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

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Supplements

Summary

Bone infections are characterised by progressive inflammatory destruction of the bone, rapidly progressing to chronic osteomyelitis. For proper treatment, it is generally necessary to combine surgical intervention with local administration of antibiotics. This provides the rationale behind the aim of this thesis (chapter I): to explore the possibilities of poly(trimethylene carbonate) (PTMC) - used as a biodegradable antibiotic delivery system – in the possible treatment of osteomyelitis.

Many different local delivery devices exist, either made of degradable or non-degradable materials (chapter II). Non-degradable delivery devices are frequently constituted by polymethylmethacrylate (PMMA) based carriers. Drawbacks are the need to remove the carrier (as the carrier itself may provide a substratum for bacterial colonisation), inefficient release kinetics and incompatibility with certain antibiotics. These drawbacks have led to the quest for degradable alternatives, but also devices made of biodegradable calcium sulphate, collagen sponges, calcium phosphate or polylactic acids have their specific disadvantages. Antibiotic treatment of osteomyelitis with the current degradable and non-degradable delivery devices is effective in the majority of cases. Degradable carriers have an advantage over non-degradable carriers that they do not require surgical removal. Synthetic PTMC may be preferred in the future over currently approved lactic/glycolic acids, because it does not yield acidic degradation products.

Experimental studies included in this thesis started by focusing on release kinetics of gentamicin and vancomycin from PTMC (chapter III).

Degradation behaviour and corresponding release profiles of gentamicin and vancomycin from slowly degrading $PTMC_{168}$ and faster degrading $PTMC_{339}$ discs were compared in the absence and presence of a lipase solution. Gentamicin release in the absence of lipase was diffusion-controlled, while vancomycin release was limited. Surface erosion of PTMC only occurred in the presence of lipase. Both antibiotics were released in high concentrations from PTMC in the presence of lipase through a combination of surface erosion and diffusion. This illustrates a major advantage of surface-eroding biodegradable polymers, allowing release of larger antibiotic molecules like vancomycin.

Additionally, antibiotic release and concurrent biofilm inhibition of PTMC with that of clinically used non-degradable bone cement (PMMA) was investigated (chapter IV). This study addressed two separate attributes of PTMC: (1) the release kinetics of gentamicin-loaded PTMC and (2) its behaviour in inhibiting biofilm formation. Both of these characteristics were compared with those of commercially available gentamicin-loaded PMMA beads, which are commonly used in the local treatment of osteomyelitis. In a lipase solution that mimics the *in vivo* situation, PTMC discs with gentamicin incorporated were degraded by surface erosion and released 60% of the gentamicin incorporated within 14 days. This is similar to the gentamicin release from clinically used PMMA beads. Moreover, biofilm formation by *Staphylococcus aureus* was inhibited by approximately 80% over at least 14 days in the presence of gentamicin-loaded PTMC discs. This is similar to the effect of gentamicin-loaded PMMA beads. In the absence of the lipase, surface erosion of PTMC discs did not occur and gentamicin release and biofilm inhibition were limited. Since gentamicin-loaded PTMC discs show antibiotic release characteristics and biofilm inhibition characteristics similar to those of gentamicin-loaded PMMA beads, PTMC

appears to be a promising biodegradable carrier in the local treatment of osteomyelitis.

Finally, new antibiotic regimens for incorporation in PTMC to target treatment recalcitrant osteomyelitis were explored (chapter V). In patients suffering from osteomyelitis, standard antibiotic therapy is of little value when methicillin-resistant *S. aureus* (MRSA), *Staphylococcus epidermidis* (MRSE), or small-colony variants (SCV) are present. Far better results could be obtained by local drug delivery of antibiotic combinations including rifampicin, using a suitable carrier. We therefore investigated *in vitro* biofilm inhibition of MRSA, MRSE, and *S. aureus* SCV strains in the course of 24, 72 and 168 h treatment by biodegradable PTMC, either unloaded, gentamicin-loaded, loaded with rifampicin and fosfomycin or rifampicin and vancomycin. Biofilm colony forming units and metabolic activity measurement (MTT assay) demonstrated statistically significant inhibition for all strains when PTMC loaded with rifampicin and vancomycin was employed, especially after 168 h treatment. Confocal laser scanning microscopy images showed similar qualitative results. PTMC loaded with only gentamicin did not show any inhibition. This exemplifies that PTMC loaded with rifampicin and vancomycin holds promise for the treatment of recalcitrant osteomyelitis.

In the quest for a degradable alternative to PMMA, this thesis comprises *in vitro* research into antibiotic loaded PTMC degradation, release and staphylococcal biofilm inhibition for the treatment of chronic osteomyelitis. The thesis was concluded by reflecting on its content from an overall perspective (chapter VI). It was demonstrated that the molecular weight of the polymer, the antibiotic (combination) incorporated and the geometrical shape are critical to the antibiotic release rate from PTMC. Our results were

built upon the assumption that lipase is an adequate agent for *in vitro* degradation modelling, but the exact mechanism of *in vivo* PTMC degradation and antibiotic release in osteomyelitis remains unclear, and needs to be further investigated. Different molecular weight PTMC and methods used to test biofilm response have been used along the chapters, each providing insights from a different perspective. The antibiotics we have tested are amongst the most clinically desired agents available, and regimens containing rifampicin are especially potent against staphylococcal biofilms. New antibiotics, other antimicrobial agents or combinations of these will be examined in the future. Prompted by clinical situations such as emerging antibiotic resistance, it will be a laborious but enticing quest to determine whether PTMC is more than the very promising carrier it currently is.

Samenvatting

Botinfecties kenmerken zich door een progressieve inflammatoire vernietiging van botweefsel, die al snel leidt tot chronische osteomyelitis. Voor een adequate behandeling is het in het algemeen noodzakelijk om chirurgisch ingrijpen te combineren met de lokale toediening van antibiotica. Hieruit voortvloeiend is het doel van dit proefschrift (hoofdstuk I): Het in kaart brengen van de potentie van poly(trimethyleen carbonaat) (PTMC) - gebruikt als biologisch afbreekbaar antibioticum-afgiftesysteem - in de mogelijke behandeling van osteomyelitis.

Er zijn verschillende lokaal werkende antibioticum-afgiftesystemen, gemaakt van afbreekbaar of niet-afbreekbaar materiaal (hoofdstuk II). Niet-afbreekbare antibioticum-afgiftesystemen bestaan veelal uit polymethylmethacrylaat (PMMA). Nadelen hiervan zijn de noodzaak dit polymeer weer te verwijderen (omdat dit materiaal zelf een substraat voor bacteriële kolonisatie kan vormen), een inefficiënt afgifteprofiel en de onverenigbaarheid met bepaalde antibiotica. Deze nadelen hebben geleid tot een zoektocht naar alternatieve, afbreekbare afgiftesystemen. Gemaakt van biologisch afbreekbaar calciumsulfaat, collageen, calciumfosfaat of polymelkzuur, bevatten deze antibiotica-afgiftesystemen als materiaal echter ook specifieke nadelen. Antibiotische behandeling van osteomyelitis is - met de huidige afbreekbare en niet-afbreekbare afgiftesystemen - in de meeste gevallen effectief. Afbreekbare afgiftesystemen hebben als voordeel ten opzichte van niet-afbreekbare afgiftesystemen dat ze geen chirurgische verwijdering vereisen. Synthetisch PTMC kan in de toekomst de voorkeur

hebben boven het momenteel aanbevolen melkzuur/glycolzuur, omdat het eerste niet resulteert in zure afbraakproducten.

Dit proefschrift bevat experimentele studies welke zich eerst toespitsen op het afgifteprofiel van gentamicine en vancomycine uit PTMC (hoofdstuk III). In de af- en aanwezigheid van een lipase-oplossing werden afbraakkinetiek en overeenkomstige afgifteprofielen van gentamicine en vancomycine vergeleken vanuit langzaam degradeerbare $PTMC_{168}$ schijfjes en snel degradeerbare $PTMC_{339}$ schijfjes. In afwezigheid van lipase was gentamicine-afgifte afhankelijk van diffusie, terwijl vancomycine-afgifte gelimiteerd bleek. Alleen in de aanwezigheid van lipase deed zich oppervlakte-erosie van PTMC voor. Hierbij gaf PTMC antibiotica in hoge concentraties af door een combinatie van oppervlakte-erosie en diffusie. Hiermee wordt een belangrijk voordeel van oppervlakte-eroderende biologisch afbreekbare polymeren aangetoond, omdat het de afgifte van grote moleculen zoals vancomycine mogelijk maakt.

De antibiotica afgifte en de daaruit voortvloeiende biofilm-inhibitie vanuit PTMC en het klinisch gebruikelijke niet-afbreekbare botcement (PMMA) werden vervolgens onderzocht (hoofdstuk IV). Het onderzoek richtte zich op twee kenmerken van PTMC: (1) het afgifteprofiel van PTMC geladen met gentamicine en (2) diens waarde in de inhibitie van biofilmvorming. Deze kenmerken werden vergeleken met die van commercieel verkrijgbare PMMA kralen geladen met gentamicine, welke vaak worden toegepast bij de lokale behandeling van osteomyelitis. In een lipase oplossing die de *in vivo* situatie nabootst, braken PTMC schijfjes met daarin gentamicine af door middel van oppervlakte-erosie en werd 60% van de gentamicine afgegeven binnen 14 dagen. Dit is vergelijkbaar met de afgifte van gentamicine uit de klinisch veelgebruikte PMMA kralen. Bovendien werd, in aanwezigheid van PTMC schijfjes geladen met gentamicine, biofilmvorming door

Staphylococcus aureus geremd met circa 80% gedurende tenminste 14 dagen, overeenkomend met het effect gevonden bij PMMA kralen geladen met gentamicine. In de afwezigheid van lipase vindt oppervlakte-erosie van PTMC schijfjes niet plaats en is gentamicine afgifte en de inhibitie van biofilms beperkt. PTMC lijkt een veelbelovend biologisch afbreekbaar antibioticum-afgiftesysteem voor de lokale behandeling van osteomyelitis, aangezien de PTMC schijfjes geladen met gentamicine een met PMMA kralen vergelijkbare antibiotica-afgifte en biofilm-inhibitie vertonen.

Tot slot werden nieuwe antibiotica regimes onderzocht, geïncorporeerd in PTMC ter bestrijding van therapieresistente osteomyelitis (hoofdstuk V). Bij patiënten met osteomyelitis is de normale antibiotische therapie van weinig waarde wanneer er methicilline-resistente *S. aureus* (MRSA), *Staphylococcus epidermidis* (MRSE) of kleine-kolonie varianten (SCV) aanwezig zijn. Betere resultaten zouden kunnen worden verkregen door een geschikt afgiftesysteem met antibiotica combinaties waar rifampicine deel van uitmaakt. Daarom onderzochten wij *in vitro* biofilm-inhibitie van MRSA, MRSE en *S. aureus* SCV stammen gedurende 24, 72 of 168 uur behandeling middels biologisch afbreekbaar PTMC dat ongeladen, ofwel geladen was met gentamicine of rifampicine en fosfomycine of rifampicine en vancomycine. Bij alle stammen vertoonde het biofilm kiemgetal en de metabolische activiteits-meting (MTT analyse) statistisch significante inhibitie wanneer PTMC geladen met rifampicine en vancomycine werd ingezet, en dan met name na 168 uur behandeling. Vergelijkbare kwalitatieve resultaten werden verkregen met confocale laser scan microscopie. PTMC geladen met louter gentamicine vertoonde totaal geen inhibitie. Dit illustreert dat PTMC geladen met rifampicine en vancomycine veelbelovend is voor de behandeling van therapieresistente osteomyelitis.

In de zoektocht naar een afbreekbaar alternatief voor PMMA ten bate van de behandeling van chronische osteomyelitis, omvat dit proefschrift *in vitro* onderzoek naar de degradatie van PTMC en antibiotica afgifte en inhibitie van stafylokokken-biofilm door PTMC geladen met antibiotica. De dissertatie werd afgesloten door terug te kijken vanuit een allesomvattend perspectief (hoofdstuk VI). Het molecuulgewicht van het polymeer, het geïncorporeerde antibioticum (of de combinatie antibiotica) en de geometrische vorm bleken van belang voor de afgiftesnelheid van antibiotica uit PTMC. Onze resultaten zijn gestoeld op de veronderstelling dat lipase een adequaat medium is voor de *in vitro* nabootsing van degradatie. Echter, het exacte mechanisme van *in vivo* PTMC degradatie en antibiotica afgifte bij osteomyelitis blijft onduidelijk en zal verder moeten worden onderzocht. In de hoofdstukken zijn verschillende molecuulgewichten PTMC en verschillende methoden ter evaluatie van de gevolgen voor de biofilm gebruikt, welke allen inzichten bieden vanuit een andere invalshoek. De antibiotica die we hebben getest behoren tot de klinisch meest aangewezen beschikbare medicamenten, waarbij combinaties met rifampicine bijzonder krachtig zijn tegen stafylokokken-biofilms. In de toekomst zullen nieuwe antibiotica, andere antimicrobiële middelen of combinaties daartussen moeten worden onderzocht. Aangespoord door klinische uitdagingen zoals de opkomende resistentie tegen antibiotica, zal het een moeizame, maar inspirerende verdere zoektocht zijn om te bepalen of PTMC meer is dan het op dit moment is: een veelbelovende antibiotica-afgiftesysteem.

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