Drug Delivery Systems

Dr. Patricia Ingrid Sealy

Lecturer, School of Pharmacy,
Faculty of Medical Sciences,
The University of the West Indies,
Eric Williams Medical Sciences Complex,
Bldg 39, Champs Fleurs,
Trinidad and Tobago
Email: patricia.sealy2@sta.uwi.edu

Abstract

Fungal osteomyelitis is a rare and potentially life threatening condition. Current management involves the prolonged administration of antifungal agents and multiple surgical procedures. The development of a biodegradable drug delivery carrier to release therapeutic concentrations of antifungal agents at concentrations that inhibit microbial growth without altering osteoblast growth and functional morphology and might provide a better option to treat fungal osteomyelitis. Antibacterial agents impregnated into beta tricalcium phosphate are commercially available to releasetherapeutic concentrations of the antibioticto treat bacterial infection. Similarly, antifungal bone delivery systems may be used as efficient and safe options for treating fungal osteomyelitis in the clinical setting.

Key words: bone delivery system, drug delivery system, fungal osteomyelitis, beta tricalcium phosphate, biodegradable carrier

1. Introduction

Drug delivery system(DDSs) can be utilized for the delivery of antimicrobial agents, particularly those which are plagued with complications, such as Amphotericin B (AmB). These systems deliverdrugs effectively and achieve greater efficacy by avoiding fluctuations in plasma drug levels, while minimizing toxicity compared to conventional formulations. It is well known that AmB is beset with adverse side-effects. Therefore, it is desirable to have safer options for clinical use (Allen and Cullis, 2004). As a consequence, many researchers have manufactured alternative formulations (liposomes, lipid nanospheres, polymeric micelles, colloidal dispersions, lipid emulsions and highly pure formulations) to overcome AmB's nephrotoxic and infusion-related adverse side-effect profile. The use of DDSs are reviewed here as viable treatment alternatives.

1.1 Lipid Drug Delivery Systems

Liposomes are microscropic vesicles, composed of one or several lipid membranes surrounding discrete aqueous compartments (Ostro and Cullis, 1989). The vesicles are designed to encapsulate water-soluble substances in the aqueous compartment and water-insoluble substances within the membrane. The particulate nature of the liposome facilitates distribution in the body, quite unlike free drug. Liposomes interact with cells by adsorption, endocytosis, lipid exchange or fusion. Liposomes can adsorb to any cells under appropriate conditions, where the contents are slowly released into the extracellular fluid and some of the contents may traverse the cell membrane. In endocytosis, the cells internalize matter, a process that is efficiently performed by a limited number of cells. Cells that are adept in phagocytosis of liposomes include cells derived from bone marrow (monocytes and macrophages). Lipid exchange involves the transfer of the lipid content only from the liposome, without release of the aqueous interior. Thereafter, the lipid molecule could remain over a long period of time or be distributed into a variety of intracellular membranes. Lipid exchange could also occur with circulating proteins. Fusion involves the interaction of the outer membrane of the liposome with the cell membrane and the concomitant release of the aqueous content directly into the cytoplasm of the cell. Liposomes will circulate in the bloodstream until they are broken down by exchange of lipids with the serum components, degraded by phospholipases, engulfed by phagocytic cells, or filtered out of the blood.

Liposomal formulations affect the pharmacokinetic of drugs by increasing the volume of distribution in favor of diseased tissue, delaying metabolism and delaying clearance (Allen, 1997; Bekersky, 2002). The liposomal formulation of AmB, Ambisome®, has been commercially available since 1997. Amphotericin B is incorporated into the wall of the circulating lipid vesicle. No drug leakage into the systemic circulation occurs after infusion.

Colloidal dispersions and suspensions have also been used as vehicles for the dissolution of drugs. Amphotericin B (Amphotec™) has been formulated as a colloidal dispersion in an aqueous solution. These components form a bilayer in a disk shape that allows for the delivery of higher doses of AMB to cells, but lower drug concentration to end organs. An isotonic suspension of AmB complexed to two phospholipids was marketed as Abelcet®. The formulation allowed for the release of active AmB from the lipid complex at the specific site of action. These formulations are efficacious and safe, but do not attain comparable (conventional) efficacy, except at high doses. These lipid formulations are also taken up by the reticuloendothelial system (RES).

A novel lipid nano-sphere (LNS) drug carrier was formulated to achieve comparable activity of conventional AmB, but with lower uptake by RES (Tommi, 2002). Lipid nanosphere is an emulsion of lipoprotein-like particles (phospholipids and neutral lipids), 25 - 50 nm in size. Lipid nano-spheres are poorly distributed into the RES, remaining mainly in the blood circulation. *In vitro* and *in vivo studies of* LNS incorporated with AMB (a low dose delivery system) displayed reduced toxicity, but maintained similar efficacy against fungi as conventional AmB.

Polymeric micelles have been investigated as drug carriers by many researchers (Yu, Okano, Kataoka and Kwon, 1998; Adams and Kwon, 2004). Polymeric micelles are nanocarriers that water-solubilize hydrophobic drug molecules. Copolymers are designed with a branched, hydrophobic interior (core) and hydrophilic exterior (shell) to maintain physical properties characteristic of conventional micelles, but with enhanced thermodynamic stability. Adams and Kwon (2004) investigated the relative aggregation state of AmB during loading and reconstitution of polymeric micelles. They found that it was possible to encapsulate AmB in a relatively (non)aggregated state in micelles prepared from acyl derivatives depending on the polymer structure and assembly conditions. Transition of this technique into clinical practice can be therapeutically advantageous because a decreased aggregated form of AMB may potentially

be less toxic towards mammalian cells while retaining the therapeutic antifungal activity of AmB (increase the selectivity of AmB for fungal cells).

In spite of the availability of these formulations (actual and potential) that (may) afford safer administration and reduced toxicity of AmB, treating fungal osteomyelitis is still problematic and potentially fatal (depending on the causative yeast). The penetration of antifungal agents such as AmB, micafungin (MFG) and fluconazole (FCZ) into bone tissue is not known. Hence the delivery of antifungal agents as an impregnated BDS may be the most plausible approach to achieve reproducible and sustained concentration in bone.

1.2 Implants

Implant materials are popularly used in dentistry, orthopedics and surgery for their osteoconductive properties (Habraken, Woke and Jansen, 2007). These materials are broadly classified as ceramic or ceramic/polymer composites, and are appropriate for replacing missing bone and teeth or delivering individualized antimicrobial therapy. Ceramic composites consist of calcium phosphate (CaP) ceramics and cements, and bioactive glass. Ceramic and polymer composites consist of polylactic/polyglycolic acid-based polymer/ceramic composites, protein- and carbohydrate-based polymer/ceramic composites and other miscellaneous polymer/ceramic composites. Ceramics interact positively with human tissues, e.g. in dentistry CaP and calcium hydroxide-based materials are used for endodontic filling materials, while metal-ceramic alloys are used for crowns. In orthopedics and plastic surgery, ceramics are used to repair/replace invertebral discs and joints, and for cranial defects, respectively. These materials possess high mechanical strength, good body response and low or non-existing biodegradability. Biodegradability is enhanced by introducing porosity (or interconnectivity of pores) which facilitates bone ingrowth if pore size is sufficiently large (macroporous). The addition of growth factors (bone morphogenic proteins, transforming growth factor, basic fibroblast growth factor and vascular endothelial growth factor) accelerate tissue ingrowth because of their osteoinductive properties and vascularization. Although ceramics bind avidly to growth factors/proteins, the release pattern is not optimal. This inconsistency is overcome by adding high amounts of growth factors to guarantee a positive response. Problems may still occur because of nutritional deficiencies at the inner surface of the implant or limited cell-cell interaction. Other materials like (bio)polymers may be physically or chemically mixed with a ceramic compound.

1.3 Application of Implants in Health Care

Nicholas and Lange (1994) used tricalcium phosphate (TCP, granules) ceramic bone graft substitutes (BGSs) in 18 patients to fill cavitary defects after the removal of benign bone tumors. In this prospective case series, the treated patients were followed clinically with radiographic examination from 2 to 4 years. They found that healing depended on the size of the defect, with the larger lesions healing more slowly. Clinical parameters of healing were seen in advance of complete bone remodeling and all patients, except one, had unrestricted activity by 24 months. No adverse reactions were attributed to the graft material. Anker, Holdridge, Baird, Cohen and Damron (2005) examined the use of ultraporous beta-TCP (β-TCP) in small cavitary defects in an uncontrolled retrospective study review. The β-TCP material was used because it afforded gradual and complete incorporation over several months, similar to the smooth transition seen in animal models. In this study, 24 patients had bone grafting with the ultraporousβ-TCP mixed with local blood. Radio graphical examination revealed that resorption and trabeculation increased steadily with time and was more advanced (beyond 6 weeks) with smaller defects compared with larger defects. Grafts disappeared in small lesions by one (1) year (longer with larger lesions) and bone renewal gradually

replaced graft material. Complications associated with β-TCP were low, and the quality of life of the patients progressed to unrestricted activities of daily living and recreational activities within 3 months.

1.4 Antibacterial-impregnated Drug Delivery Systems

Credit is given to Buchholz for introducing the concept of local antibiotic impregnated (in polymethyl methacrylate [PMMA]) delivery devices (Buchholz and Engelbrecht, 1970). Antibiotics selected for use in a DDS should be active against a broad range of susceptible pathogens, locally released at concentrations exceeding (>10 times) the minimum inhibitory concentration (MIC) for the identified pathogen, not induce adverse effects or enter the systemic circulation; not be allergenicor stable at body temperature and water soluble to ensure diffusion from the carrier (Younger, Masri and Duncan, 1999; Kanellakopoulou et al., 2000). Yet, antibiotics must also be thermostable because of the high temperatures exposed at the time of polymerization of the bone cement, be available in powder form as it is not recommended for aqueous antibiotic solution to be mixed with bone cement (large volumes prevented the cement from setting). According to Buranapanitkit et al (2005) and Stallmann, Faber, Bronckers, NieuwAmerongen, and Wuisman (2006), an ideal non-biodegradable carrier would not act as a scaffold for microbes (biofilm formation), would release its contents in a concentration that equals or exceeds the MIC of the drug to prevent the formation of resistance, cause a discrete burst after implantation to eradicate the contaminating bacteria and be useful for prophylactic therapy, and would exceed the release rate profile of nonbiodegradable cements. Host tissue response to these delivery systems depends on the chemical composition, surface texture, porosity and density, shape, and the size of biodegradable material.

A number of DDS have been investigated to date for the delivery of antimicrobial therapeutic agents (Table 1). Korkusuz, Uchida, Shinto, Araki, Inque and One (1993) showed that parenteral antibiotics and surgical debridement alone or in combination with acrylic bone cements failed to eradicate experimentally-induced bacterial osteomyelitis in rats. However, a locally implanted antibiotic calcium HA (CHA) ceramic composite was successful in eradicating the infection without removal of the metal implant. This suggests the possible superiority of CHA over acrylic implant material. Goodell, Flick, Hebert and Howe (1986) investigated the use of extemporaneously prepared beads of bone cement (PMMA) for delivery of tobramycin in a patient with bilateral total hip arthroplasties. The devise was effective in delivering high local concentrations of tobramycin in two phases, but with a low potential for toxicity. Benoit et al., (1997) investigated the *invivo* (rabbits) and *in vitro* release characteristics of vancomycin from plaster of paris (POP) that was either coated or not coated with a biodegradable polylactide-co-glycolide polymer. The *in vitro* study revealed that the release of the antibiotic depended on the depth of the coat. The coating attenuated the burst effect and extended the release (variable, based on the number of polymer layers) of vancomycin over weeks. This pattern of release was confirmed with the *in vivo* study. The drug concentration exceeded vancomycin's MIC for the microbe, but did not exceed toxic serum levels. Winkler et al., (2000) studied the kinetics of elution of vancomycin and tobramycin from impregnated human and bovine bone for assessing the combined effects of bone repair and eradication. In this *in vitro* study they determined that properly processed bone was an excellent carrier for both drugs; cortical bone was less assessable (binding capacity) to the antibiotics initially, but was comparable with cancellous bone over the long term. The drugs were also released in two phases over weeks. They concluded that an antibiotic-graft compound could achieve eradication of pathogens and grafting of bone defects in a one-stage procedure. The release of gentamycin loaded cements was correlated to the physical characteristics of cements by van de Belt, Neut, Uges, Schenk, van Horn, van der Mei et al. (2000). They found that the initial release of gentamicin from an acrylic polymer depended on surface roughness and porosity, but the total amount released depended on bulk porosity. This implied that manipulating the composition of the polymer or the

method of mixing could prolong or limit the release of the antibiotic. A calcium hydroxyapatite (CaHa) antibiotic implant was evaluated for its efficacy as an antibiotic delivery system in an *in vivo* model by Shirtliff, Calhoun and Mader (2002). A rabbit model was infected with staphylococcus and treated after four weeks with or without debridement, systemic antibiotics, vancomycin impregnated PMMA beads or CaHa implants with or without vancomycin compared with the infected untreated control group. The rate of clearance of infection were comparable for the mice treated with antibiotics and cement (82% for CaHa vs. 70% PMMA), but all other therapies had less than 50% clearance. The authors implied that CaHa could be an appropriate alternative to PMMA. Stallmann, Faber, Slotema, Lyaruu, Bronckers, Amerongen and Wuisman (2003) studied the release of antimicrobial peptides (AMP) from CaP bone cements and granules as a DDS to treat resistant bone infection. Burst release was observed for the cements followed by low-level continuous release compared with the granules which had high burst release for the first 24 hours. For both materials, efficacy of AMP was not diminished upon release. It also appeared that the release of AMP from the surface of the carrier influenced burst-release and the second phase was influenced by gradual diffusion from deeper, porous (varies with carrier) layers. McLaren, McLaren, Nelson, Wassell and Olsen (2002) found that complete or partial exchanges influenced the release rates of tobramycin from calcium sulfate pellets. Complete exchanges caused a significantly faster release (> 50%) in the first 24 hours compared with the same extent of release that was observed in six (6) days. Faber, Stallmann, Lyaruu, de Blieck, Bervoets, van NieuwAmerongen et al., (2003) found that a proportional percentage of AMP incorporated into PMMA influenced the release kinetics of AMP, in addition to porosity and surface roughness. The release prolife of gentamicin from commercially available CaP biodegradable cements was compared by Stallmann et al., (2006) in an *in vitro* model. All the granular cements exhibited burst release in the first 24 hours (unlike the non-granular cements) and the release profile followed "square root of time" kinetics. Additionally, release in the first week ranged from 36 to 78% and 30 to 62 % for non-granular and granular cements, respectively. They postulated that these results may find clinical application for the prevention (burst release) and treatment (prolonged release) of osteomyelitis. Benghuzzi, Tucci, Russell, Ragab, Graves and Confetti (2006) showed that tobramycin incorporated into TCP Lysine (TCPL) was capable of releasing tobramycin in two (2) phases over 15 weeks, when implanted at the site of a complex fracture in male rats. The TCPL carrier was osteoconductive and animals showed evidence of osteoblast alkaline phosphatase activity. Liu, Tsai, Wen-NengUeng, and Chan (2005); and Liu, Chi, Lin, Ueng, Chan, and Chen (2007) fabricated (*in vitro*) poly-lactic-glycolic acid capsules as a solvent-free biodegradable delivery device impregnated with vancomycin for long-term local delivery with favorable release rates. They hope to replicate these results in an *in vivo model*.

1.5Antifungal-impregnated Drug Delivery System

The first case of locally impregnated antifungal loaded bone cement (AmB-PMMA) used to treated *C. albicans*osteomyelitis, subsequent to total hip arthroplasty was reported (decades after the technique was first used) by Marra et al., (2001). The fungal infection was cured, but the patient suffered from subsequent recurrent bacterial infections. Two other cases of antifungal loaded bone cement (FCZ-PMMA) to manage prosthetic joint infection were documented; patients were successfully cured of *Candida spp. (*Bruce, Kerry, Norman and Stockley, 2001). In 2002, Silverberg, Kodali, Dipersio, Acus and Askew, (2002) used an *in vitro* model to explore the potential of antifungal agents (AmB, FCZ and 5-Flucytosine, 5-FC) impregnated with PMMA to elute the impregnated agent in sufficient volumes to inhibit *C. parapsilosis*. Fungal growth was inhibited with AmB and FCZ, but not with 5-FC. Buranapanitkit, Oungbho and Ingviya (2005) investigated the efficacy of HA composite impregnated with AmB compared with PMMA. Hydroxyapatite showed significantly improved elution characteristics when impregnated with AmB over a 6-week period

compared with 5-weeks for PMMA.

Table 1. *In Vitro & In Vivo* Studies of Antimicrobial Impregnated Drug Delivery Systems.

Reference	BDD	Design	Elution	Results
Benghuzzi, 2006	TCPL capsules	*in vivo* release of TM from TCPL	Burst release of TM over 2 Ds, followed by continuous over 30 Ds	TCPL (+ TM) were both osteoconductive, TM delivered locally decreased the infection rate vs. sham
Stallman, 2006	CaP	*in vivo* release of GM from CaP	Burst & sustained release over 21 Ds was observed	Burst or sustained release of GM can be achieved by manipulation, based on cement or granule type
Liu, 2005	PLGA capsules	*in vitro* release of GM and VM	HPLC characterized release of GM and VM over 2-4 wk	Novel technique for manufacture of medicinal degradable capsules for long-term drug delivery
Buranapanitkit, 2005	HA-POP-CTS HA-POP-ALG PMMA	Compare AMB inhibition zone widths of BCs	NA	HA-POP-CTS = HA-POP-ALG > PMMA
Stallman, 2003	$Ca_3(PO_4)_2$ cements & granules	Compare biphasic release patterns of AMP	Burst release (from surface) followed by continuous release (gradual diffusion from deeper layers) for cements and granules	More AMP was incorporated into the cement vs. granule. High burst release on D1 for granules only. Release characteristics based on physical properties of the carrier
Faber, 2003	PMMA	*in vitro* release of AMP-Dhvar5 (120, 600 and 1200 µg) per PMMA beads	Initial high release for 600 & 1200 beads during 1stwk, followed by a lower level of continuous release	Total release was 9 µg per bead over 7 D for the 120 µg bead compared with 416 & 1091 µg respectively for 600 &1200 µg bead without loss of anti-microbial activity (MRSA)
Silverberg, 2002	PMMA	*in vitro* release of AMB, FCZ and 5-FC	Measurable zones of inhibition w/AMB and FCZ only	Zones of inhibition increased with doubling of concentration
McLaren, 2002	10% $CaSO_4$	*in vitro* release of TM, complete vs. partial exchange method	> 50% of TM released on D1 w/ complete vs. 50 % at D6 w/partial exchange method	The volume of exchange diluents determined the amt of drug released.
Shirfiff, 2001	CaHA vs PMMA	*in vivo* rabbit MRSA osteo-myelitic model, and release of VM	NA	CaHA = PMMA Treatments, began 2 weeks after infection, and lasted 4 wk

van de Belt, 2000	Acrylic BCs	Compared release of GM from bone cements	Rate of release after 1 wk varied from 4 to 5.3% for bone cements	Surface roughness, and porosity controled the kinetics of GM release form bone cements
Winkler, 2000	Human & bovine BG	*in vitro* release of TM & VM from cancellous & cortical BG into 5% human albumin media, exchanged q24h for 28 D	High initial rate of release w/logarithmic decrease over testing period. VM > TM initially. TM eluted steadily over a longer period compared to VM	The eluted ABX > MIC for the testing period. VM in bovine > human bone, NS. Cortical = Cancellous bone
Benoit,1997	POP coated with PLA-GA polymers (multiple layers)	*in vitro* release of VM from POP coated polymer in rabbit femoral condyle	Burst and continuous release of VM was evident and was > MIC for S. aureus	VM release from POP depended on coating depth – which decreased the burst effect and extended release to > 5 wk
Korkusuz, 1993	CHA ceramic composite	*in vivo* release ofGM in experimen-tally produced osteomyelitis in rats	High concentrations of GM at site of infection and bacteria were eradicated without removal of implant	Parenteral antibiotics & surgical debridement alone or in combination with acrylic bone cement failed to eradicate infection
Goodell, 1986	PMMA	*in vitro* release of TM from PMMA in multiple electrolyte solutions	High burst release on D1 followed by gradual release over 28 Ds	Extemporaneously prepared beads were effective for delivering high conc. w/low toxic effects

Key:
ALG - alginate
AMB - amphotericin B
AMP - antimicrobial peptide
BG - bone graft
$CaSO_4$ - calcium sulfate
CaHA, CHA – calcium hydroxyapapite
CaP - calcium phosphate
CTS - chitosan
D - day(s)
FCZ - fluconazole
5FC - Flucytosine
GM - gentamycin
h - hour
MRSA - Methicilin resistant*staphylo-ccoccusaerus*
NA -not applicable
NS - not significant
PMMA - polymethy-lmethacrylate
PLA-GA, PLGA - polylactic acid glycolic acid
POP- plaster of Paris
q- every
TM - tobramycin
TCPL - tricalcium phosphate lysine
VM - vancomycin
w - with
vs -versus

1.6.Conclusion

These limited stories have not encouraged the pharmaceutical industry to commercially explore the use of local delivery devices as a therapeutic option for treating fungal osteomyelitis, possibly because of the rare prevalence of such infections to date. Pharmaceutical companies generally do not expend time, effort and scarce resources to bring a drug to market, if the potential of financial gain is dim. However, current advances in medical technology are responsible for the increased human life expectancy (especially in the developed world), coupled with an increasing aged (over 65 year old) population. Of all the risk factors that may predispose to fungal infection, we can postulate that a corresponding increase in the number of total joint arthroplasties (for the aged population) may be inevitable. For these reasons, DDSs should be explored as treatment alternatives for fungal infection.

2. References

Adams, M., & Kwon, G.S. (2004). Spectroscopic investigation of the aggregation state of amphotericin B during loading, freeze-drying and reconstitution of polymeric micelles. *Journal of Pharmaceutical Science*, 7 (SI): 1-6. Retrieved from http://www.ualberta.ca/

Allen, T.M. (1997) Liposomes: Opportunities in drug delivery. *Drugs*, 54 Suppl 4, 8-14.

Anker, C.J., Holdridge, S.P., Baird, B., Cohen, H., &Damron, T.A. (2005). Ultraporous beta-tricalcium phosphate is well incorporated in small cavitary defects. *Clinical Orthopaedics and Related Research*, 434, 251-257.

Benghuzzi, H., Tucci, M., Russell, G., Ragab, A., Graves, M., &Conflitti J. (2006). Targeted sustained delivery of tobramycin at the site of a femoral osteotomy. *Biomedical Sciences Instrumentation*, 27, 531-535.

Benoit, M.A., Mousset, B., Delloye, C., Bouillet, R., & Gillard, J. (1997). Antibiotic-loaded plaster of Paris implants coated with poly lactide-co-glycolide as a controlled release delivery system from the treatment of bone infections. *International Orthopaedics*, 21, 403-408.

Bekersky, I., Fielding, R.M., Dressler, D.E., Lee, J.W., Buell, D.N., &Walsh,T.J. (2002). Pharmacokinetics, excretion, and mass balance of liposomal amphotericin B (AmBisome) and amphotericin B deoxycholate in humans. *Antimicrobial Agents and Chemotherapy*, 46, 828-833.

Bruce, A.S., Kerry, R.M., Norman, P., &Stockley, I. (2001). Fluconazole-impregnated beads in the management of fungal infection of prosthetic joints. *Journal of Bone and Joint Surgery* (Br), 83, 183-184.

Buchholz, H.W., &Engelbrecht, H. (1970). Depot effects of various antibiotics mixed with Palacos resins. *Chirurg*, 41, 511-515.

Buranapanitkit, B., Oungbho, K., &Ingviya, N. (2005). The efficacy of hydroxyapatite composite impregnated with amphotericin B. *Clinical Orthopaedics and Related Research*, 437, 236-241.

Faber, C., Stallmann, H.P., Lyaruu, D.M., de Blieck, J.M., Bervoets, T.J., van NieuwAmerongen, A., et al. (2003). Release of antimicrobial peptide Dhvar-5 from polymethylmethacrylate beads. *Journal of Antimicrobial Chemotherapy*, 51, 1359-1364.

Goodell, J.A., Flick, A.B., Hebert, J.C., & Howe, J.G. (1986). Preparation and release characteristics of tobramycin-impregnated polymethylmethacrylate beads. *American Journal of Hospital Pharmacist*, 43, 1454-1461.

Habraken, W.J., Wolke, J.G., & Jansen J.A. (2007). Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering. *Advanced Drug Delivery Reviews*, 30, 59, 234-248.

Kanellakopoulou, K., &Giamarellos-Bourboulis, E.J., (2000). Carrier systems for the local delivery of antibiotics in bone infections. *Drugs*, 59, 1223-1232.

Korkusuz, F., Uchida, A., Shinto, Y., Araki, N., Inoue, K., & Ono, K. (1993). Experimental implant-related osteomyelitis treated by antibiotic-calcium hydroxyapatite ceramic composites. *Journal of Bone and Joint Surgery*, British volume, 75, 111-114.

Liu, S.J., Tsai, Y.E., Wen-NengUeng, S., & Chan, E.C. (2005). A novel solvent-free method for the manufacture of biodegradable antibiotic-capsules for a long-term drug release using compression sintering and ultrasonic welding techniques. *Biomaterials*, 22, 4662-4669.

Liu, S.J., Chi, P.S., Lin, S.S., Ueng, S.W., Chan, E.C., & Chen, J.K. (2007). Novel solvent-free fabrication of biodegradable poly-lactic-glycolic acid (PLGA) capsules for antibiotics and rhBMP-2 delivery. *International Journal of Pharmaceutics*, 330, 45-53.

Marra, F., Robbins, G.M., Masri, B.A., Duncan, C., Wasan, K.M., Kwong, E.H., et al. (2001). Amphotericin B-loaded bone cement to treat osteomyelitis caused by *Candida albicans*. *Canadian Journal of Surgery*, 44, 383-386.

McLaren, A.C., McLaren, S.G., Nelson, C.L., Wassell, D.L., & Olsen K.M.. (2002). The effect of sampling method on the elution of tobramycin from calcium sulfate. *Clinical Orthopaedics and Related Research*, 403, 54-57.

Ostro, M.J., &Cullis, P.R. (1989). Use of liposomes as injectable-drug delivery systems. *American Journal of Hospital Pharmacists*, 46, 1576-1587.

Shirtliff, M.E., Calhoun, J.H., &Mader, J.T. (2002). Experimental osteomyelitis treatment with antibiotic-impregnated hydroxyapatite. *Clinical Orthopaedics and Related Research*, 401, 239-47.

Silverberg, D., Kodali, P., Dipersio, J., Acus, R., & Askew, M. (2002). In vitro analysis of antifungal impregnated polymethylmethacrylate bone cement. *Clinical Orthopaedics and Related Research*, 403, 228-231.

Stallmann, H.P., Faber, C., Slotema, E.T., Lyaruu, D.M., Bronckers, A.L., Amerongen, A.V., et al., (2003). Continuous-release or burst-release of the antimicrobial peptide human lactoferrin 1-11 (hLF1-11) from calcium phosphate bone substitutes. *Journal of Antimicrobial Chemotherapy*, 52, 853-855.

Stallmann, H.P., Faber, C., Bronckers, A.L., Nieuw Amerongen, A.V., &Wuisman P.I. (2006) In vitro gentamicin release from commercially available calcium-phosphate bone substitutes influence of carrier type on duration of the release profile. BMC Musculoskeletal Disordisorders. Retrieved from http://www.biomedcentral.com/content/pdf/1471-2474-7-18.pdf.

Tomii, Y. (2002). Lipid Formulation as a drug carrier for drug delivery. *Current Pharmaceutical Design*, 8, 467-474.

van de Belt, H., Neut, D., Uges, D.R., Schenk, W., van Horn, J.R., van der Mei, H.C., et al. (2000). Surface roughness, porosity and wettability of gentamicin-loaded bone cements and their antibiotic release. *Biomaterials*, 21, 1981-1987.

Winkler, H., Janata, O., Berger, C., Wein, W., &Georgopoulos, A. (2000). In vitro release of vancomyicn and tobramycin from impregnated human ad bovine bone graft. *Journal of Antimicrobial Chemotherapy*, 46, 423-428.

Yu, B.G., Okano, T., Kataoka, K., & Kwon, G. (1998) Polymeric micelles for drug delivery: solubilization and haemolytic activity of amphotericin B. *Journal of Control Release*, 53, 131-136.

Younger, A.E., Masri, B.A., & Duncan, C.P. (1999). *Infected total hip replacement*. In E. V. Craig (Ed). Clinical orthopedics. Maryland: Lippincott Williams and Wilkins.