INTRODUCTION

The aim of this thesis was to develop and characterize local drug delivery formulations composed of calcium phosphate bone cement, modified with biodegradable microspheres for the treatment and prevention of bone infections. The opening chapter gives a general overview regarding bone infections and common treatment strategies to give a general overview. Some facts on bone morphology and histology will be introduced for comperison. The idea to use local drug delivery systems consisting of biodegradable polymers and calcium phosphate based cement matrices for the treatment of bone diseases, will be briefly discussed. In Chapter 2, microencapsulation of antibiotics using different technologies will be explained in-depth, especially the spray drying method, which was the focus of this thesis.

BONE INFECTIONS

In spite of progress in surgical techniques and antibiotic prophylaxis, postoperative infections still account for many surgical complications, frequently caused by nosocomial infections, resulting in an increase in duration and cost of hospitalization [1]. Bone infections are playing the main role and were already classified as osteomyelitis by Waldvogel et al. 1970 [2]. Osteomyelitis describes an infection of bone marrow, cancellous and compact bone [3] and can be classified into different subtypes, like acute haematogenous, posttraumatic or chronic osteomyelitis [4]. Primary infection processes are starting after bacteria inoculation, such as with *S. aureus* at the surgery site, reaches the medullar cavity and encroach compacta and spongiosa. Thereby increasesing intra osseous pressure, leads to pain and destruction of blood vessels (*Figure 1*). The Volkmann channels, responsible for guiding blood vessels, are used contribute to spreading of microorganism [1]. Necrosis is the consequence, and the bone dies due to disruption of blood transport and increase of pressure inside of the bone. High

local doses of different antibiotic drugs at the infection side, may eradicate the pathogen and thereby the infection [5].

THERAPY STRATEGIES TO TREAT BONE INFECTIONS

The objectives for treatment of osteomyelitis are to eliminate the infection and to prevent the development of chronic infections. Intravenous applications of antibiotics have to begin early during the infection process. Surgical removal of necrotic bone tissue is usually necessary. The open space left by the removed bone tissue may be filled with bone graft to promote the growth of new bone tissue [6]. Antibiotic therapy continued for at least four to six weeks is essential, which causes the risk of side effects and requires a prolonged hospital stay of the patient [7]. Apart from these aspects, the resection of infected material is as important as adequate blood circulation to promote the healing process. Additional to these strategies, local antibiotic therapy could be useful to increase the concentration of antimicrobial agents at the infection site and decrease systemic toxicity. Advantages and disadvantages of different strategies to cure bone infections are given in *Table 1*.

BONE MORPHOLOGY

Bone is one of the most differentiated tissues and hardest substances of the human body. The supporting function and locomotion of the human, the skeleton deals with the mineral balance of the body, and is controlled by hormones [8]. The stability of the skeleton, against pressure, tension, bending, and torsion, is based upon inclusion of inorganic components in the organic intercellular matrix. Mainly component is hydroxyapatite with 60 to 70 %, but also fluoroapatite, carbonated apatite, calcium carbonate and magnesium carbonate can be found in the mineral phase of bones [9].

Strategy	Advantage	Disadvantage
• Systemic application of antibiotics	• successful eradication of bacteria	• high side effects • expensive hospital stay • low antibiotic concentration leads to resistances
• Surgical removal of dead bone and infected tissue	• successful method • no antibiotic application, no toxic side effects	• additional surgery • addition infection risk • increasing costs
• Local antibiotic delivery	• low systemic side effects • application in first surgery feasible • shorter hospital stay • lower costs	• low antibiotic concentration leads to resistances

Table 1: Advantages and disadvantages for common strategies to treat bone infections

Hence, hydroxyapatite is often used as a bone filling material and takes a leading part in this study as matrix for new locally active drug delivery systems. Hydroxyapatite is arranged by side the collagen fibrills every 25 to 30 nm and is stabilized throughout the surrounding matrix. Thus high compressive strength and elasticity are achieved. For the admission of traction forces collagen fibrils are used. Human bones can be subdivided into two main types,

1) Cortical bones, which have a high stiffness due to the tubular constitution and a low porosity of 5 to 10 % and
2) Trabecular bone, which do have a high porosity of up to 60 % and a sponge like structure, therefore a low stiffness.

An advantage of this special bone type is the high metabolic turnover and a high concentration of bone cells [9]. For this reason, we have chosen this bone type to test biocompatibility of the composites (*Chapter 5*).

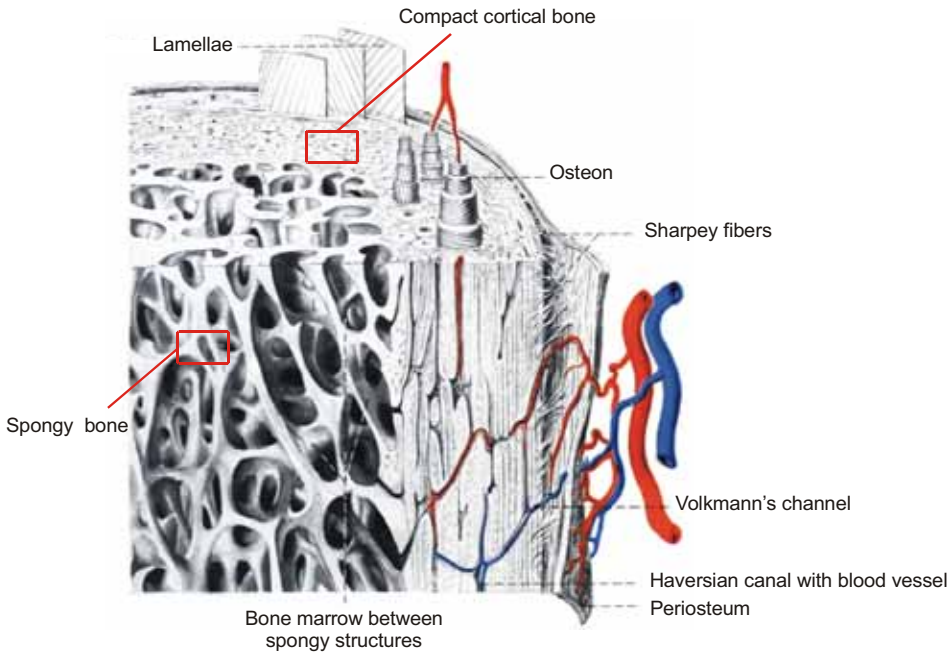

Figure 1: Schematic drawing of the construction of compact and spongy (cancellous) bone (Adapted from [8])

Different cell types can be identified in the human bone, mainly osteocytes, like osteoblasts and osteoclasts. Osteoblasts are responsible for the osteogenesis and are locally generated by differentiating connective tissues. Osteoblasts are secreting collagen, and glyco-protein, while forming new bone matrix. This so produced substance, called osteoid, will be mineralized with the help of osteoblasts to form osteocytes. These specific cells reside in caves, named lacunae. These caves are connected by channels, called canaliculi. Another drawback of bone infections is the possibility to use these canaliculi to spread the infection to other bone areas. Different osteocytes are connected via gap junctions for intercellular communication, like it is known from nerve cells. Bone remodelling is one function, which can be lead by the osteocytes. Therefore, these cells are stimulated mechanically, e.g. by walking or jumping, which can also be called mechanical loading.

For research experiments the loading can be used to incubate bone tissue *ex-vivo* and to observe the performance of bone [10].

The remodelling of the human bone matrix is a physiological process, where the degradation of old bone and the building of new bone matrix is in balance [11]. Different markers, like alkaline phosphatase, osteocalcin or inflammatory mediators can be used for identifying bone degradation or assembling.

CALCIUM PHOSPHATE-BASED BONE SUBSTITUTION

Bone defects were often filled with calcium phosphate based bone substitutes. A first paper about bone substitution was published in 1892, and focused on the use of plaster of Paris for the filling of bone defects [12]. After eradication of dead bone and infected tissue it is necessary to refill the defect to prevent ingrowth of connective tissue, and to reconstruct the functionality. Golden standard for this procedure remains autologous bone transplantation with little side effects, as be seen with allogenic bone material [13]. Synthetic materials were classified, regarding their application load-bearing and non-load-bearing materials implantation sites. For load-bearing defects often metals, such as titanium or cobalt-chromium alloy are used and fixed with bone cements based on polymethylmethacrylates (PMMA) [14]. We will concentrate on calcium phosphate materials, which are used for defect filling in the non-load bearing bones [15]. In this application, materials like collagen [16], biodegradable polymers [17], and various calcium phosphate ceramics were used [13]. Generally, two main reaction products according to the pH of the cement paste can be distinguished. On one hand hydroxyapatite, if the pH is higher then 4.2 and brushite cements for pH lower then 4.2 on the other hand. Due to their excellent biocompatibility, synthetic calcium phosphates have gained acceptance for various dental or medical applications, which include fillers for periodontal defects, maxillofacial reconstruction or spine fusion [13].

Brand name	Company	Cement type	Ref.
BoneSource	Stryker-Leibinger	HA	[18]
Calcibon®	Biomet Merck	HA	[19; 20]
Cerasorb®	Curasan	β-Tricalciumbisortho-phosphate	[21; 22]
Collapat ®II	Biomet Merck	synthetic hydroxyapatite 0,7 g, native collagen Type I.	
Endobon®	Biomet Merck	Pentacalcium-hydroxid-(tris)-phosphate	[23]

Table 2: Products on the market of calcium phosphate bone substitutes for defect filling

Table 2 gives a short overview of some commercially available bonesubstitute products. Synthetic bone substitutes, such a β-tricalcium phosphate (β-TCP) and hydroxyapatite (HA) are commonly used as blocks, cements, pastes, powders or granules. In this thesis, calcium phosphate cement (CPC) was used. It combines the advantage of being freely mouldable and adaptable to bone defects with the excellent biocompatibility of calcium phosphate compounds. Additionally, the cement has the benefit of low-temperature setting compatible with organic molecules and living cells [24]. Brown et al. were the first o demonstrate the formation of an apatitic cement consisting of a mixture of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) [25]. According to the literature, CPCs are obtained by mixing one or several reactive calcium phosphate powders with an aqueous solution to form a paste that hardens within a defined period of time [26]. The strength of CPC is highly dependent on their porosity; thus, reducing the amount of water used in mixing the paste can increase strength. This effect will be determined in detail in *Chapter 4* [24].

With modification of CPCs, the potential use increased recently. Different substances, such bone morphogenetic proteins [27], antibiotics or polyacrylic acid were added to improve perfomrance [28]. For the treatment of bone infections antibiotics were added as solution, but

this affected te mechanical strength of bone tissue due to disturbance of the nanostructure of calcium phosphate molecules [29]. To avoid this problem, microencapsulation of antibiotic drugs in an inert matrix polymer was investigated via spray drying in this thesis, with the aim to modify CPC without loss of mechanical strenth.

LOCAL ANTIBIOTIC DRUG DELIVERY FOR BONE INFECTIONS

Local antibiotic delivery system has become an accepted treatment for a variety of reasons. The primary reason for using these local delivery vehicles is the ability to achieve very high local concentrations of antibiotics without associated systemic toxicity [30]. Because bone regeneration often is required as a part of the treatment plan, a recent trend is to provide a framework of osteoinductive and osteoconductive materials together with antibiotic delivery [31]. The idea of using antimicrobial agents for the local treatment of infections is not new. In 1979 Wahlig et al. published studies in dogs with Septopal® chains consisting of polymethylmethacrylate (PMMA) loaded with gentamicin for the treatment of osteomyelitis [32]. By implanting Septopal® chains at the infection site, high local gentamicin concentrations were reached, and systemic side effects were avoided. Now 30 years later, Septopal® chains are still the golden standard for the prevention and treatment of bone infections, although some problems were noted, like resistances because of unreleased antibiotic drugs [33; 34], a second surgery because of no biodegradation, applying necrosis due to high exothermal setting reaction and at last toxic side effects resulting from released monomers [35]. In addition to gentamicin, which was used very often in the past [36-38; 28], also cefazolin [39], vancomycin [40], teicoplanin [41] and tetracycline [42] were applied via the local administration route.

Author	Year	Antibiotic drug	Application from	Ref.
Armstrong	2002	Gentamicin Flucloxacillin Vancomycin	Palacos®	[43]
Bohner	2000	Gentamicin	CaP cement + poly(acrylic acid)	[28]
Burd	2001	Tobramycin	Polycaprolactone beads	[44]
Castro	2003	Ciprofloxacin	HA cement + TCP	[45]
Conceicao	2000	Gentamicin+ Norfloxacin/ Ciprofloxacin	HA – Anionic collagen composites	[46]
Fernandez	2002	Vancomycin	Acrylic-phosphate glasses	[47]
Hendriks	2003	Gentamicin	Acrylic bone cement	[48]
Joosten	2004	Gentamicin	HA cement	[49]
Lucke	2003	Gentamicin	Titanium coated with PDLLA	[50]
Ratier	2004	Tetracycline	Cement (Cementek®)	[42]
Sanchez	2001/ 2	Gentamicin	HA cement + DL-PLA	[51; 52]
Soriano	2000	Gentamicin	CaP + DL-PLA	[53]
Wahlig	1979	Gentamicin	PMMA chains	[32]

Table 3: Local antibiotic delivery for the treatment of osteomyelitis from the aspect of different cement matrices

Table 3 gives an overview of research efforts for the treatment of osteomyelitis with local antibiotic delivery systems. In addition, *Table 4* shows products on the market of combinations between bone cements and antibiotics. To prevent the above mentioned side effects of PMMA, also hydroxyapatite cements can be used for the delivery of drugs to the bone [45]. The advantages are the biocompatibility, due to its analogy to the hydroxyapatite of the human bone, the ease of adhesion of bone cells on the surface [54] and its injectability through a commonly used syringe [55].

Brand name	Company	Cement	Drug
CMW ® bone cement with gentamicin	DePuy Orthopädie (Johnson & Johnson)	PMMA	Gentamicin sulfate
Copal®	Biomet Merck	PMMA	Gentamicin sulfate, Clindamycin-HCl
Palamed®	Biomet Merck	PMMA	Gentamicin sulfate
Refobacin®Palacos ®R	Biomet Merck	PMMA	Gentamicin sulfate
Septopal ® Chains	Biomet Merck	PMMA	Gentamicin sulfate
SmartSet® GHV bone cement	DePuy Orthopädie (Johnson & Johnson)	PMMA	Gentamicin sulfate

Table 4: Products on the market of antibiotic modified bone cements

The idea to investigate a new local drug delivery system, based on a blend of biodegradable microspheres and calcium phosphate bone cement, was driven by different advantages, we hoped to combine in such a composite material. First of all, antibiotic delivery can be controlled, by using biodegradable microspheres and additionally the formation of resistances against antibiotic drugs can be prevented. Using a biodegradable polymer matrix, which degrades during a defined period, can be achieved by the choice of a suitable polymer. Additionally, a second surgery to remove non-degradable polymer matrices, like PMMA chains, can be avoided. Thus, hospitalization of the patient can be reduced and therapy costs can be decreased. The microencapsulation of the antibiotic drug in PLGA microspheres, prevent an interaction of the drug with the cement setting reaction, as it will be demonstrated in *chapter 3*. Calcium phosphate cement was used in this thesis, due to its biocompatibility, injectability and ease of handling.