Antibiotic-loaded poly(trimethylene carbonate) degradation, release and staphylococcal biofilm inhibition

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Chapter I

General introduction

Background

As a human being, one of our greatest challenges is to achieve a harmonious relationship with other forms of life around us. Reading this, many will - rightfully - linger over important interactions with loved ones and other influential people, maybe even think about the food we eat each day and our role in the conservation of nature. But unbeknown to our awareness, there is another battle fought each day of our lives. Dedicated to maintain a delicate balance with the smallest of life forms living in and around our bodies, we deal with microorganisms every day.

Because of natural selection in the human race, the construction of sewers and waterworks, and knowledge about infections and treatment, we are far better in dealing with infections than our far ancestors. Nowadays, most of the microorganisms we own or encounter cause benefit or simply do us no harm. Still, others continuously attack us by trying to harvest nutrients and colonise space available in our bodies. To do so these infection causing microorganisms, or pathogens, will however need to bypass our many defences. Our primary, physical barrier is only breached once a pathogen penetrates our skin or body orifice mucosa. Immediately, our innate and adaptive immune systems take over. These guardians generally deal with any intruder, but nevertheless sometimes fail.

In the case of infection localised to bone (osteomyelitis), the pathogen reaches its destination directly during trauma or surgery, or by transport through body fluid or cells. Additional pathogen and patient specific factors associated with osteomyelitis are shown in Table 1. Importantly, surgical implants are frequently colonised during placement

[Knobben et al. 2006], despite the use of advanced methods promoting sterility [Hooper et al. 2011]. Once located on and in bone, pathogens can be particularly tenacious, especially when the infection progresses to chronic disease.

In the case of a patient suffering from osteomyelitis and natural defences failing, the final defence usually employed at this time is medical treatment. Prior to the discovery of penicillin, osteomyelitis was characterised by high mortality rates (approximately 33%) due to the occurrence of sepsis [uptodate.com]. Owing to antibiotic therapy, mortality is nowadays low, but considerable morbidity is still unfortunately common. Both the pathogenesis of chronic osteomyelitis and shortcomings in therapy cause patients suffering from osteomyelitis harm, because when infection is localised within the confined space of bone, the inflammatory process compresses vascular channels, leading to poor blood supply, consequent bone necrosis and local shortage of administered antibiotics. Several strains such as *S. aureus* and *S. epidermidis* frequently form organised communities attached to necrotised bone or foreign materials, a so-called biofilm. In these communities, the bacteria are further protected from host defences and antibiotic therapy [Costerton et al. 2003]. Additionally, *S. aureus* can evade neutralisation by residing inside human cells [Baumert et al. 2002]. Finally, increasingly prevalent antibiotic resistance poses a new difficulty in osteomyelitis.

Therefore, chronic osteomyelitis is treated by surgical decompression and removal of dead tissue, followed by systemic and/or local application of antibiotics. With local application, an antibiotic delivery device is employed to ascertain high local antibiotic concentrations [Walenkamp et al. 1986]. Historically, such devices are made out of

polymethylmethacrylate (PMMA; bone cement), but more recent research is primarily focussed on biodegradable alternatives.

Table 1: Classification, location, patient specifics, and microorganisms (ranked according to prevalence) commonly associated with osteomyelitis. Partly adapted from Lew & Waldvogel [Lew & Waldvogel 2004].

Classification & Location	**Patient specifics**	**Microorganisms**
Osteomyelitis All	Immunosuppression (e.g. drug-induced, malignancy, age, malnutrition, diabetes, post splenectomy, AIDS), Nearby infection, Foreign body	1. *Staphylococcus aureus* 2. *Staphylococcus epidermidis* 3. Streptococci 4. Gram-negative aerobic bacilli
Post-traumatic Long bones	All ages	1. *Staphylococcus aureus* 2. Gram-negative aerobic bacilli 3. Anaerobes
Implant-related Lower-, upper extremities, Sternum, Pelvis, Jaw	Prosthesis population (predominantly elderly)	1. *Staphylococcus epidermidis* 2. *Staphylococcus aureus* 3. Streptococci 4. Gram-negative aerobic bacilli
Diabetic Lower extremities	Diabetic population (predominantly elderly)	1. *Staphylococcus aureus* 2. Streptococci 3. Enterococci 4. *Staphylococcus epidermidis* 5. Gram-negative aerobic bacilli 6. Anaerobes
Haemato-genous Vertebrae, Growth plates (long bones)	Elderly, Youth, Sickle cell population	1. *Staphylococcus aureus* 2. Gram-negative aerobic bacilli 3. Streptococci 4. *Mycobacterium tuberculosis* 5. Salmonella spp.

Aim of the thesis

The aim of this thesis is to explore the possibilities of poly(trimethylene carbonate) (PTMC) - used as a biodegradable antibiotic delivery system – in the possible treatment of osteomyelitis. Specifically, the studies included in this thesis are *in vitro* studies focusing on: 1) release kinetics of various antibiotics from PTMC, 2) comparing antibiotic release and concurrent biofilm inhibition of PTMC with that of clinically used non-degradable bone cement (PMMA), and 3) evaluating new antibiotic regimens for incorporation in PTMC to target treatment recalcitrant osteomyelitis.