review described the heterogeneity of the results obtained concerning the TBF dependence in temperature, and the authors concluded that the TBF changes are not predictable as they might be influenced by several parameters including both pathophysiological status and hyperthermia techniques used. These complex multi-parameter effects lead to a non-uniform response to heat treatment and thus make it difficult to predict how tumors will respond to heating [139]. It is now well known that tumor tissues soak in hypoxia conditions [146]. Upon hyperthermia, the oxygen supply was mostly seen as evolving in a temperature-dependent manner. Two main reasons are usually evoked: i) increases in TBF and ii) heat-induced decrease in tumor oxygen consumption. Thus, at temperature ranging from 40-42.5°C, an improvement of TBF in rodents was suggested to explain the three-fold increase in tissue oxygenation [147, 148]. However, as for the TBF effects induced by hyperthermia, further investigations are required to define the real effects (short- or long-lasting oxygenation) as well as the underlying mechanisms [139]. Concerning tumor pH, one has to differentiate the extracellular pH (pH_e) from the intracellular one (pH_i) [136, 149]. The effects of heating seemed to favor a decrease of both pH_i and pH_e, which in turn leads to an improvement of hyperthermia by sensitizing tumor cells [139]. However, some contradictory outcomes raise the need for further studies in order evaluate the impact of heat on pH.

Unfortunately, this pathophysiological heterogeneity in MME responses is rarely taken into account for a specific antitumor treatment protocol, although it may improve outcome. For example, it is widely accepted that resistance to radiotherapy is mostly due to hypoxia conditions within the tumors, whereas ineffective systemic chemotherapy is most probably due to poor tumor blood perfusion which in turn leads to low local concentration of anticancer agents. Thus, combination of hyperthermia and other established cancer treatment modalities finds its rationale through the plethoric effects of heat on tumor cells. An improved local drug bioavailability along with an increased up-take of antineoplastic drugs is thought to occur under heat as the TBF as well as the membrane permeability are enhanced. Moreover, the DNA repair system impairment by heat suggested that anticancer drug acting on the DNA and radiotherapy should be more effective.

2.4. Biologic rationale for hyperthermia in combination with chemotherapy

As previously mentioned, mild and moderate hyperthermic treatments (40-47°C) are mostly used in combination with established therapy modalities (e.g., surgery, chemotherapy, radiotherapy). Through its plethoric effects, hyperthermia modulates the cytotoxicity of radiotherapy – so-called "thermal radiosensitization" - and many antineoplastic agents - "thermal chemosensitization". To quantify and compare the extent of heat effects on anticancer drugs activity both *in vitro* and *in vivo*, the concept of "thermal enhancement ratio" (TER) has been introduced. The TER describes the pharmacodynamic aspect of heat-drug-interaction and is defined as the quotient of tumor cell survival when treated with antiblastic drug either at normal temperature or under hyperthermia conditions. Obviously, TER is depending on the nature and concentration of drug, the temperature achieved and the time of exposure as well as the tumor cells treated [150]. The sequence in which the treatment is applied (i.e. sequential *vs.* synchronous) appears also to be determinant for a therapeutic

benefit [151-154]. Moreover, for a sequential heat-drug or drug-heat treatment the delay between them is also critical [155]. Urano and coworkers reviewed *in vitro, in vivo* and clinical trials of combined thermo- and chemotherapy. The authors concluded that: i) the cytotoxicity enhancement is optimized in temperatures ranging from 40.5°C to 43°C, ii) high variability in TER was observed for the same antineoplastic drug depending on the cell lines studied, iii) at physiological temperatures the drug of choice is not necessary the same as at elevated temperatures, iv) high local drug concentrations along with homogenous localized tumor heat are necessary to obtain a sufficient TER [156]. The heat-drug combination results mainly in three different interaction types named "(supra-)additive", "threshold" or "independent". The "additive" or "supra-additive" interactions reflect a TER linearly correlated to the temperature and are mostly observed for alkylating agents and platinum compounds. The "threshold" behavior, as observed for doxorubicin and mitomycin, shows a small enhancement in drug activity at low temperature followed by a significant improvement above a threshold temperature. Finally, when no relationship between heat and drug activity is observed, the drug is classified as "independent" [157]. Although several clinical trials including hyperthermia and chemotherapy alone [158-160] or along with radiotherapy and/or surgery and/or immunotherapy were carried out [25, 26, 29, 161-164], few of them were really performed to evaluate the impact of hyperthermia on chemotherapy [165, 166]. To date, the only phase III clinical trials performed in this view demonstrated that regional hyperthermia increases the benefit of chemotherapy for the treatment of localized high-risk soft-tissue sarcoma [30]. Several studies in cell cultures or in animals were published showing an increase in neoplastic agents' activity through different suggested mechanisms including heat-increased membrane permeability, antiproliferative effects by modulating the expression of cell cycle regulatory proteins or cell repair system inhibition [151, 167, 168]. Consequently, reduced doses of drug could be used leading to lower systemic toxicity [150,

153, 154, 167, 169]. Finally, the tumor growth delay was significantly increased by heat-drug combination [170, 171].

The major cause of failure in the treatment of human malignancies is certainly the multifactorial phenomenon of drug resistance. Several mechanisms are involved, such as "multidrug resistance" (MDR) and the activation of NF-κB. MDR is mostly dominated by the overexpression of ATP-binding cassette (ABC) drug transporters. This family of ATPase transmembrane efflux pumps – including the glycoprotein p170 (PGP170) – transports actively several drugs from the cytosol to the extracellular milieu and thus is able to decrease the intracellular drug concentration and consequently the cytotoxic effect [172]. The transcriptional nuclear factor NF-κB is responsible of an anti-apoptotic mechanism and its activation through chemotherapy, radiotherapy, TNF-α production and other stimuli results in gene expression related to cell proliferation, angiogenesis and anti-apoptotic processes [173]. In a recent study, the cytotoxic effect of gemcitabine on human pancreatic carcinoma cell lines (AsPC-1 and MIAPaCa-2) was enhanced by heat treatment at 43°C for 1 hour applied 24 hours before drug addition. Gemcitabine alone activated NF-κB expression in both cell lines, thus creating drug resistance, whereas heat treatment inhibited this gemcitabine-induced NF-κB expression. [174]. In a MDR Chinese hamster ovary cell line the combination of heat and cyclosporine A was correlated to an increase in melphalan uptake as well as a decreased drug efflux accompanied by modified membrane permeability, suggesting that MDR was bypassed [175]. The cellular cisplatin resistance was overcome by association with heat through several potential mechanisms. Indeed, intracellular accumulation was suggested to be due to an increase in membrane fluidity and membrane and cytoplasmic protein denaturation, whereas altered DNA conformation and inhibition of DNA repair probably resulted in a better DNA accessibility and a less effective repair of cisplatin-DNA adducts [176-180]. More recently, the cisplatin resistance reduction was also observed in several studies including

phase I/II and III clinical trials. These studies concluded that the combination of cisplatin and hyperthermia, eventually accompanied by radiotherapy, has a great impact on the response rate and toxicity [159, 181-184].

Thus, the combination of heat treatment and drug administration represents a relevant strategy as it can enhance the cytotoxic effects of drugs but also overcome the drug resistance response. However, at this stage, one has to keep in mind that the heat shock response is a physiological reaction of cells to many internal and external stresses including radiation, oxidative stress and toxic compounds such as chemotherapeutic substances. Consequently, thermotolerance could also result in chemoresistance [65]. From a clinical point of view, this fact is of critical importance and has to be clarified in order to avoid treatment failure. Thus, more efforts are still needed to understand the underlying mechanisms by which heat and drug interact according to the hyperthermia technique used, anticancer substances administered, the schedule applied and tumor treated.

3. Delivering heat and chemotherapy

In view of thermal enhancement, devices able to deliver heat and antiblastic drugs are of great interest for cancer treatment. In particular, magnetizable nanoparticles functionalized with suitable coatings and loaded with drugs have shown promise. Iron oxide nanoparticles (SPIONs) coated with poly(N-isopropyl acrylamide) and loaded with doxorubicin to obtain "composite nanoparticles" (CNPs) could, *in vitro*, release doxorubicin and rise temperature to the hyperthermic range (i.e. 41°C to 47°C) [185, 186]. In rats bearing human hepatocellular carcinoma (HCC), intra-arterially injected CNPs were deposited in HCC and delivered doxorubicin and heat (up to 48°C) under exposure to an alternating magnetic field [187]. SPIONs coated with starch polymers coupled with phosphate functional end groups were also loaded with cisplatin and tested on BP6 rat sarcoma cells. The authors concluded that

delivering heat and cisplatin resulted in markedly decreased survival of cells, eventually due to synergistic effects [188]. A recent review covering advances in surface-modified SPIONs for drug delivery reported that the main limitations of SPIONs are to target interior body regions without targeting surrounding tissues, and to control nanoparticle residence time at the targeted site [189]. Reaching a high local iron oxide content required to achieve therapeutic temperature still remains a major hurdle for targeting approaches. Moreover, although these approaches may be relevant for soft tissue cancers, they possess some shortcomings for solid tumors and specifically for bone tumor treatment, such as a poorly vascularized access and a weakened diseased bone tissue.

4. Intraosseous forming implants

Delivering heat and chemotherapeutic agents intra-osseously to treat bone metastases may be more efficiently achieved by an *in situ* forming implant. The implant would entrap SPIONs, avoiding distant migration and delivering locally antiblastic drugs. It should also provide mechanical support to weakened bone either through its own mechanical strength in case of non-biodegradable implants, or through its osteoinductive ability to form load-bearing bone, for the biodegradable counterpart. As the axial skeleton is the most common site affected by bone metastases [54, 55], percutaneous vertebroplasty (VP) was developed in the 1980s [190, 191]. This image-guided, cement injection technique is mainly used to treat vertebral hemangioma, bone metastases, osteoporotic fractures and vertebral myeloma [192]. Today, cementoplasty combines the two approaches of vertebroplasty and its more recent variant, kyphoplasty (KP). Both have in common the same filling materials, but in KP an inflatable balloon first restores some of the vertebral height prior to cement injection [62]. Intraosseously forming cements are categorized according to three types of material: i) injectable poly(methyl methacrylate) (PMMA) cements, ii) calcium phosphate cements

(CPCs) or iii) calcium sulfate cements (CSCs). Each of these cements has different biomechanical properties, which in turn will lead to different behaviors within the vertebral body as well as within adjacent vertebrae [193]. Acrylic cement is used in KP and VP mainly to support mechanically the fractured vertebrae and consequently relieve pain [194]. PMMA cements are prepared by mixing a solid phase consisting of poly(methyl methacrylate) and a liquid phase containing the methyl methacrylate monomer, in the presence of an activator and an initiator, i.e. benzoyl peroxide and N,N-dimethyl-p-toluidine, respectively. The *in situ* implant formation is the result of a polymerization and occurs by exothermic reaction. The temperature achieved depends on the cement formulation. Measurements using eleven different commercially available acrylic cements were achieved during *in vivo* VP and suggested that PMMA cements may be divided into three groups according to the temperatures achieved: i) below 50°C, ii) from 50°C to 60°C and iii) above 60°C [195]. Thus, care should be taken to avoid thermal drug degradation during polymerization. Moreover, during the polymerization monomer radicals are formed and drug chemical inactivation must be avoided during this step [196]. The second class of bone-implantable materials is represented by the biodegradable calcium phosphate cements (CPCs) [197]. Based on different calcium phosphate salts, mainly described by their Ca/P ratio (by increasing order, brushite, tri-, tetra-, octo-calcium phosphates and hydroxyapatite), CPCs offer the possibility to restore new bone tissue. Thanks to their chemical similarity to apatite, a major inorganic bone component, CPCs have suitable biocompatibility and bioactivity properties [198, 199]. They are also prepared by mixing a powder phase, containing the calcium phosphate salts, with a liquid phase, consisting of an aqueous solution, which will result in hardening of the cement. In contrast to PMMA cements, CPC formation takes place by hydrolytic and redox reactions instead of a polymerization [200]. After hardening, the kinetic of CPC degradation and resorption is mostly dictated by the Ca/P ratio and consequently it becomes possible to

control the rate of bone formation by changing this ratio [199]. Calcium sulfate cements (CSCs) are prepared by mixing calcium sulfate powder with a diluent to obtain a solid or partially solid structure. CSCs are biodegradable devices that enhance both angiogenesis and osteogenesis [201], albeit possessing relatively weak strength. Finally, another class of bone-implantable materials is represented by the group of ceramics, i.e. ceramics, glasses and glass-ceramics, and were recently reviewed according to their structures, properties, processing and clinical applications elsewhere [199].

4.1. Drug-releasing intraosseous implants

Several studies reported the feasibility of loading therapeutic drugs such as antibiotics into different bone cements and are reviewed in recent papers [202-204]. Local antibiotic elution exceeded the therapeutic concentrations (i.e. the minimum inhibitory concentrations) and systemic adverse effects were largely avoided. Thus, intraosseous delivery systems providing a localized and targeted treatment of bone infections may avoid or reduce the need for intravenous and/or oral antibiotics. Several clinical trials with favorable outcomes using PMMA-antibiotic beads currently support this approach as a standard practice for musculoskeletal diseases. Extending these outcomes to cancer treatment, the previously cited intraosseously forming implants were also loaded with antiblastic drugs in order to improve the local growth control of BM as well as to decrease the systemic circulating drug concentration (Table II). PMMA cement was mixed with daunorubicin (DNR) at various concentrations and injected either subcutaneously in athymic nude mice or into the femur of Wistar rats. The *in vitro* DNR release profile reached only 16% of the initial DNR amount (1mg) after approximately 8 days, whereas during the first 6 hours 6% were already released. In order to modify the kinetics of drug elution patterns a pore generator, i.e. mannitol at different concentrations, was mixed with the DNR-PMMA cement. The results showed a

markedly improved release, as it attained 57% and 90% – DNR concentration of 0.2 mg – after 15 days with a mannitol concentration at 2% and 10%, respectively. DNR was proven to be thermostable and the exothermic cement hardening did not influence DNR structure. Moreover, no significant influence on the mechanical properties of the cement was measured after adding mannitol at 2%. Thus, rate and duration of elution were tailored without affecting the cement's mechanical properties. In nude mice implanted with either soft tissue sarcoma, bronchial sarcoma or renal cell carcinoma a significant reduction in local recurrence was obtained, whereas this effect was not achieved with breast cancer. In the Wistar rat femur model, the tumor growth was delayed from 22.6 days for the control group (pure PMMA cement) to 37.8 days for the PMMA cement group containing 1 mg DNR. Finally, biodistribution of radiolabeled DNR showed that DNR concentration was the highest at the injected site, i.e. within the tumor tissue, whereas it was very low in others sensitive organs (e.g., brain, heart, lung), supporting the authors' conclusion that systemic side effects were minimized by this local drug delivery [205]. Acrylic cement was also loaded with methotrexate (MTX) and, as for DNR, the exothermic polymerization did alter neither MTX structure nor MTX elution, which was measured for as long as six months. The survival rate of dogs was improved along with a reduction of local recurrence [206]. Kim et al. loaded MTX at various amounts ranging from 5 mg to 50 mg, within commercially available PMMA cements (Simplex P bone cement, Howmedica, USA). Drug elution reached a plateau by the third week. Depending on the drug loading, the concentrations eluted were 130 to 4000-fold higher than the minimum inhibitory concentration (MIC) required for DNA synthesis inhibition. The cytotoxic effects of MTX eluted on two osteosarcoma cell lines (SaOS2 and MG63) showed a significant decrease of viable cells after at least 3 days of culture [207]. Mechanical spine support is a key property of these cements in order to obtain pain relief. Using MTX-cement as presented above, subsequent drug release did not affect the

compressive properties of the cement, and bioactivity was shown up to 30 days [208]. Similar findings were demonstrated by loading custom-made PMMA cement with MTX (4.5 % w/w) since the authors did not detect any changes in the compression modulus of the cement before or after drug elution [209]. Using two commercially available acrylic bone cements (Vertebroplastic^{TM} and Confidence Ultra^{TM}) loaded with methotrexate at varying amounts, the same authors found a correlation between MTX elution and a decrease in flexural modulus as well as cement strength. This decrease was not proportional to the initial amount of MTX added [210]. Different concentrations of DOX and pamidronate were incorporated within surgical Simplex™ acrylic cement in order to study their influence on cement mechanical properties and their *in vitro* release. It was found that as much as 2g of either DOX or pamidronate or both can be added to the cement without significantly altering its strength. The amounts of drug released were determined to be biologically active [211]. The mechanical properties were difficult to predict and should be determined for each cement-drug combination as well as for each drug amount loaded before further *in vivo* studies or clinical trials. In rabbits bearing VX2 tumor within the tibiae, an intraosseous injection of acrylic cement loaded with MTX was correlated with a decrease in osteoclast proliferation and bone destruction, although the latter was not totally eliminated. The authors suggested that MTX-loaded acrylic cement could diminish tumor-induced osteolysis [212]. Interestingly, the same group performed another study with MTX-loaded PMMA cement and a correlation between amounts of MTX and a significant reduction in pulmonary metastases was reported compared to the drug-free cement [213]. An *in vitro* evaluation of the cytotoxic effect of a combination of PMMA cement (Simplex P bone cement, Howmedica, USA) and four different concentrations of either MTX, doxorubicin (DOX) or cisplatin (CDDP) was performed on stromal giant-cell tumor cells (GCT) obtained from five patients. The *in vitro* release rate decreased for all drugs after the third day, reaching a plateau after one week. The maximum

eluted percentage of drug incorporated in the cement was 15% and corresponded to the lowest dose of DOX (0.12 % w/w), whereas the highest drug load corresponded to the smallest eluted percentage. The release pattern was dose-independent. The cytotoxic effects on GCT for four different doses of each drug showed that at the lowest doses for each drug and at day 14 the cell death still corresponded to 45% for DOX, 34% for CDDP and 31% for MTX. A comparison of the cytotoxic effects on GCT and osteoblasts (healthy cells) was also performed and a difference in the growth of the two tissues was detected at day 14, as the osteoblasts were more sensitive to the cytotoxic effects than the GCT cells [214]. Rosa and coworkers studied the release and the cytotoxic effects of acrylic cements loaded with MTX, DOX or CDDP on MCF-7 human breast cancer cells. The authors observed that the inhibitory effect on MCF-7 cells became smaller with time for all three drugs. Although CDDP was seen at cement surface using energy-dispersive X-ray, the duration of release suggested an elution from inside the cement [215]. The PMMA polymerization was not disturbed or inhibited by doxorubicin or cisplatin loading, and both drugs remained active post-elution on sensitive neoplastic cells [216]. Finally, zoledronic acid-loaded acrylic cement was prepared and its cytotoxic effect studied on giant cell tumor, multiple myeloma and renal cell carcinoma cell lines. The results showed that the drug could be loaded, and was released in a biological active form, which resulted *in vitro* an *in vivo* tumor growth inhibition [217].

Calcium phosphate cements (CPCs) were also used as anticancer drug carriers for intra-osseous application in a few studies. Doxorubicin-loaded CPC was prepared and incubated with RMT-1 E4 rat breast cancer cells showing a marked decrease in cell proliferation compared to pure CPC. DOX-loaded CPC was injected into rat femur, inducing neither fracture nor necrosis, although diffuse edematous changes occurred in the medullary space. In mouse air-pouch model bearing sarcoma 180 cells, the injected formulation inhibited cell proliferation and improved significantly the survival rate [218]. The viability of osteosarcoma

(U20S) and metastatic breast cancer (MDA-MB-231) cell lines decreased under contact with CPC loaded with paclitaxel. The drug kept its bioactivity post-elution and diminished the viability of osteosarcoma cells, whereas it merely stopped cell proliferation of MDA cells [219]. An association of cisplatin and caffeine charged with CPC was reported to release both drugs in a sustained manner and the eluted drugs inhibited SOSN2 cell proliferation *in vitro*. The combined drug-loaded CPC, injected within rats tibiae, decreased the 6-weeks tumor volume and significantly improved the survival rate up to 100 days [220]. In another study, local cisplatin delivered by a CPC was compared to its systemic administration in adult rabbits. The systemic cisplatin concentration was lower for implanted rabbits compared to the systematically administered group. Moreover, bone formation occurred within 12 weeks after cisplatin-loaded CPC implantation [221]. Taken together these studies indicate the feasibility of delivering anticancer drug to bone with bioactive CPC, although the low tensile strength compared to bone may limit their use. Finally, calcium sulfate cement supplemented with hydroxyapatite and a binder (alginate or chitosan) was loaded with MTX. These cements were able to release MTX *in vitro* for over one month and to kill up to 80% of human mammary carcinoma cells [222].

4.2. Thermotherapy through intraosseous implants

Besides the feasibility of loading bone cements with anticancer agents, studies were also performed to deliver heat through cements (Table III). PMMA cement was loaded with magnetite particles (Fe_3O_4) and once exposed to an alternating magnetic field (120 Oe=12 mT and 100 kHz), the surface temperature of PMMA cement containing 50% (w/w) Fe_3O_4 increased up to 48°C. The authors measured an increased compressive strength and setting time of the cement both correlated to the increase of Fe_3O_4 concentration. As expected, the maximum temperature achieved during the setting reaction was reduced as the magnetite

particles amounts increased [223]. A clinical trial including patients receiving CPC loaded with Fe_3O_4 in combination with intramedullary nail or plate and bone lesions curettage was performed, some patients also received systemic chemotherapy. The hyperthermic sessions lasted for 15 minutes and the temperature achieved on the surface of cortical bone was around 42°C. A better radiographic outcome was obtained for the "hyperthermic group" than for patients receiving only palliative surgery, but no significant differences were observed compared to patients receiving palliative surgery plus radiotherapy [224]. Using ceramics made of a filler-resin mixture, where filler components consisted of silica glass powder and magnetite particles and resin components corresponding to bis-α-glycidylmethacrylate (bis-GMA), rabbits bearing VX2 tumors within their tibia were treated. Following intra-tibial injections, the animals were exposed to an external alternating magnetic field (100 kHz), raising the temperature to around 43°C in the tissues. The maximum circumference of the operated leg was significantly decreased in animals treated by heat compared to the control group, suggesting that hyperthermia diminished both tumor growth and inflammatory response of the surrounding tissue. Histological examination confirmed the regressive change of tumor tissue [225]. Ceramic composed of magnetite and silica glass powders as fillers and bis-GMA based resin was also studied in term of heat-generation, bone heat-distribution and mechanical properties. Rabbits were implanted within their tibia and exposed to a high amplitude magnetic field (300 Oe=30mT and 100 kHz), raising the temperature up to 60°C, 43-45°C and 40°C for the ceramic, the cortical surface and at 1 cm distance from the ceramic in the medullary canal, respectively; similar results were obtained using human cadaver tibiae. This illustrated the rapid spatial temperature decrease found around hyperthermic implants. This study also demonstrated that heat-generation is improved by an increase of magnetite content [226]. Glass-ceramics containing magnetite (SiO_2-CaO-Fe_2O_3-B_2O_3-P_2O_5) were implanted into rabbit tibiae and evaluated according to their ability to bond to bone tissue and

deliver heat. After 8 weeks of implantation, the authors reported a tight bonding to bone through a calcium phosphate-rich layer. Moreover, when exposed to a magnetic field (300 Oe=30mT and 100 kHz) the surrounding bone temperature rose to more than 42°C for 30 minutes, suggesting a potential use for hyperthermic treatment [227]. Another ferrimagnetic glass-ceramic based on SiO_2-Na_2O-CaO-P_2O_5-FeO-Fe_2O_3 was prepared and studied in terms of bioactive properties and specific heat power when exposed to a magnetic field of high amplitude and frequency (40 kA/m (~50mT) and 440 kHz), without reporting the temperature achieved. Soaked during two weeks in a simulated body fluid, the material had a bioactive behavior shown by the hydroxyapatite layer deposited at its surface [228]. Bioactivity of glass-ceramic was also studied by Ebisawa and coworkers and the same observations concerning the formation of apatite layer on the glass-ceramic surface when immersed in a simulated body fluid were made [229]. Ceramic prepared by mixing a powder system (SiO_2-Al_2O_3-Fe_2O_3-MgO-CaO-SO_3-K_2O) with water was tested with respect to different parameters such as its specific heating power. The heat produced when it was exposed to an alternating magnetic field (10 kHz) corresponded to a specific heat power of 2.11 W/g *in vitro* [230]. Finally, mice bearing breast carcinoma and implanted with a glass-ceramic were exposed to an alternating magnetic field (500 Oe=50mT and 10 kHz) and the authors observed a significant tumor growth delay without determining the local temperature achieved [231]. The available data indicate not only the feasibility, but also the potential of hyperthermic implants to treat bone tumors, despite too frequent lacunar characterization of the heating parameters. In addition, the feasibility of delivering anticancer drugs through intraosseously forming devices was also demonstrated in several studies. Since the combination of thermo- and chemotherapy is known to be effective at a lower temperature than hyperthermia alone, it is surprising that no studies were found where both drugs and heat were delivered to treat locally bone metastases. In order to investigate implants capable of multimodal,

thermochemotherapy, we formulated acrylic cement loaded with different anticancer drugs and SPIONs [232]. This cement was shown to achieve hyperthermic temperatures when exposed to alternating magnetic fields using clinical conditions while releasing bioactive anticancer drugs. Further studies on the potential additional effects on tumor cells both *in vitro* and *in vivo* are ongoing.

5. Conclusion

Treatment of tumors by induced hyperthermia has gained clinical acceptance as an adjuvant in oncology protocols, combined with radio- or chemotherapy. Indeed, through its plethoric effects, hyperthermia potentiates the cytotoxicity of established treatment modalities. Moreover, as a physical therapy, hyperthermia is less limited by its side effects than radio- or chemotherapy. However, most hyperthermia techniques still suffer of lack in selectively targeting deep-seated tumor and preserving healthy surrounding tissues. Magnetic fluid hyperthermia (MFH) addresses these shortcomings and hence offers the possibility to target superficial as well as deep-seated tumors in a selectively manner. In view of a better localized treatment of bone metastasis, technological advances may offer promises. On one hand, novel *in situ* forming drug depots compatible with bone have shown their ability to fight cancer in preclinical models. On the other hand, compounds bearing magnetic particles can generate local temperature rises expected to produce an antitumor effect. Although distinct bone tumors treatment using local chemotherapy or hyperthermia had shown great outcomes, the approach of combined local therapy of bone metastases remains to be explored.

Tables and figures

Table I: Clinical techniques of hyperthermia

	Sources of energy	***Limitations***	***Applications***
Local Hyperthermia	MW RF US	therapeutic depth of only few cm limited in regions with irregular surface ultrasound may cause patients discomfort	superficial tumors 3-10 cm depth with single sources < 20 cm depth with multiple sources
Interstitial Hyperthermia		Positioning and orientation of applicators and antennas Invasive technique	tumors less than 5 cm in diameter and in an accessible location for applicators implantation (e.g., head and neck, prostate)
Endocavitary Hyperthermia			Natural hollow openings (esophagus, vagina, cervix, rectum…)
Regional and body-part Hyperthermia		Heating of healthy surrounding tissue Systemic side effects (e.g. malaise, tachycardia)	Deep seated tumors (pelvis, abdomen…)
Magnetic Fluid Hyperthermia	EM field and magnetizable particles	Localized approach with limited range of action Potential particle toxicity	Superficial and deep seated tumors Mild or moderate hyperthermia or thermoablation
Whole-body Hyperthermia	IR	Maximum 42°C Superficial overheating resulting in thermal lesions Systemic toxicity (cardiac, coagulation…) Deep analgesia and sedation or general anesthesia	Cancer with distant metastases
Hyperthermic isolated limb perfusion	Extracorporeal heat exchange	Invasive techniques	Arms, legs, lung and liver cancer

Abbreviations: MW: microwaves; RF: radiofrequency; US: ultrasound; EM field: electromagnetic field; IR: infrared

Table II: Bone implants for drug release

	Cement	*Drugs*	*In vitro assays*	*In vivo/in vitro models*	*Main findings*	*Ref*
AC	Palacos® and Refobacin-Palacos®	DNR	16% released over 8 days	• Nude mice • Wistar rats	• Improved *in vitro* release (57% and 90% over 15 days) by mannitol addition at 2% and 10% respectively • Reduction in local recurrence in nude mice • Tumor growth delayed in Wistar rats • Systemic side effects minimized	[205]
	Custom made	MTX	10% released after 18 hours	• Rat bearing osteosarcoma • Spontaneous osteosarcoma in dogs	• Slow *in vitro* release maintained after six months • Both local and general effects in rat • Increased survival and reduced local recurrence in dogs	[206]
	Simplex P®	MTX	Maximum of 11.7% released over 4 weeks	• Human osteosarcoma cell lines (SAOS2 and MG63)	• Concentrations eluted largely higher than the minimum inhibitory concentration of DNA synthesis • Progressive reduction of the viable cells from the third day of incubation for both cell lines until the 14^{th} day.	[207]
	Simplex P®	MTX	Constant diffusion up to 30^{th} day	• MCF-7 breast cancer cell lines	• No alteration of cement compressive properties by elution • Proliferation of cells inhibited strongly during first days followed with a decrease thereafter	[208]
	Vertebroplastic®	MTX	$50ng/mm^2/h$ for 6 hours then decreased rate to $10ng/mm^2/h$ by 36 hours	-	• No change in the cement compression modulus pre- and post-elution • Decrease in the diffusion rate after 6 hours • Predicted diffusion model developed	[209]
	Confidence Ultra® Vertebroplastic®	MTX	~ 40% released over 28 days	-	• Influence of MTX amount loaded on elution profile, flexural strength and flexural modulus • Decrease of flexural strength and modulus after storage in saline media for 45 days	[210]
	Simplex®	DOX PDT	Burst release during 24hours followed by small amount released during several weeks	-	• Influence of DOX and PDT amount loaded on elution profile • Cements Young modulus and tensile strength not affected by as much as 4 g drug added • Compressive and tensile strength retained after 6 months of wet storage	[211]
	Palacos-R®	MTX	-	• Half-lop rabbits inoculated with VX_2 cell lines within the tibiae	• Decrease in osteoclast proliferation and bone destruction • Decrease in tumor-induced osteolysis	[212]
	Simplex P®	MTX DOX CDDP	9.3% MTX 11% DOX 7.6% CDDP released after 4 weeks	• Stromal Giant-cell tumor cells from patients • Osteoblasts from patients	• Lowest dose of each drug (75µg) corresponded to percentage of tumor cells survival of 55%, 66% and 69% for DOX, CDDP and MTX respectively • At day 14 and dose of 100 µg of each drug, osteoblasts were slightly more sensitive than tumor cells	[214]
	Simplex P®	MTX DOX CDDP	-	• MCF-7 human breast cancer cells	• Higher cytotoxic effects obtained for DOX during 2 days • No cytotoxic effects after 15 days for DOX and CDDP • Diffusion of CDDP from inside the cement suggested	[215]
	Simplex P®	ZA	Burst release during 24hours and plateau reached after 4 days	• Giant-cell tumor cells • Human multiple myeloma (NCI-H929) • Human renal cell carcinoma (RBM1-IT4)	• ZA decreased the number of viable tumor cells in a dose-dependent manner • Giant-cell tumor cells and human renal cell carcinoma are more susceptible to ZA	[217]
CPC	TCP:TeCP:DCP (7.5:2:0.5) CS:SS:W (0.5:1.2:8.3)	DOX	-	• RMT-1 E4 rat breast cancer cells • Healthy rat femur • Mouse air-pouch model bearing sarcoma 180 cells	• No significant difference in compressive strength with or without drug • *In vitro* cell proliferation rate significantly decreased during 7 days • Neither fracture nor necrosis occurred when injected within rat femur; 24 weeks after implantation in femur, large bone formations detected • 75% of mice survived for 16 weeks *vs.* 12.5% in control group	[218]
	α-TCP:DCP:HA (8.5:1:0.5) 2% Na_2HPO_4	PTX	-	• Osteosarcoma cells (U_2OS) • Metastatic breast cancer cells (MDA-MB-231)	• Decreased viability of osteosarcoma cells • Cell proliferation stopped for the metastatic breast cancer cells	[219]
	Cerapaste®	CDDP Caffeine	14.1% CDDP 82.4% caffeine released after 8 weeks	• Rat osteosarcoma SOSN2 cells • Fischer 344/NS1c rats inoculated with SOSN2 into tibiae	• Caffeine turned SOSN2 cells more sensitive to CDDP • Cell proliferation decreased starting at 48hours and no proliferation observed from 5^{th} days • Tumor volume at 6 weeks decreased by caffeine-CDDP association • 85% of rats survived for 14 weeks *vs.* 0% in control group	[220]
	TCP:TeCP:DCP (7.5:2:0.5) CS:SS:W (0.5:1.2:8.3)	CDDP	62% released after 4 weeks	• Healthy Japanese white rabbits	• Systemic CDDP concentrations lower for implanted rabbits than for systemically administrated group • Strongly higher bone concentrations for implanted group • Bone formation observed after 12 weeks post-implantation	[221]
CSC	CS:HA + alginate or chitosan	MTX	Release for over 1 month	• Human mammary carcinoma cells	• Higher release using alginate as binder • Up to 80% of cell death	[222]

Abbreviations: AC: Acrylic cements; CPC: calcium phosphate cements; CSC: calcium sulfate cements; CS: calcium sulfate; CDDP: cisplatin; DNR: daunorubicin; DOX: doxorubicin; MTX: methotrexate; PDT: pamidronate; PTX: paclitaxel; ZA: zoledronic acid; TCP: tricalcium phosphate; α-TCP: α-tricalcium phosphate; TeCP: tetracalcium phosphate; DCP: dicalcium phosphate; HA: hydroxyapatite; CS: chondroitin sulfate; SS: sodium succinate; W: water

Table III: Bone implants for hyperthermia

	Composition	*AMF parameter**	*In vitro assays*	*In vivo/in vitro models*	*Main findings*	*Ref*
AC	AC added by maximum of Fe_3O_4 50% (w/w)	12 mT 100 kHz	Cement surface temperature up to 48°C	-	• 60% Fe_3O_4: no uniform cement obtained • At 30mT: surface temperature reached up 70°C	[223]
CPC	CPC loaded with Fe_3O_4 40% (w/w)	n.r. 1.5 MHz	-	Clinical trials 15 patients with 16 metastatic bone tumors in the extremities	• Comparison of hyperthermia (HT) group (15), operative (Op) group (8) and operative + radiotherapy (Op+RT) group (22) • 50% of HT group: reduction in bone lesions with visible bone formation • 87% of HT group: effective treatment outcome (significant difference with Op group but not with Op+RT group) • Temperature reached around 43°C on the surface of cortical bone	[224]
Ceramic	Filler 90%: magnetite powder and silica glass (SiO_2) Resin10%: bis-GMA	n.r. 100 kHz	-	Rabbits inoculated with VX_2 within tibiae	• Decrease significantly the tumor growth and inflammatory response • Cortical bone destruction and pathological fractures diminished • Temperature reached around 43°C within tissue	[225]
	Filler 90%: magnetite powder and silica glass (SiO_2) Resin10%: bis-GMA Magnetite content 50% (w/w)	Max. 30 mT 100 kHz	-	Healthy rabbit and human cadaver implanted within tibiae	• Implant temperature: 50-60°C • Bone-muscles interface: 43-45°C • Cortical surface at a distance 10mm from cement: 42°C • Medullary canal at a distance 10mm from cement: 37°C • Fibrous changes of the adjacent bone but no change in the cortical bone	[226]
Glass-ceramic	$CaO\text{-}SiO_2\text{-}Fe_2O_3\text{-}B_2O_3\text{-}P_2O_5$ Iron oxide content 40% (w/w)	Max. 30 mT 100 kHz	-	Healthy rabbit implanted within tibiae	• Implant temperature: 45°C • Bone-muscles interfaces temperature: 42°C • Tight bond to bone after 8 weeks	[227]
	$CaO\text{-}SiO_2\text{-}FeO\text{-}Fe_2O_3\text{-}Na_2O\text{-}P_2O_5$ Iron oxide content 45% (w/w)	~ 50 mT 440 kHz	Glass-ceramic soaked in SBF for 14 days	-	• HA layer formed at the surface when soaked in SBF	[228]
	$CaO\text{-}SiO_2\text{-}FeO\text{-}Fe_2O_3$ Iron oxide content 36% (w/w)	n.r.	Glass-ceramic soaked in SBF for 7 to 30 days	-	• HA layer formed at the surface when soaked in SBF when glass-ceramic is added by Na_2O and/or P_2O_5 and/or B_2O_3	[229]
	$SiO_2\text{-}Al_2O_3\text{-}Fe_2O_3\text{-}P_2O_5\text{-}Li_2O$	50 mT 10 kHz	-	Sprague-Dawley rats for toxicology study and mice bearing subcutaneously breast carcinoma tumors	• Well tolerated • *In situ* temperature achieved: 43-44°C • 12% totally cured after 100 days and 50% significant tumor growth delay	[231]
Ferrimagnetic silicate cement	$10SiO_2\text{-}2Al_2O_3\text{-}52Fe_2O_3\text{-}0.6MgO\text{-}33CaO\text{-}(SO_3+K_2O)R$	n.r. 10 kHz	-	Wistar rat implanted within muscle for biocompatibility study	• Maximum specific heating power = 2.11W/g • Biocompatible (9 weeks)	[230]

Abbreviations: AMF: alternating magnetic field; AC: Acrylic cements; CPC: calcium phosphate cements; bis-GMA: bis-α-glycidylmethacrylate; HA: hydroxyapatite; SBF: simulated body fluid n.r.: not reported

* Magnetic field amplitude is converted to milltesla (10 Oe = 1 mT)

6. References

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