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

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Chapter VI

General discussion

Introduction

In the treatment of chronic osteomyelitis, the use of a carrier is currently the only feasible way to achieve high amounts of antibiotics for an extended period at the infection site. The ideal antibiotic carrier for the treatment of osteomyelitis is characterised by good biocompatibility, controllable degradation kinetics, the ability to incorporate and release any antibiotic desired for treatment for an extended, but well-defined period in sufficiently high amounts, preferably by a constant drug release profile over time.

The clinically most used antibiotic carrier is non-degradable polymethylmethacrylate (PMMA), which is capable of curing or supressing chronic osteomyelitis in many cases, but does not degrade. It therefore has to be removed in order to prevent its colonisation by the target pathogens. Leaving a carrier like PMMA in place is especially dangerous due to the remaining sub-therapeutic levels of release, which possibly potentiate antibiotic resistance among possible colonizing microorganisms. In addition, PMMA exhibits sub-optimal release characteristics for large molecular size antibiotics (e.g. vancomycin), while not being compatible with others (e.g. tetracycline, rifampicin).

Biodegradable carriers can release incorporated antibiotics either by diffusion, degradation, or a combination of the two (chapter II). Only when degradation is the major mechanism involved, high initial release can be tempered in favour of effective long-term antibiotic release. In commercially available degradable carriers however (e.g. collagen fleeces, calcium sulphates and phosphates), release is determined primarily by

diffusion. Moreover, collagen fleeces and calcium sulphates absorb large amounts of water, stimulating seroma formation and increasing the risk of secondary infection. Among synthetic polylactic/polyglycolic acid carriers, especially the co-polymer polylactic-co-glycolic acid (PLGA) shows good release profiles primarily based on bulk erosion, but these carriers are known to produce acidic degradation products that can lead to bone resorption.

In the quest for degradable alternatives, we have therefore focussed on poly(trimethylene carbonate) (PTMC). PTMC shows excellent biocompatibility and degrades through surface erosion [Zhang et al. 2006b], which theoretically yields constant antibiotic release over time. Accordingly, this thesis comprises *in vitro* research into antibiotic loaded PTMC degradation, release and staphylococcal biofilm inhibition for the treatment of chronic osteomyelitis.

Degradation and release profile

Using a lipase solution, *in vivo* surface erosion of PTMC was modelled to study the degradation and antibiotic release from PTMC. We have shown that, as well as the molecular weight of PTMC, the antibiotic (combination) incorporated in PTMC determines both the degradation speed of PTMC (chapter V) and the mechanism and kinetics of release (chapter III). Additionally, the geometrical shape of the PTMC carrier further determines the way in which the release profile can be modulated into an actual day-to-day release. Therefore, a high molecular weight PTMC carrier loaded with large antibiotic particles and moulded into a slab will probably provide a longer lasting constant release compared to the discs equipped in this thesis.

In the literature, it has been suggested that PTMC degrades by surface erosion *in vivo* via macrophage-mediated enzymatic (cholesterol esterase) and/or oxidative degradation as a result of frustrated phagocytosis [Chapanian et al. 2009; Bat et al. 2009]. Recent research however, demonstrated that only enzymatic interaction on the surface appears responsible for the more rapid degradation of high molecular weight PTMC [Vyner et al. 2014]. Knowledge of the mechanism responsible for degradation - and ultimately antibiotic release - is important, since this thesis was built upon the assumption that lipase is an adequate agent *for in vitro* degradation modelling. Although in hindsight, it might have been more preferable if we would have used cholesterol esterase in this thesis, the exact mechanism of degradation in humans remains unclear, as macrophages secrete over a hundred different agents that can modulate the

inflammatory response to biomaterials and influence their degradation [Laskin & Pendino 1995]. It is impossible to fully predict the degradation of PTMC without application of an *in vivo* osteomyelitis model, because the presence of infection will have major implications on macrophage availability and expression of enzymes responsible for the degradation of PTMC.

Clinical perspective

For the sake of animal well-being, extensive *in vitro* testing is required prior to initiating animal experiments. However, each method for *in vitro* research has its own disadvantages. Methods used to test biofilm response vary along the chapters to provide new insights. Crystal violet staining of an entire biofilm yields data that are completely different in nature than data derived from metabolic testing (MTT assay), colony forming unit counting or confocal imaging, but taken together may provide an adequate justification for doing animal experiments.

Obviously, eventually *in vivo* testing has to be executed to fully establish the usefulness of PTMC loaded with antibiotics for the treatment of osteomyelitis. Although our results are very promising, it will be interesting to see whether macrophage mediated degradation of PTMC has an influence on the severity and treatment of infection, or if the infection influences PTMC degradation and thereby antibiotic release. Many challenges have to be overcome. For instance, sterilisation for *in vivo* use by gamma irradiation influences PTMC molecular weight [Foks et al. 2005] and may therefore interfere with favourable high molecular weight associated release characteristics.

Furthermore, new antibiotics, other antimicrobial agents or combinations of these must be examined, although the antibiotics we have tested are amongst the most clinically desired agents available [Rao & Ziran 2011]. Regimens containing rifampicin are especially potent against staphylococcal biofilms as this antibiotic also targets sessile pathogens [Rao & Ziran 2011], including when methicillin-resistant species are involved

[Perlroth et al. 2008], and have been tested in PTMC (chapter V). For osteomyelitis due to methicillin-resistant *Staphylococcus aureus*, vancomycin is still the preferential antibiotic, and is successfully released by PTMC (chapters III and V). Although minimum inhibitory concentrations to vancomycin among *S. aureus* strains have increased over the past decade, aiming for slightly higher concentrations usually suffices in treatment. New agents suitable for methicillin-resistant *S. aureus*, but not yet tested in PTMC, are linezolid (although bacteriostatic rather than bactericidal) and daptomycin [Fraimow 2009].

Within the coming decades, antibiotic resistance will increase the need for alternative carriers like PTMC. Clinical situations will prompt researchers to focus on new treatment modalities, favouring locally acting biodegradable carriers. Versatile, user- and patient-friendly products will need to be available at low cost. It will be a laborious but highly enticing process to find out whether PTMC will meet all these demands and prevail as more than the very promising carrier it currently is.