Bio-optical imaging of host–bacteria interactions in biomaterial-associated infection

Daghighi, Seyedmojtaba

Chapter 6

General discussion

Implanted biomaterials and devices provide a niche for bacterial adhesion in which growth of these bacteria into a biofilm may lead to biomaterial-associated infection (BAI), the primary cause of biomaterial implant and device failure. Among the various strategies which have been developed to inhibit bacterial adhesion and subsequent biofilm formation and to reduce the incidence of BAI, antimicrobial coatings have been shown to provide the most promising solution. In order to advance the development of new antimicrobial coatings, a better understanding of the interaction between the host immune response, bacteria and biomaterial surfaces during the course of BAI is urgently required.

Recently, increasing attention has been given to techniques which can be used for whole body imaging in small animals in a wide area of biomedical applications (1). Imaging technologies such as positron emission tomography, magnetic resonance imaging and computed tomography are commercially available for pre-clinical studies and offer a high spatial resolution (magnetic resonance imaging and computed tomography) and deep tissue penetration. However, these techniques are costly and time consuming and, more importantly, are hardly able to directly detect bacterial presence with respect to infection diseases. In contrast, bioluminescence and fluorescence imaging enable simultaneous, longitudinal monitoring of bacterial persistence and the associated host immune response. In addition, such methods are relatively rapid, cost effective and easy to use. Bio-optical imaging techniques not only provide comprehensive data during the course of an infection, but also allow statistically significant conclusions to be drawn with relatively low numbers of animals because one and the same animal can be followed longitudinally throughout the duration of an experiment without sacrifice. In this thesis, we have studied host-bacteria interactions in BAI using bio-optical imaging techniques *in vitro* and in murine models.

In vivo bioluminescence imaging of BAI

In vivo bio-optical imaging of BAI using bioluminescently-engineered strains allows the infecting pathogen to be monitored longitudinally and non-destructively, providing an accurate assessment of the bacterial persistence throughout the duration of an experimentally induced infection. Bacterial bioluminescence-based *in vivo* models, mimicking infection developed from peri- or post-operative contamination of a biomaterial implant, require a pathogen with a luciferase based reporter system capable of emitting visible light that can be detected through live tissues using a highly sensitive CCD camera.

Previously, it has been shown that a strong correlation exists between bioluminescence arising from bacterially contaminated biomaterials in animals and *ex vivo* bacterial counts after culturing organisms from explanted materials after sacrifice (2). This seems to be in contradiction with the results as presented in Chapter 4, showing that staphylococcal bioluminescence activity is mainly controlled by bioluminescence co-factors, like NADPH rather than by expression of *Lux*-genes. This implies that changes in bioluminescence may be attributed to bacterial growth and to changes in the metabolic activity of the bacteria present, largely affected by the growth-state of the individual bacteria. This raises questions, not so much about the usability of the technique, but much more about the interpretation of its results. Several interpretations are valid. The first option of bioluminescent data interpretation from animal BAI-models is to trust bioluminescence signals to be indicative of the total number of viable bacteria in a biofilm, as shown experimentally, because in a biofilm these bacteria are considered to form a heterogeneous community of organisms in different growth phases all contributing to the bioluminescence signal. A second interpretation of bioluminescence data explains reductions in bioluminescence over time not in terms of a reduction in the number of colony forming units (see e.g. Chapters 3 and 5), but as a result of bacterial inactivation due to macrophage encapsulation or nutritional shortages in a biofilm (3,4). A third interpretation, and probably the

best, is a combination of both interpretations, and it advocates the combined use of bioluminescence and post-sacrifice culturing. Both colony forming units and bioluminescence are considered to be complementary to each other, not only providing numbers of viable bacteria, but also providing information on their metabolic state. A combination of low bioluminescence and high numbers of colony forming units, for instance, may indicate low efficacy of antibiotics and high numbers of inactivated bacteria in peri-implant tissue. Further research on this topic, using the combination of bioluminescence imaging and culturing might shed light on processes leading to bacterial inactivation in biofilms.

In vivo fluorescence imaging of BAI

One of the drawbacks of using bioluminescence BAI models is that it lacks the possibility to translate the model to clinical settings, for the simple reason that BAI in patients originates from non-bioluminescent bacteria. Thus, interest of clinicians is drawn to other bio-optical techniques, such as fluorescence imaging using intra-operative fluorescence cameras (5,6) and opto-acoustic imaging (7), which allows imaging in deeper tissues. Both techniques use excitation light as the source of energy for detection, rather than the energy source of the bacteria themselves, which makes fluorescence independent from the bacterial metabolic state and alleviates the requirements of high camera sensitivity. A number of fluorescence techniques have been evaluated within the context of this thesis, a few of which have been extensively described in Chapter 5. In BAI studies, fluorescence imaging can be categorized into two main modalities: fluorescence imaging of bacterial presence and fluorescence imaging of the immune system activity. Within each of these two categories, several working mechanisms were evaluated, of which some are presented in this thesis:

a) Expression of fluorescence proteins within bacterial cells.

b) Activatable fluorescent probes, i.e. quenched fluorophores becoming fluorescent when the probe is cleaved by a specific enzyme (Chapter 5).

c) Targeted fluorescent probes, which accumulate at molecular moieties towards which specific binding is subsequently accomplished (Chapter 5).

d) Vascular probes, which circulate in the blood and accumulate at sites with increased vascular permeability.

Fluorescence imaging, however, seldom allows direct bacterial detection as with bioluminescence imaging, unless fluorescent bacteria are used. Fluorescent bacteria may express fluorescence, but such protein-derived fluorescence frequently does not exceed the auto-fluorescence of animal tissue according to the experiences gained with murine models in this thesis. Moreover, the problem of the use of genetically modified organisms, clinically never occurring, persists.

Direct detection of bacterial presence was shown to be feasible *in vitro* by using probes which turn into a fluorescent state after specific cleavage by bacterial toxins or other bacterial proteases, such as LasA from *Pseudomonas aeruginosa*. Similar probes for a broader spectrum of bacterial strains were described earlier (8). In the experiments with LasA probes however, efficacy was shown only when high numbers of bacteria were present and it is yet unclear whether the immune system is able to cleave these probes as well. Other activatable probes such as MMPsense® and Prosense® are activated by the immune system rather than by bacteria themselves (Chapter 5). This may leave probe development as a clear objective for the future: finding and validating probes that are activated by clinically relevant numbers of bacteria. A promising probe for this purpose has recently been described: maltodextrin-based imaging probes can detect bacteria *in vivo* with a high sensitivity, independent of the host response (9). The combination of bacteria-specific probes with the probes that can detect local inflammatory responses, may enable the separation of the inflammation due to a sterile implant and BAI. Further validation of probe combinations may pave the way for clinical application of fluorescence imaging in image-guided implant debridement and may support decision-making regarding antibiotic treatment of BAI or immediate implant replacement.

Antimicrobial coatings and biodegradability

Antimicrobial coatings have been shown to be the most promising strategy for prevention of BAI. For instance, local delivery of antibiotics from implant coatings such as cardiovascular stents (10), surgical meshes (11), urinary catheters (12) and in orthopedic appliances (13) has been described extensively. Other strategies effective in preventing BAI may be provided by non-adhesive coatings, preventing initial bacterial adhesion, and coatings with contact bactericidal activity.

This thesis shows that the persistence of BAI on and around an implanted biomaterial is influenced by degradation of the biomaterial. In Chapter 3, we show that infecting bacteria disappeared completely from fully degraded bacterially contaminated surgical meshes, whereas bioluminescence increased in case of partially degraded meshes. This potential of biodegradable materials in reducing or potentially preventing BAI, encouraged us to review the potential mechanisms of infection resistance of degradable biomaterials (Chapter 2). It appeared that there is limited knowledge on how degradable materials may reduce infection risk and how such materials will perform implant coatings or scaffolds in tissue-engineering. This might be an important question to address in future research.

References

1. Studwell AJ, Kotton DN. A shift from cell cultures to creatures: *in vivo* imaging of small animals in experimental regenerative medicine. Mol Ther 2011;19:1933-41.
2. Kadurugamuwa JL, Sin L, Albert E, Yu J, Francis K, DeBoer M, et al. Direct continuous method for monitoring biofilm infection in a mouse model. Infect Immun 2003;71:882–90.
3. Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, Williams SH, et al. *Staphylococcus aureus* biofilms prevent macrophage phagocytosis and attenuate inflammation *in vivo*. J Immunol 2011;186:6585-96.
4. Boelens JJ, Dankert J, Murk JL, Weening JJ, Van der Poll T, Dingemans KP, et al. Biomaterial-associated persistence of *Staphylococcus epidermidis* in pericatheter macrophages. J Infect Dis 2000;181:1337–49.
5. Crane LM, Themelis G, Buddingh KT, Harlaar NJ, Pleijhuis RG, Sarantopoulos A, et al. Multispectral real-time fluorescence imaging for intraoperative detection of the sentinel lymph node in gynecologic oncology. J Vis Exp 2010; 44:2225.
6. Van Dam GM, Themelis G, Crane LM, Harlaar NJ, Pleijhuis RG, Kelder W, et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-alpha targeting: first in-human results. Nat Med 2011;17:1315-9.
7. Razansky D, Distel M, Vinegoni C, Ma R, Perrimon N, Köster RW, et al. Multispectral opto-acoustic tomography of deep-seated fluorescent proteins *in vivo*. Nat Photonics 2009;3:412-7.
8. Zhou J, Loftus AL, Mulley G, Jenkins AT. A thin film detection/response system for pathogenic bacteria. J Am Chem Soc 2010;132:6566-70.
9. Ning X, Lee S, Wang Z, Kim D, Stubblefield B, Gilbert E, et al. Maltodextrin-based imaging probes detect bacteria *in vivo* with high sensitivity and specificity. Nat Mater 2011;10:602-7.
10. Elieson M, Mixon T, Carpenter J. Coronary stent infections: a case report and literature review. Tex Heart Inst J 2012;39:884-9.
11. Engelsman AF, Van der Mei HC, Ploeg RJ, Busscher HJ. The phenomenon of infection with abdominal wall reconstruction. Biomaterials 2007;28:2314-27.
12. Falagas ME, Kapaskelis AM, Kouranos VD, Kakisi OK, Athanassa Z, Karageorgopoulos DE. Outcome of antimicrobial therapy in documented biofilm-associated infections: a review of the available clinical evidence. Drugs 2009;69:1351-61.
13. Costerton JW. Biofilm theory can guide the treatment of device-related orthopaedic infections. Clin Orthop Relat Res 2005;437:7-11.

Summary

Despite the fact that the number of patients receiving biomaterial implants and devices is increasing, biomaterials-associated infection (BAI) is still the major causes of implant failure. The consequences of a BAI are significant and include increased hospitalization times, high treatment costs, and often revision surgery, while not seldom yielding morbidity and even death. In order to develop new antimicrobial strategies, an accurate understanding of the host-bacterial interaction in BAI is essential. The interplay between antimicrobial strategies applied in the form of coatings and the host immune response to BAI is difficult, if not impossible, to mimic in *in vitro* assays. As a result, the use of animal models is indispensable for assessing the immune-compatibility and antimicrobial efficacy of antimicrobial coatings applied on biomaterials.

Chapter 1 gives a brief introduction to bacterial adhesion, biofilm formation and the consequences of BAI in patients. Chapter 1 also briefly introduces the host inflammatory processes in the context of a foreign body reaction and BAI. Although host-bacterial interactions in BAI have a great impact on developing antimicrobial strategies, the complex interaction between the immune system and bacteria is not well characterized. Therefore the first aim of this thesis is to gain a better understanding of the biological events that take place during the interaction of the host immune system with bacteria and implant surfaces during the course of BAI. As the second aim of this thesis, *in vivo* implant BAI models based on bioluminescence and fluorescence imaging of BAI will be developed and validated.

Depending on the application, implants and devices can be made of degradable or non-degradable biomaterials. **Chapter 2** gives an overview of the potential mechanisms of infection resistance of degradable *versus* non-degradable biomaterials. Degradable biological materials demonstrate increased resistance to bacterial infection compared to non-degradable synthetic biomaterials. Current knowledge about the specific mechanisms of how degradable biological materials are afforded increased resistance to infection is

limited. Therefore, in this Chapter a number of hypotheses to explain the decreased infection risk associated with the use of degradable *versus* non-degradable biomaterials are evaluated and discussed with reference to the present state of knowledge.

In **Chapter 3** the decreased infection risk associated with degradable *versus* non-degradable biomaterials is evaluated *in vivo*. Biomaterials are increasingly used for the restoration of human function, but can become infected as a result of peri- or early post-operative bacterial contamination, although BAIs can also initiate at any time from haematogenous spreading of bacteria from an infection elsewhere in the body. Infecting bacteria in BAI not only seek shelter in their own protective biofilm matrix, but also hide in surrounding tissue. This Chapter compares staphylococcal persistence on and around a degradable and non-degradable surgical mesh through the use of longitudinal bioluminescence imaging in a murine model, including histological evaluation of surrounding tissue after sacrifice. Surgical meshes were first contaminated with bioluminescent *Staphylococcus aureus* Xen29 and subsequently subcutaneously implanted in mice. Bioluminescent staphylococci persisted on and around non-degradable meshes during the 28-day course of the study, whereas bioluminescence returned to control levels and bacteria disappeared from surrounding tissues once a degradable mesh had fully dissolved. Thus the application of degradable biomaterials yields major advantages with respect to the prevention of BAI, as dissolution of the implant not only is associated with elimination of the protective biofilm mode of growth of the infecting organisms, but also allows the immune system to clear the surrounding tissue from infecting organisms.

Bioluminescence-imaging is often used for longitudinal evaluation of bacterial presence in live animals. Although linear relations exist between numbers of bacteria in biofilms and their bioluminescence, such relations cease to exist under antibiotic pressure. In **Chapter 4** we have evaluated the influence of antibiotic pressure on staphylococcal bioluminescence. To this end,

bioluminescent flux from bioluminescent *S. aureus* Xen29 was quantified in absence and presence of different antibiotics. Staphylococcal bioluminescence was inhibited by antibiotics at sub-inhibitory (sub-MIC) concentrations, but seemed to be enhanced when measured 24 h after incubation, for all antibiotics evaluated. Similar enhancements of bioluminescence at sub-MIC antibiotic concentrations were observed in E-tests®. Therefore, antibiotic pressure may impact the relationship between bacterial numbers and their bioluminescence. Under antibiotic pressure, staphylococcal bioluminescence enhancement was not associated with an increased level of *LuxA*-gene expression, suggesting that staphylococcal bioluminescence activity profiles in the presence of antibiotics are mainly controlled by bioluminescence co-factors, like NADPH rather than by *Lux*-genes.

Biomaterial implants increase the risk of infections due to the biofilm mode of growth of bacteria adhering on implant materials, in which bacteria are protected against antibiotic treatment and the local immune system. Matrix-metalloproteinases (MMPs) and cell surface integrin receptors facilitate transmigration of inflammatory cells toward infected or inflamed tissue. In **Chapter 5** we have investigated the relationship between MMP- and integrin-expression and the clearance of infecting *S. aureus* around implanted biomaterials in a murine model. MMP- and integrin αvβ3-expression were monitored in mice with and without subcutaneously implanted catheter sections in absence and presence of bioluminescent *S. aureus* Xen36. Staphylococcal persistence was imaged longitudinally over time using bioluminescence imaging. The activatable MMPSense®680 and integrin-targeted IntegriSense®750 probes were injected on different days after implantation and their signal intensity and localization monitored using fluorescence imaging. After sacrifice 7 or 16 days post-implantation, staphylococci from catheters and surrounding tissues were cultured on agar-plates and presence of host inflammatory cells was histologically evaluated. MMP- and integrin-expression were equally enhanced in presence of

staphylococci or biomaterials up to 7 days post-implantation, but their localization along the catheter sections differed. Bacterial clearance from tissue was higher in absence of biomaterials. Although MMP- and integrin-expression were enhanced in presence of both staphylococci and biomaterial, it is of clinical relevance that the immune system remained hampered in eradicating bacteria during the first 7 days post-implantation in presence of biomaterials.

In **Chapter 6** different subjects of our findings in Chapters 2 to 5 including the discrepancies between bioluminescence data and numbers of viable bacteria are discussed. It is advocated that bioluminescence and post-sacrifice culturing provide complementary information about numbers of viable bacteria and their metabolic state. In addition, it is concluded that fluorescence imaging as an additional modality to bioluminescence imaging, enables the assessment of the progress of inflammation. In order to translate our pre-clinical studies to clinical research however, more effort is needed to develop fluorescent probes for bacterial detection.

Samenvatting

Biomateriaal geassocieerde infecties (BAI) zijn nog steeds de belangrijkste reden voor het falen van een implantaat, terwijl het aantal patiënten dat een biomateriaal krijgt stijgt.. De gevolgen van BAI zijn significant en gaan gepaard met een langer verblijf in het ziekenhuis, hoge behandelingskosten en vaak noodzaak tot een revisie-operatie met morbiditeit en soms zelfs overlijden als gevolg. Om nieuwe antimicrobiële strategieën te ontwikkelen, zoals antibacteriële coatings, is een goed begrip van de interactie tussen gastheer (het menselijk lichaam) en bacterie bij BAI essentieel. Het samenspel tussen de antimicrobiële werking van coatings en de immuunrespons van de gastheer ten opzichte van BAI is moeilijk, zo niet onmogelijk, *in vitro* na te bootsen. Vandaar dat het gebruik van diermodellen onmisbaar is om de immuuncompatibiliteit en antimicrobiële efficiëntie van antimicrobiële coatings vast te stellen.

Hoofdstuk 1 is een korte introductie en gaat in op bacteriële hechting, biofilm formatie en de klinische gevolgen van BAI. Hoofdstuk 1 introduceert ook kort de ontstekingsprocessen in de context van een vreemd-lichaamsreactie en BAI. Hoewel gastheer-bacterie-interacties in BAI een grote impact hebben op de ontwikkeling van antimicrobiële strategieën, is de interactie tussen het afweersysteem en bacteriën nog onvoldoende beschreven. Vandaar dat een beter begrip van die interactie het eerste doel van dit proefschrift is. Als tweede doel van dit proefschrift worden *in vivo* BAI modellen, die gebaseerd zijn op bioluminescentie- en fluorescentiebeeldvorming ontworpen en gevalideerd.

Voor de ontwikkeling van implantaten kan afhankelijk van de toepassing gebruik gemaakt worden van afbreekbare of niet-afbreekbare biomaterialen. **Hoofdstuk 2** geeft een overzicht van de potentiele mechanismen van infectie-resistentie van afbreekbare biomaterialen. Afbreekbare biomaterialen laten een verhoogde resistentie tegen bacteriële infectie zien vergeleken met niet-afbreekbare synthetische biomaterialen. De huidige kennis over de specifieke mechanismen waardoor afbreekbare biologische materialen een verhoogde resistentie tegen infecties vertonen is beperkt. Daarom wordt in dit hoofdstuk een

aantal hypotheses besproken en geëvalueerd waarmee geprobeerd wordt het verlaagde infectierisico van afbreekbare biomaterialen ten opzichte van niet-afbreekbare biomaterialen te verklaren.

In **Hoofdstuk 3** wordt het risico op infectie van afbreekbare biomaterialen vergeleken met niet-afbreekbare biomaterialen in een *in vivo* onderzoek. Biomaterialen worden in toenemende mate gebruikt om de functie van het menselijk lichaam te herstellen. Biomaterialen kunnen geïnfecteerd raken door peri- of vroege postoperatieve bacteriële contaminatie, maar BAI kan ook op elk willekeurig moment ontstaan als gevolg van de hematogene spreiding van bacteriën als gevolg van een infectie elders in het lichaam. Bacteriën die BAI veroorzaken zoeken niet alleen bescherming in hun eigen biofilmmatrix, maar ook in het omliggende weefsel en zijn daarom lastig te verwijderen. Dit hoofdstuk vergelijkt de hardnekkigheid van stafylokokken-infecties op en rond afbreekbare en niet-afbreekbare chirurgische matjes door het gebruik van longitudinale bioluminescentiebeeldvorming in een muismodel en door histologische evaluatie van biopsieën van omliggend weefsel die zijn verkregen na opoffering. Chirurgische matjes werden eerst besmet met bioluminescente *Staphylococcus aureus* Xen29 bacteriën en vervolgens subcutaan geïmplanteerd in muizen. Bioluminescente stafylokokken bleven aanwezig op en rond niet-afbreekbare matjes gedurende de 28 dagen die het onderzoek duurde, terwijl bioluminescentie terugviel naar controlewaarden. Nadat een afbreekbaar matje eenmaal volledig was opgelost verdwenen de bacteriën echter uit omliggend weefsel. Het toepassen van afbreekbaar biomateriaal biedt dus grote voordelen op het gebied van preventie van BAI, aangezien het oplossen van het implantaat niet alleen eliminatie van de beschermende biofilm inhoudt, maar ook het immuunsysteem de kans geeft het omliggende weefsel vrij te maken van infecterende organismen.

Bioluminescentie wordt vaak gebruikt voor longitudinale evaluatie van de aanwezigheid van bacteriën in levende dieren. Hoewel er lineaire relaties bestaan

tussen het aantal bacteriën in biofilms en hun bioluminescentie, gelden deze relaties niet in aanwezigheid van antibiotica. In **Hoofdstuk 4** hebben we de invloed van antibiotica op de bioluminescentie van stafylokokken bestudeerd. Hiervoor hebben we de bioluminescentieflux van bioluminescente *S. aureus* Xen29 bacteriën gekwantificeerd in af- en aanwezigheid van verschillende antibiotica. Bij lage concentraties van antibiotica, waarbij de groei van bacterien alleen geremd wordt maar niet gestopt (zogenaamde sub-MIC concentraties) daalt aanvankelijk de bioluminescentie als functie van de concentratie. Na 24 uur incubatie echter, laat het een maximum zien: een effect dat onafhankelijk was van het type antibiotica. Soortgelijke verhoging van bioluminescentie als functie van de sub-MIC concentraties werd ook waargenomen in E-testen®. Dit geeft aan dat de toediening van antibiotica mogelijk effect heeft op de relatie tussen het aantal bacteriën en hun bioluminescentie. Na toediening van antibiotica was de verhoging van de bioluminescentie in stafylokokken niet gerelateerd aan een verhoogde *LuxA*-gen expressie. Dit suggereert dat de bioluminescentie van stafylokokken in de aanwezigheid van antibiotica voornamelijk gecontroleerd wordt door bioluminescentie-cofactoren, zoals NADHP in plaats van door *Lux*-genen.

Biomateriaal implantaten verhogen het risico op infecties doordat bacteriën biofilms vormen op implantaat materialen, waardoor bacteriën beschermd worden tegen antibiotica en het lokale immuunsysteem. Matrixmetalloproteinases (MMPs) en celoppervlakte-integrinereceptoren faciliteren de transmigratie van immuun cellen naar geïnfecteerd of ontstoken weefsel. In **hoofdstuk 5** hebben we de relatie onderzocht tussen MMP- en integrine-expressie en het opruimen van infectie veroorzakende *S. aureus* bacteriën rondom geïmplanteerde biomaterialen in een muismodel. MMP- en integrine αvβ3-expressie werden bekeken in muizen met en zonder subcutaan geïmplanteerde kathetersecties in de af- en aanwezigheid van bioluminescente *S. aureus* Xen36 bacteriën. De aanwezigheid van stafylokokken werd zichtbaar

gemaakt door middel van tijdsafhankelijke longitudinale bioluminescentie-beeldvorming. De activeerbare MMPSense®680 en integrinespecifieke IntegriSense®750 probes werden op verschillende dagen na implantatie geïnjecteerd en de signaalsterkte en locatie werd gevolgd met behulp van fluorescentiebeeldvorming. Na opoffering, 7 of 16 dagen na implantatie werden de stafylokokken van de katheters en het omliggende weefsel gekweekt op agar-platen en werd de aanwezigheid van ontstekingscellen van de gastheer histologisch geëvalueerd. MMP- en integrine expressie waren evenveel verhoogd in de aanwezigheid van stafylokokken en biomateriaal tot 7 dagen na implantatie, maar de locatie op de kathetersecties verschilde. Hoewel MMP- en integrine-expressie extra verhoogd waren in de aanwezigheid van zowel stafylokokken als biomateriaal, is het klinisch relevant dat het immuunsysteem niet in staat was de bacteriën op te ruimen gedurende de eerste 7 dagen na implantatie in de aanwezigheid van biomateriaal.

In **hoofdstuk 6** worden verschillende onderwerpen van onze bevindingen uit hoofdstuk 2 t/m 5 besproken, inclusief de discrepanties tussen bioluminescentie data en aantal levende bacteriën. Het wordt bepleit dat naast het meten van bioluminescentie ook bacteriën gekweekt worden om zo zowel informatie te verkrijgen over het aantal bacteriën en de metabolische staat waarin ze zich bevinden. Daarnaast wordt de conclusie getrokken dat fluorescentiebeeldvorming als aanvulling op bioluminescentiebeeldvorming de mogelijkheid geeft om de voortgang van een ontsteking te beoordelen. Echter, om onze preklinische studie naar klinisch onderzoek te kunnen vertalen zullen ook fluorescente probes ontwikkeld moeten worden waarmee bacteriën rechtstreeks kunnen worden aangetoond.

Acknowledgements

Thanks God for helping me to finish this stage of my carrier. I owe my deepest appreciation to all people who help and support in different ways, made this thesis possible and who have made my graduate experience one that I will cherish forever.

I'm infinitely grateful to my promotors, Prof. Henk Busscher and Prof. Henny van der Mei. Dear Henk and Henny, I am very thankful for giving me the opportunity to join the group of Biomedical Engineering as a PhD candidate. It was an honor and pleasure to work under your supervision and I'm sure that without your constant commitment, this thesis would have never come to this point. I am also indebted to my co-promotor, Dr. Jelmer Sjollema. Dear Jelmer, your door has always been open for questions and discussions. Thank you for all your help and guidance you have granted me all these years.

I sincerely acknowledge my thesis reading committee: Prof. Ruud Bank, Prof. Jan Maarten van Dijl and Prof. Geert Walenkamp for taking their time to read my thesis. Words of gratitude are addressed to following NANTICO members whom I have collaborated and shared joyful discussions: Dr. Bas Zaat, Dr. Jim Odekerken, Dirk van Asseldonk, Heidrun Keul, Dr. Tim Welting, Rik Cleophas, Dr. Ton Dirks and Prof. Ton Loontjens.

I would like to address special thanks to my paranymphs: Sara Ehsani and Jan Swartjes, who agreed to step in and helped me with organizing my defense day. Jan, thank you for translating the summary of my thesis. I also would like to thank Rene Dijkstra, Marja Slomp, Valery Jasper, Martijn Riool, Betsy van de Belt-Gritter and Leonie de Boer, who kindly helped me with in vivo experiments.

Wya, I would like to say thank you for all the help with the administrative and financial issues. Ina, Willy and Ellen, thank you for all the assistance with secretarial and paper work. I would like to extend my thanks to past and present BME colleagues and my friends especially Hossein Zadegi, Mohammad Eslami, Mihaela Crismaru, Abas Halajnia, Shahriar Sharifi, Anton, Katya, Song, Bingran and dear Farhad.

My deepest thank to my parents and my parents-in-law for their help, support and encouragements.

My beautiful flower, Golnar, my life has never been so beautiful since you came into my life. Everything without you is priceless.

And finally, no words can describe my gratitude I have for my dearest, Sona. Thank you seems very inadequate here. I have been infinitely blesses by God that He gave you to me. It was your unflinching patience, support and immeasurable love that helped me in all these years.