Peri-prosthetic interface tissue around aseptic loosened prostheses: not waste, but a potential therapeutic target?

Schoeman, A.E.; Schoeman A.E.

SUMMARY AND GENERAL DISCUSSION

7

The research in this thesis represents part of a project aimed at developing a minimally invasive treatment for aseptic loosened orthopaedic implants (e.g. hip-, knee implants). In this thesis the biology of the process of aseptic loosening of implants was explored. Ultimately, these findings may lay down the foundation for the identification of biological targets to counteract the aseptic implant loosening process. Eventually a biological refixation approach for loosened orthopaedic implants may be developed. In **Chapter 1**, the process of aseptic loosening as well as aspects of the peri-prosthetic interface tissue were introduced. Aseptic loosening is the most common reason for complex implant revision surgeries, which affects on average 10-20% of total hip and knee replacement patients 10-20 years after the primary joint replacement.[1-3] During the implant loosening process a fibrous-like tissue, the so-called peri-prosthetic interface tissue, is formed at the bone-implant interface. This interface tissue usually contains a wide variety of cells, which produce cytokines and chemokines triggering bone resorption around the implant. This leads to mechanically unstable implants and ultimately a need for revision surgery due to incapacitating pain or even a peri-prosthetic fracture. Aseptic loosening of implants occurs secondary to a biological response to implant wear-debris particles, originating from the articulating joint, of which the most important cellular target is believed to be the macrophage. Nevertheless, the precise biological mechanisms underlying the loosening process have still to be elucidated. Consequently, biologic targets for successful treatment of aseptic loosening have not been found yet. Therefore, the aim of this thesis was to increase the knowledge on the biological responses around aseptic loosened prostheses by (1) characterizing the cellular content of the peri-prosthetic interface tissue, (2) studying the osteogenic potential of peri-prosthetic interface tissue cells, and (3) exploring the individual host-immune responses in relation to early migration of prostheses.

Does the cellular content of peri-prosthetic interface tissue shed new light on the mechanism of implant loosening?

Characterization of the peri-prosthetic interface tissue is one of the ways of studying the underlying mechanisms of aseptic loosening and potential therapeutic targets for treatment. Numerous studies have provided information about the cellular content of peri-prosthetic interface tissue as well as the cytokines and chemokines it produces.[4-6] In a recent review, on histological and immunological aspects of aseptic loosening, a predominance of macrophages, multinucleated cells and signs of inflammation was reported in the peri-prosthetic interface tissue.[5] However, a lack of information on the role of fibroblasts and osteocytes amongst others was noted. Since only studies based on histological evaluation (e.g. cell/tissue/organ cultures) were included in that review, important cellular and molecular mechanisms associated with aseptic implant loosening may have been missed. Therefore, in **Chapter 2**, we conducted a systematic literature search

aimed at summarizing the characteristics of peri-prosthetic interface tissue based on *in vitro* findings, in order to provide an overview of the currently proposed cellular mechanisms involved in implant loosening. Besides macrophages also fibroblasts, derived from the peri-prosthetic interface tissue, were shown to be actively involved in osteoclastogenesis with subsequent pathologic bone resorption through production of inflammatory cytokines, chemokines, matrix degrading enzymes, osteoclastogenic factors and angiogenic factors. Moreover, fibroblasts were reported to be considered as potential therapeutic target in treating aseptic loosening. Again, only a few papers addressed the involvement of osteoblasts/osteogenesis in the process of implant loosening. Two papers suggested that the peri-prosthetic interface tissue of some patients with aseptic loosening exhibits osteogenic characteristics.[7, 8] Another paper showed a potentially disturbed osteogenic signalling in these patients.[9] Thus, (effects on) osteoblasts do seem to play a role in the process of aseptic loosening. Therefore, we concluded that besides the well-known role of macrophages and osteoclasts in the mechanism of loosening, the role of fibroblasts and osteoblasts should be taken into account.

These findings led us to perform a tissue characterization study of peri-prosthetic interface tissue samples collected during revision surgery for aseptic loosened hip prostheses. In that study both gene expression analysis and (immuno)histochemistry were combined to evaluate the presence of various cell types, including fibroblasts and osteoblasts (**Chapter 3**). Results showed that peri-prosthetic interface tissue samples express macrophage-, fibroblast-, osteoblast- and endothelial cell-related genes. Exploring gene expression patterns between samples revealed two components, clustering osteoblast- and fibroblast-related genes in another component than macrophage- and endothelial cell-related genes. Overall, a high inter-tissue sample variability in factor loading scores of the components was observed, which could not be explained by patient- or prosthesis characteristics. (Immuno) histochemical staining of the tissue samples showed predominance of both fibroblasts and macrophages with high inter- and intra-tissue sample variation in stained area and staining location. No significant associations were found between the stained area and patient- or prosthesis characteristics or gene expression data. Besides studying the macrophage in general, we also investigated the presences of macrophage subtypes in the tissue samples. In one-third of the samples M1(pro-inflammatory phenotype) and M2 (anti-inflammatory, pro-tissue healing phenotype) macrophages were present in comparable amounts, whereas almost two-third showed the predominance of M2 macrophages. In conclusion, fibroblasts and osteoblasts seem to be at least as important as macrophages in the aseptic loosening process. In addition, in particular M2 macrophages were present in our peri-prosthetic tissue samples.

The observed clustering of fibroblast- and osteoblast-related genes in a different component than macrophage- and endothelial cell-related genes, underscores the

different roles these (clusters of) cell types have in the process of implant loosening. Furthermore, differences in component scores between tissue samples indicate that the implant loosening process as such is based on a variety of cellular mechanisms between patients but also within patients. Morowietz et al. showed that the (proportional) presence of distinct cell types within peri-prosthetic interface tissue characterize different mechanisms of loosening (i.e. wear-debris induced, inadequate initial fixation).[10] Both fibroblasts and macrophages are the prominent cell types within the peri-prosthetic interface tissue (**Chapter 3**) and can produce similar osteolytic factors in response to wear-debris particles (**Chapter 2**). However, fibroblasts might also be involved in another mechanism of loosening, where inadequate initial mechanical fixation of the implant results in an expanding fibrous tissue (like scar tissue) with subsequent micromovement of the implant. The latter preventing a close interconnection between bone and implant. [10, 11] Fibroblasts can therefore significantly influence prosthesis survival because of their inflammatory response to wear debris particles and their capacity to produce and remodel the extracellular matrix around implants.[12] The observed differences in cellular content between patients might be the reason why therapeutic strategies which (only) interfere with the osteolytic process of aseptic loosening (e.g. non-steroidal anti-inflammatory drugs (NSAIDs), antibodies to specific osteolytic mediators) [13-19] showed inconclusive results. A combined therapy, which not only targets the inflammatory response to wear debris particles and the subsequent osteolytic process but also targets the extracellular matrix production and modulation, might therefore be necessary to successful interfere with the aseptic loosening process.

Although the role of macrophages within the peri-prosthetic interface tissue has been extensively studied, recently, the role of macrophage polarization showed seemingly contradictive results. Based on retrieval and *in vitro* studies, it has been shown that the presence of macrophages of the M1 phenotype (pro-inflammatory) in the peri-prosthetic interface tissue outweighs the presence of macrophages of the M2 phenotype (anti-inflammatory, pro-tissue healing).[13] Additionally, inflammatory wear debris responses are aggravated in M1-macrophages but are suppressed in M2-macrophages.[14, 15] However, other studies reported either a predominance of M2-macrophage activation or presence of equal proportions of both macrophage subtypes. [9, 16] In our study samples, the presence of M2-macrophages outweighed the presence of the M1 phenotype, although some of our samples showed no differences between both subtypes. Koulouvaris et al. proposed that in the final stages of loosening (i.e. revision is required), the pro-inflammatory response to wear debris particles might not be decisive anymore, resulting in a more prominent presence of M2-macrophages.[9] Moreover, a local dynamic shift in the macrophage phenotype from the inflammatory M1 to the anti-inflammatory M2 phenotype is presumed a transition from a state of inflammation to tissue regeneration.[17-19] Therefore, the

abundance of M2-macrophages within the peri-prosthetic interface tissue might be indicative for a regenerative capacity of this tissue and proposes the prospective use of macrophage modulation as an potential early therapeutic strategy in the loosening process. Local modulation of the macrophage phenotype, which was already pointed out and investigated by several other authors[13, 20-22], might limit the wear particle-induced inflammation with subsequent less peri-prosthetic osteolysis.

In general, a large variability in outcome measures as well as study population characteristics exists, which makes comparison between studies almost impossible. In the context of patient heterogeneity, most *in vitro* studies make use of selected cell types of animal or human origin (i.e. cell lines or specific primary cell types) to reduce heterogeneity. Although, this approach facilitates interpretation of the results, it ignores the complexity of the variety of cellular mechanisms in aseptic prosthesis loosening. As aseptic loosening involves the cross-talk between a variety of cells and the subsequent production of a wide-range of inflammatory mediators and matrix degrading factors, such basic experimental approaches are not representative of the in vivo situation. Therefore, despite the relatively high variability, more research should be focused on studying the complete peri-prosthetic interface tissue instead of selected cells, like in our study in **Chapter 3**. Another general limitation of (characterization) studies on peri-prosthetic interface tissue is the relatively small sample size. For that matter, to increase the ability to compare study results, studies with a sufficient sample size and more detailed and comparable description of study and tissue characteristics should be conducted.

Do peri-prosthetic interface tissue cells have potential osteogenic capacity?

The current strategy for the treatment of aseptic implant loosening (i.e. revision surgery) is successful with 10-year survival rates of 70-80%.[1-3, 23] However, the burden to the patient is large due to the duration of the surgical procedure, (e.g. risks for infection) and extensive blood loss causing morbidity (e.g. cardiovascular, renal) to the patient in the postoperative period.[24] Minimally invasive percutaneous implant refixation procedures have therefore been developed in order to reduce morbidity, while stabilizing the primary prosthesis with bone cement either or not in combination with eradication of the peri-prosthetic interface tissue.[25-28] Other strategies aimed at reducing the prevalence of aseptic loosening by improving the quality of the primary implant (e.g. design and material) or on interfering with the osteolytic process of aseptic loosening (e.g. the use of non-steroidal anti-inflammatory drugs (NSAIDs), bisphosphonates and antibodies to specific osteolytic mediators).[29-36] Bone resorption (osteolysis) is part of the bone remodelling process which also involves bone formation. Surprisingly, only limited attention has been paid to the role of bone formation (osteogenesis) in aseptic loosening and even less is reported on the role of osteogenesis in treatment of aseptic loosening.

Osteogenesis can be stimulated in a variety of ways, for example, growth factors can stimulate signalling pathways that are involved in osteoblast differentiation. Important osteogenic signalling pathways include the bone morphogenetic protein (BMP) and the Wingless (Wnt) signalling pathway and several studies have even shown interactions between these two pathways.[37-41] However, little is known about uncoupling these pathways and their intrinsic inhibitors (like sclerostin) and the effect on osteoblast differentiation. Since, sclerostin, acts physiologically as a downstream molecule of BMP signalling to inhibit Wnt signalling and negatively regulates bone mass [42, 43], interference with both pathways and sclerostin might prolong the effect of BMPs on osteoblast differentiation or bone formation. Therefore, in **Chapter 4**, the possibility to enhance bone regeneration by interference with BMP and Wnt signalling pathways was studied in human and murine cell lines. The interaction of both pathways on the effect on expression of SOST (the gene encoding sclerostin) was investigated using human osteosarcoma cells. Results showed that SOST expression could be either decreased with increasing Wnt signalling or increased by stimulation of BMP signalling. However, GIN (a specific inhibitor of GSK3β, which is an important part/member in Wnt signalling) could significantly decrease the BMP4-induced SOST expression and thereby uncouple BMP signalling and SOST expression. In addition, using the murine pre-osteoblastic cell line KS483, combined BMP4 and GIN stimulation could enhance osteoblast differentiation (increase both ALP activity and matrix mineralization) compared to BMP4 alone. Altogether, this study showed that uncoupling BMP signalling and SOST expression (using GIN) leads to an enhanced BMP4-induced osteoblast differentiation. This effect has potential to be used in clinical practice to induce local bone formation, e.g. osseointegration of implants or fracture healing.

Until now, stimulation of osteogenesis within the peri-prosthetic interface tissue itself has never been studied. Therefore, in **Chapter 5**, the actual capacity of peri-prosthetic interface tissue cells to differentiate into the osteoblast lineage was investigated. In addition, the potential to (further) increase osteoblast differentiation using the bone formation enhancing factors tested in **Chapter 4** was examined. Results showed that culturing peri-prosthetic interface tissue cells in osteogenic medium increased ALP staining as well as gene expression levels and resulted in production of a mineralized matrix in the majority of the donors, when compared to cells cultured in normal culture medium. In general, addition of BMPs, GIN or a combination of BMPs and GIN to the osteogenic culture medium could not significantly further increase the studied osteogenic characteristics, although in some donors it could be increased. Overall, a high inter- and intra-donor variability in response to different osteogenic stimuli was observed, which hampered the identification of a standard formula inducing osteogenic differentiation. Nonetheless, peri-prosthetic interface tissue cells were proven to possess osteogenic potential and as such stimulation of osteogenesis within the peri-prosthetic interface tissue could possibly counteract or slow down osteolysis

in the aseptic loosening process. Several animal studies already showed that BMP2 can enhance neo-bone formation to fill critical-sized bone defects.[44-46] In addition, a case-report study showed that implantation of a construct with BMP2 in combination with bone marrow derived mesenchymal stem cells could fill the critical bony defect after revision surgery.[47] Furthermore, preclinical studies showed the potential of inhibiting sclerostin via a monoclonal antibody in enhancing bone formation and preventing implant loosening.[48, 49]

The ability of peri-prosthetic interface tissue to respond to osteogenic stimuli suggests that a population of cells is (already) committed to the osteoblastic lineage. As we did not find an association between the responsiveness to osteogenic stimuli and the cell content of the tissue, we were not able to pinpoint the exact cell type(s) responsible for the osteogenic capacity of peri-prosthetic interface tissue. Both our characterization study and the systematic review showed macrophages and fibroblasts to be the main cell type in peri-prosthetic interface tissue. Therefore, likely one of these cell-types, or a specific subpopulation of these cell types, is able to increase ALP production and matrix mineralization in response to osteogenic stimuli. In a study by Heinemann et al., the main cell type found in granulomas obtained from prosthetic revisions was shown to stain positive for ALP as well as CD68 (a macrophage marker).[50] In a study by Zreiqat et al, foamy macrophages but not spindle-shaped mesenchymal cells, both obtained from loosened prostheses, were shown to express and produce several osteoblastic genes and proteins.[7] On the other hand, literature also shows fibroblasts to be able to express several osteoblast related genes.[51] Interestingly, several studies have showed that specific human fibroblasts (i.e. dermal and periodontal fibroblasts) are able to differentiate into osteoblasts.[52-54] Thus, finding a population of cells within the interface tissue, which can differentiate into the osteoblastic lineage is likely to provide a new opportunity to interfere with the altered balance in bone remodelling and bone resorption in aseptic loosening.

Almost all tissue samples responded to either one of the applied osteogenic stimuli, however, there was not one single, nor a combination of factor(s) that effectively induced osteogenic differentiation in all donors. Studies investigating the osteogenic differentiation of human bone marrow derived mesenchymal stem cells also showed inter-individual variation in the osteogenic capacity of these cells.[55, 56] Moreover, in a study using adipose derived mesenchymal stem cells inter-individual variation in response to BMP2 was shown.[57] Therefore, stimulation of the osteogenic capacity of peri-prosthetic interface tissue cells warrants either further research into one universal stimulus for all patients or requires a more personalized medicine approach. In addition, further research into the influence of clinical parameters (e.g. patient- and prosthesis characteristics) on the osteogenic capacity of peri-prosthetic interface tissue cells could also be necessary to be able to develop such a universal or personalized approach.

Does the individual host immune response relate to prosthesis migration?

Cellular responses to wear debris particles play a key role in the progression of osteolysis around aseptic loosened prostheses. Phagocytosis of wear debris particles triggers the release of (pro-)inflammatory cytokines, activating pathways which lead to bone resorption. The extent of osteolysis may vary between patients due to, amongst others, differences in individual host-immune responses to wear debris, which can be related to an individual genotype.[58-60] Therefore, evaluation of differences in host-immune responses between patients could possibly help to predict the risk of aseptic loosening of the prosthesis. Currently, loosening can be detected by measuring sub millimetre migration of the prosthesis relative to the host bone using simultaneous two standard radiographs with radiostereophotogrammatric analysis (RSA).[61-64] However, RSA measurements require the insertion of 1 mm tantalum beads in the patient's bone, thus (early) assessment of loosening using RSA can only be used in patients that have been included in clinical RSA studies. Because of the genetic nature of host-immune responses it might be possible to detect the risk of loosening early, even before the patient needs joint replacement surgery. Therefore, in **Chapter 6**, the relation between innate immune responses and implant migration, using RSA, was investigated. For this purpose, whole-blood of TKA patients, with different designs of knee prostheses, was stimulated with a peptide inducing the Toll-like receptor 2 (TLR2) immune response. As a result, a variety of cytokines was produced, which were shown to cluster in two components. The component containing (primarily) pro-inflammatory cytokines correlated inversely with migration. Further analysis showed IFNγ to have the highest contribution to this association. The latter was only observed in those patients who had a prosthesis other than the Nexgen knee prosthesis, for which we have no explanation yet. In conclusion, this study shows that patients with high levels of IFNγ upon stimulation of TLR2 are at lower risk of early migration of their knee prosthesis.

Several studies have shown that progressive early migration, as measured with RSA, is associated with an increased risk at revision of knee and hip prostheses at the long term.[64, 65] Therefore, the observed association between specific cytokines and early migration of knee prostheses (**Chapter 6**), indicates that the host-immune responses might potentially be used as predictor for implant loosening. Since IFNγ showed the highest contribution to the observed association, this might be a potential "loosening" biomarker. Some studies [66-71] have reported on the role of IFNγ in bone remodelling, a continuous dynamic process, which is present around implants, leading to either implant fixation or loosening. Nevertheless, the exact role of IFNγ in these responses has not yet been fully established, since both *in vitro* studies investigating the effect of IFNγ on osteoblastogenesis or osteoclastogenesis and in vivo studies on IFNγ and bone remodelling have reported inconclusive results.[66-71] For that matter, biomarker panels instead of single biomarkers, are likely to have far greater potential to predict, diagnose and monitor the progression of peri-prosthetic osteolysis

which ultimately results in aseptic implant loosening.[72] Future studies using biomarker panels might be able to identify patients with a specific cytokine profile upon stimulation of their innate immune system, which could potentially predict a patient's susceptibility to aseptic loosening. Eventually, identification of patients' specific host-immune responses to implant material could help to develop a preoperative prediction model for implant failure. This may also guide patient's follow-up moments (i.e. more frequent if at risk in order to prevent gross bone loss and subsequent peri-prosthetic fracture) as well as patient's assessment preoperatively, taking patients' specific host-immune responses into the shared decision making process for choosing for joint replacement surgery or a more conservative approach in osteoarthritis patients.

As the study in **Chapter 6** was the first study to investigate the relation between an individual innate immune response and early migration of knee prostheses, future studies have to confirm our found association. In the current study, the host-immune response was determined using stimulation of TLR2, which has been shown to be easily reproducible and not to suffer from possible contamination of endotoxins on wear debris particles.[73, 74] However, particle size or type of material influences the secreted inflammatory cytokine profile [58] and as such stimulation with wear debris particles would provide a more realistic approach investigating the association of host-immune responses and prosthesis migration. Therefore, future studies using wear debris particles should be conducted. Due to heterogeneity in patient- and prosthesis characteristics within our study, pin-pointing possible confounders in the observed association was impossible. Nevertheless, the absence of the association between IFNγ and early migration in patients receiving the Nexgen type of knee prosthesis is an indication that prosthesis characteristics might be important in the observed association. Previous studies already showed that type of fixation or prosthesis design are of influence on migration and failure rate of prostheses.[62, 75] Future association studies, with more homogenous patient and prosthesis characteristics, are therefore essential to clarify the link between individual host immune responses and prosthesis migration and ultimate implant failure due to loosening.

General conclusions and future perspectives

The findings in this thesis support the significant role of macrophages in the aseptic loosening process of orthopaedic implants. Variation regarding the presence of M1- and M2-macrophages within the peri-prosthetic interface tissue implies a potentially important contribution of macrophage polarization in the loosening process. Nevertheless, besides macrophages, the role of fibroblasts and osteoblasts in the mechanism of loosening should not be underestimated and warrants further investigation. Furthermore, the demonstrated osteogenic potential of peri-prosthetic interface tissue cells accentuates the regenerative capacity of this tissue, which provides a (new) opportunity to interfere with the altered balance (i.e. imbalance) in bone remodelling in aseptic loosening.

The large inter-tissue differences observed at all levels in all studies within this thesis, as well as described in other studies investigating the loosening process, clearly show the complexity of the loosening process. Nevertheless, this thesis provides clues for alternative therapeutic strategies to interfere with the loosening process as such and shows the potential for the use of specific host-immune responses for (early) detection and possibly prediction of loosened implants. However, further research into these directions is warranted.

General recommendations for future research are to mimic as much as possible in vivo like experimental settings, for that matter the use of the complete peri-prosthetic interface tissue, for example in organ cultures, in combination with the presence of wear particles, inflammatory cells and resorptive cells might be an option to be explored. Moreover, a sufficient sample size and detailed information on patient- and prosthesis characteristics are needed to improve the generalizability of the results.

Identification of the specific cell type(s) which possess(es) osteogenic capacity within the peri-prosthetic interface tissue might help to more specific stimulate osteogenesis. In addition, pro-osteogenic compounds other than BMP and/or GIN, like compounds influencing the Hedgehog or IGF signalling, might induce a more potent stimulation of osteogenesis of the peri-prosthetic interface tissue. Ultimately one universal osteogenic stimulus could be found, although it is more likely, to find different patient more specific osteogenic stimuli in a personalized medicine approach.

The effect of modulation of macrophage subtypes should be delineated into more detail. Converting pro-inflammatory M1-macrophages to an anti-inflammatory pro-tissue healing M2 phenotype [22, 76] might reduce the inflammatory response to wear debris particles and is thus a possible target for an early intervention in the loosening process. In this respect, specialized pro-resolving lipid mediators (SPMs)[77, 78] could be interesting to investigate, as SPMs are able to modulate the inflammatory response to biomaterials through M2 macrophage polarization.[79]

Early intervention can only be realized when loosening is detected at an early stage. With this respect, the use of biomarker panels to predict a patient's susceptibility to aseptic loosening should be studied. In addition, large prospective association studies are needed to further elucidate the link between individual host immune responses and the failure, due to aseptic loosening, of implants.[59, 80, 81]

Continuing research on the biological responses around aseptic loosened prostheses will provide a rationale for better understanding aseptic loosening of orthopaedic implants. Ultimately this knowledge provides the basis for the development of therapeutic strategies for the treatment of loosened prostheses at an earlier stage, before gross loosening due to severe osteolysis is present. In the end, this will hopefully result in the redundancy of extensive revision surgery, with high risk of morbidity to the patient might be prevented.

References

1. *SKAR*. Annual reports. The Swedish knee arthroplasty register. Available from: http://www.myknee.se/en/publications/annual-reports.
2. *SHAR*. Annual reports. The Swedish hip arthroplasty register. Available from: http://www.shpr.se/en/Publications/Documents-Reports.
3. *LROI*. Annual reports. Insight into quality of orthopaedic care in the Netherlands. Available from: http://www.lroi.nl/en/annual-reports.
4. *2007 AAOS/NIH osteolysis and implant wear: biological, biomedical engineering, and surgical principles.*J Am Acad Orthop Surg, 2008. 16(Supplement 1): p. 128.
5. Gallo, J., et al., *Contributions of human tissue analysis to understanding the mechanisms of loosening and osteolysis in total hip replacement.* Acta Biomater, 2014. **10**(6): p. 2354-66.
6. Goodman, S.B., et al., *Cellular profile and cytokine production at prosthetic interfaces. Study of tissues retrieved from revised hip and knee replacements.* J Bone Joint Surg Br, 1998. **80**(3): p. 531-9.
7. Zreiqat, H., et al., *Macrophages at the skeletal tissue-device interface of loosened prosthetic devices express bone-related genes and their products.* J Biomed Mater Res A, 2003. **65**(1): p. 109-17.
8. Al-Saffar, N., et al., *Assessment of the role of GM-CSF in the cellular transformation and the development of erosive lesions around orthopaedic implants.* Am J Clin Pathol, 1996. **105**(5): p. 628-39.
9. Koulouvaris, P., et al., *Expression profiling reveals alternative macrophage activation and impaired osteogenesis in periprosthetic osteolysis.* J.Orthop.Res., 2008. **26**(1): p. 106-116.
10. Morawietz, L., et al., *Proposal for a histopathological consensus classification of the periprosthetic interface membrane.* J Clin Pathol, 2006. **59**(6): p. 591-7.
11. Mavrogenis, A.F., et al., *Biology of implant osseointegration.* J Musculoskelet Neuronal Interact, 2009. **9**(2): p. 61-71.
12. Buckley, C.D., *Why does chronic inflammation persist: An unexpected role for fibroblasts.* Immunol Lett, 2011. **138**(1): p. 12-4.
13. Rao, A.J., et al., *Revision joint replacement, wear particles, and macrophage polarization.* Acta Biomater, 2012. **8**(7): p. 2815-23.
14. Trindade, M.C., et al., *Interferon-gamma exacerbates polymethylmethacrylate particle-induced interleukin-6 release by human monocyte/macrophages in vitro.* J Biomed Mater Res, 1999. **47**(1): p. 1-7.
15. Pajarinen, J., et al., *The response of macrophages to titanium particles is determined by macrophage polarization.* Acta Biomater, 2013. **9**(11): p. 9229-40.
16. Jamsen, E., et al., *Characterization of macrophage polarizing cytokines in the aseptic loosening of total hip replacements.* J Orthop Res, 2014. **32**(9): p. 1241-6.
17. Murray, P.J. and Wynn, T.A., *Protective and pathogenic functions of macrophage subsets.* Nat Rev Immunol, 2011. **11**(11): p. 723-37.
18. Mosser, D.M. and Edwards, J.P., *Exploring the full spectrum of macrophage activation.* Nat Rev Immunol, 2008. **8**(12): p. 958-69.
19. Martinez, F.O., et al., *Macrophage activation and polarization.* Front Biosci, 2008. **13**: p. 453-61.
20. Goodman, S.B., et al., *Novel biological strategies for treatment of wear particle-induced periprosthetic osteolysis of orthopaedic implants for joint replacement.* J R Soc Interface, 2014. **11**(93): p. 20130962.
21. Mantovani, A., Sica, A. and Locati, M., *Macrophage polarization comes of age.* Immunity, 2005. **23**(4): p. 344-6.
22. Rao, A.J., et al., *Local effect of IL-4 delivery on polyethylene particle induced osteolysis in the murine calvarium.* J Biomed Mater Res A, 2013. **101**(7): p. 1926-34.

23. *NJR*. Annual reports. National joint Registry. Available from: http://www.njrcentre.org.uk/njrcentre/Reports,PublicationsandMinutes/Annualreports.
24. Strehle, J., et al., *The outcome of revision hip arthroplasty in patients older than age 80 years: complications and social outcome of different risk groups.* J Arthroplasty, 2000. **15**(6): p. 690-7.
25. de Poorter, J.J., et al., *Gene therapy and cement injection for restabilization of loosened hip prostheses.* Hum Gene Ther, 2008. **19**(1): p. 83-95.
26. Andreykiv, A., et al., *On stabilization of loosened hip stems via cement injection into osteolytic cavities.* Clin Biomech (Bristol, Avon), 2012. **27**(8): p. 807-12.
27. Malan, D.F., Valstar, E.R. and Nelissen, R.G., *Percutaneous bone cement refixation of aseptically loose hip prostheses: the effect of interface tissue removal on injected cement volumes.* Skeletal Radiol, 2014. **43**(11): p. 1537-42.
28. Raaijmaakers, M. and Mulier, M., *Percutaneous in situ cementation of a loose femoral stem.* J Arthroplasty, 2010. **25**(7): p. 1169 e21-4.
29. Hannouche, D., et al., *Ceramics in total hip replacement.* Clin Orthop Relat Res, 2005. **430**: p. 62-71.
30. Bragdon, C.R., et al., *The 2012 John Charnley Award: Clinical multicenter studies of the wear performance of highly crosslinked remelted polyethylene in THA.* Clin Orthop Relat Res, 2013. **471**(2): p. 393-402.
31. Johanson, P.E., et al., *Highly crosslinked polyethylene does not reduce aseptic loosening in cemented THA 10-year findings of a randomized study.* Clin Orthop Relat Res, 2012. **470**(11): p. 3083-93.
32. Joyce, T.J., *CORR Insights(R): the John Charnley Award: highly crosslinked polyethylene in total hip arthroplasty decreases long-term wear: a double-blind randomized trial.* Clin Orthop Relat Res, 2015. **473**(2): p. 439-40.
33. Lavigne, P., et al., *Modulation of IL-1beta, IL-6, TNF-alpha and PGE(2) by pharmacological agents in explants of membranes from failed total hip replacement.* Osteoarthritis Cartilage, 2002. **10**(11): p. 898-904.
34. Ong, S.M. and Taylor, G.J., *Doxycycline inhibits bone resorption by human interface membrane cells from aseptically loose hip replacements.* J Bone Joint Surg Br, 2003. **85**(3): p. 456-61.
35. Syggelos, S.A., et al., *In vitro effects of non-steroidal anti-inflammatory drugs on cytokine, prostanoid and matrix metalloproteinase production by interface membranes from loose hip or knee endoprostheses.* Osteoarthritis Cartilage, 2007. **15**(5): p. 531-42.
36. Shanbhag, A.S., *Use of bisphosphonates to improve the durability of total joint replacements.* J Am Acad Orthop Surg, 2006. **14**(4): p. 215-25.
37. Bain, G., et al., *Activated beta-catenin induces osteoblast differentiation of C3H10T1/2 cells and participates in BMP2 mediated signal transduction.* Biochem Biophys Res Commun, 2003. **301**(1): p. 84-91.
38. Mbalaviele, G., et al., *Beta-catenin and BMP-2 synergize to promote osteoblast differentiation and new bone formation.* J Cell Biochem, 2005. **94**(2): p. 403-18.
39. Chen, G., Deng, C. and Li, Y.P., *TGF-beta and BMP signaling in osteoblast differentiation and bone formation.* Int J Biol Sci, 2012. **8**(2): p. 272-88.
40. Fukuda, T., et al., *Canonical Wnts and BMPs cooperatively induce osteoblastic differentiation through a GSK3beta-dependent and beta-catenin-independent mechanism.* Differentiation, 2010. **80**(1): p. 46-52.
41. Miclea, R.L., et al., *Inhibition of Gsk3beta in cartilage induces osteoarthritic features through activation of the canonical Wnt signaling pathway.* Osteoarthritis Cartilage, 2011. **19**(11): p. 1363-72.
42. Kamiya, N., et al., *Wnt inhibitors Dkk1 and Sost are downstream targets of BMP signaling through the type IA receptor (BMPRIA) in osteoblasts.* J Bone Miner Res, 2010. **25**(2): p. 200-10.

43. Krause, C., et al., *Distinct modes of inhibition by sclerostin on bone morphogenetic protein and Wnt signaling pathways.* J Biol Chem, 2010. **285**(53): p. 41614-26.
44. Muller, C.W., et al., *BMP-2-transduced human bone marrow stem cells enhance neo-bone formation in a rat critical-sized femur defect.* J Tissue Eng Regen Med, 2015.
45. Ishack, S., et al., *Bone regeneration in critical bone defects using three-dimensionally printed beta-tricalcium phosphate/hydroxyapatite scaffolds is enhanced by coating scaffolds with either dipyridamole or BMP-2.* J Biomed Mater Res B Appl Biomater, 2015.
46. Park, J., et al., *Bone regeneration in critical size defects by cell-mediated BMP-2 gene transfer: a comparison of adenoviral vectors and liposomes.* Gene Ther, 2003. **10**(13): p. 1089-98.
47. Jager, M., et al., *Saving Implants BMP-2 Application in Revision Total Hip Surgery.* Int J Biomed Sci, 2006. **2**(2): p. 187-95.
48. Liu, S., et al., *Sclerostin antibody prevents particle-induced implant loosening by stimulating bone formation and inhibiting bone resorption in a rat model.* Arthritis Rheum, 2012. **64**(12): p. 4012-20.
49. Suen, P.K., et al., *Sclerostin monoclonal antibody enhanced bone fracture healing in an open osteotomy model in rats.* J Orthop Res, 2014. **32**(8): p. 997-1005.
50. Heinemann, D.E., et al., *Alkaline phosphatase expression during monocyte differentiation. Overlapping markers as a link between monocytic cells, dendritic cells, osteoclasts and osteoblasts.* Immunobiology, 2000. **202**(1): p. 68-81.
51. Ducy, P., Schinke, T. and Karsenty, G., *The osteoblast: a sophisticated fibroblast under central surveillance.* Science, 2000. **289**(5484): p. 1501-4.
52. Choe, Y., et al., *Continuously generated H2O2 stimulates the proliferation and osteoblastic differentiation of human periodontal ligament fibroblasts.* J Cell Biochem, 2012. **113**(4): p. 1426-36.
53. Yamamoto, K., et al., *Direct conversion of human fibroblasts into functional osteoblasts by defined factors.* Proc Natl Acad Sci U S A, 2015. **112**(19): p. 6152-7.
54. Phillips, J.E., Guldberg, R.E. and Garcia, A.J., *Dermal fibroblasts genetically modified to express Runx2/Cbfa1 as a mineralizing cell source for bone tissue engineering.* Tissue Eng, 2007. **13**(8): p. 2029-40.
55. Glueck, M., et al., *Induction of Osteogenic Differentiation in Human Mesenchymal Stem Cells by Crosstalk with Osteoblasts.* Biores Open Access, 2015. **4**(1): p. 121-30.
56. Siegel, G., et al., *Phenotype, donor age and gender affect function of human bone marrow-derived mesenchymal stromal cells.* BMC Med, 2013. **11**: p. 146.
57. Vanhatupa, S., et al., *Bone Morphogenetic Protein-2 Induces Donor-Dependent Osteogenic and Adipogenic Differentiation in Human Adipose Stem Cells.* Stem Cells Transl Med, 2015. **4**(12): p. 1391-402.
58. Matthews, J.B., et al., *Comparison of the response of primary human peripheral blood mononuclear phagocytes from different donors to challenge with model polyethylene particles of known size and dose.* Biomaterials, 2000. **21**(20): p. 2033-44.
59. Gordon, A., et al., *Individual susceptibility to periprosthetic osteolysis is associated with altered patterns of innate immune gene expression in response to pro-inflammatory stimuli.* J Orthop Res, 2010. **28**(9): p. 1127-35.
60. Wilkinson, J.M., et al., *Polyethylene wear rate and osteolysis: critical threshold versus continuous dose-response relationship.* J Orthop Res, 2005. **23**(3): p. 520-5.
61. Ryd, L., et al., *Roentgen stereophotogrammetric analysis as a predictor of mechanical loosening of knee prostheses.* J Bone Joint Surg Br, 1995. **77**(3): p. 377-83.
62. Pijls, B.G., et al., *RSA prediction of high failure rate for the uncoated Interax TKA confirmed by meta-analysis.* Acta Orthop, 2012. **83**(2): p. 142-7.

63. Valstar, E.R., et al., *Guidelines for standardization of radiostereometry (RSA) of implants.* Acta Orthop, 2005. **76**(4): p. 563-72.
64. Nieuwenhuijse, M.J., et al., *Good diagnostic performance of early migration as a predictor of late aseptic loosening of acetabular cups: results from ten years of follow-up with Roentgen stereophotogrammetric analysis (RSA).* J Bone Joint Surg Am, 2012. **94**(10): p. 874-80.
65. Pijls, B.G., et al., *Early migration of tibial components is associated with late revision: a systematic review and meta-analysis of 21,000 knee arthroplasties.* Acta Orthop, 2012. **83**(6): p. 614-24.
66. Fox, S.W. and Chambers, T.J., *Interferon-gamma directly inhibits TRANCE-induced osteoclastogenesis.* Biochem Biophys Res Commun, 2000. **276**(3): p. 868-72.
67. Takayanagi, H., et al., *T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-gamma.* Nature, 2000. 408(6812): p. 600-5.
68. Madyastha, P.R., et al., *IFN-gamma enhances osteoclast generation in cultures of peripheral blood from osteopetrotic patients and normalizes superoxide production.* J Interferon Cytokine Res, 2000. **20**(7): p. 645-52.
69. Gao, Y., et al., *IFN-gamma stimulates osteoclast formation and bone loss in vivo via antigen-driven T cell activation.* J Clin Invest, 2007. **117**(1): p. 122-32.
70. Duque, G., et al., *Autocrine regulation of interferon gamma in mesenchymal stem cells plays a role in early osteoblastogenesis.* Stem Cells, 2009. **27**(3): p. 550-8.
71. Duque, G., et al., *Interferon-gamma plays a role in bone formation in vivo and rescues osteoporosis in ovariectomized mice.* J Bone Miner Res, 2011. **26**(7): p. 1472-83.
72. Mertens, M.T. and Singh, J.A., *Biomarkers in arthroplasty: a systematic review.* Open Orthop J, 2011. **5**: p. 92-105.
73. Greenfield, E.M., et al., *Bacterial pathogen-associated molecular patterns stimulate biological activity of orthopaedic wear particles by activating cognate Toll-like receptors.* J Biol Chem, 2010. **285**(42): p. 32378-84.
74. Maitra, R., et al., *Endosomal damage and TLR2 mediated inflammasome activation by alkane particles in the generation of aseptic osteolysis.* Mol Immunol, 2009. **47**(2-3): p. 175-84.
75. Nelissen, R.G., Valstar, E.R. and Rozing, P.M., *The effect of hydroxyapatite on the micromotion of total knee prostheses. A prospective, randomized, double-blind study.* J Bone Joint Surg Am, 1998. **80**(11): p. 1665-72.
76. Wang, Y., et al., *Inhibitory effects of recombinant IL-4 and recombinant IL-13 on UHMWPE-induced bone destruction in the murine air pouch model.* J Surg Res, 2013. **180**(2): p. e73-81.
77. Norling, L.V., et al., *Proresolving and cartilage-protective actions of resolvin D1 in inflammatory arthritis.* JCI Insight, 2016. **1**(5): p. e85922.
78. Serhan, C.N., et al., *Lipid mediators in the resolution of inflammation.* Cold Spring Harb Perspect Biol, 2014. **7**(2): p. a016311.
79. Vasconcelos, D.P., et al., *Modulation of the inflammatory response to chitosan through M2 macrophage polarization using pro-resolution mediators.* Biomaterials, 2015. **37**: p. 116-23.
80. Wilkinson, J.M., et al., *Variation in the TNF gene promoter and risk of osteolysis after total hip arthroplasty.* J Bone Miner Res, 2003. **18**(11): p. 1995-2001.
81. Malik, M.H., et al., *Genetic susceptibility to total hip arthroplasty failure--positive association with mannose-binding lectin.* J Arthroplasty, 2007. **22**(2): p. 265-70.

APPENDIX

Nederlandse samenvatting
List of publications
Curriculum Vitae
Dankwoord

8