Cellular and molecular players in vascular and left ventricular remodeling

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Summary

This thesis addresses 3 important areas of ischemic heart disease; 1) Developing novel methods for patient diagnosis and stratification, 2) Understanding the monocytic response, 3) Identifying novel therapeutic targets based on genetic heterogeneity of patients.

Molecular imaging of atherosclerotic plaques

Traditional methods of assessing atherosclerotic disease severity have involved calculating the percentage of vessel stenosis using angiography. Nevertheless, this is an oversimplification that does not include the complex molecular and cellular composition of the plaque. Plaque composition is more reflective of vulnerability of a lesion to rupture. In **Chapter 2** we have developed a novel radiotracer to visualize atherosclerotic lesions with active remodeling by targeting matrix metalloproteinases (MMPs) 2 and 9. We examined various MMP subtype-selective inhibitors that could successfully target MMP2 and 9. The compound with highest inhibitory potency was radiolabeled with [^{123}I], and showed uptake in mouse atherosclerotic lesions. Radiotracer uptake in plaques co-localized with MMP2 and 9 distributions. We concluded to have developed a novel radiotracer suitable for single photon emission computed tomography (SPECT) imaging that could successfully target MMP2 and 9 in mouse atherosclerotic lesions.

In **Chapter 3**, we resynthesized the compound developed in **Chapter 2** by radiolabeling with [^{18}F], a radioisotope suitable for positron emission tomography (PET). In vivo biodistribution experiments in wildtype mice revealed rapid stabilization of the compound in blood. Unfortunately substantial uptake of the radiotracer was seen in bone, suggesting defluorination of the compound. Administration of the radiotracer to atheroprone mice (ApoE-/-) demonstrated localization of the radioligand in lesions corresponding to regions expressing MMP2 and 9. Greater uptake was seen in aortic arches of atheroprone mice than wild type mice and atheroprone mice treated with a selective MMP blocker. Biospecificity of the radiotracer for human MMP2 and 9 was confirmed based on successful binding in *ex vivo* human carotid plaques and storage phosphor imaging. Immunostaining of human carotid lesions showed co-localization of the radiotracer with MMP2 and 9 expression. Binding of the radioligand to MMP2/9 in human carotid lesions was significantly diminished with a selective MMP blocker. We concluded the development of a novel radiotracer suitable for PET that can target MMP2 and 9 in atherosclerotic lesions of both humans and mice.

Progressive coronary artery occlusion due to atherogenesis can result in myocardial ischemia. Monocytes play an important role in both myocardial repair as well as adverse remodeling. In **Chapter 4** we review the factors that modulate monocyte polarization

toward different macrophage phenotypes in the healing myocardium. Modulation of macrophage polarization can have a great impact on myocardial healing following acute myocardial infarction (AMI). Furthermore, monocyte/macrophage subsets have distinct phenotypic and functional roles, whereby pro-inflammatory macrophages can even exacerbate myocardial ischemic damage. Thus, in this chapter we review (pre)clinical studies demonstrating key molecules involved in macrophage polarization which can affect cardiac healing if modulated.

In **Chapter 5**, we developed a novel method for three dimensional ex vivo imaging of infiltrating monocyte subsets relative to the entire coronary vasculature. Using an imaging cryomicrotome, we achieved three-dimensional visualization of monocytes relative to the entire coronary network. Methods to quantify exogenous cells in the entire myocardium were presented. We further demonstrated a novel method to detect multiple cell populations with the use of multi-color fluorescence imaging. Methods to quantify the transmural distribution of infiltrating exogenous monocytes in ischemic myocardium were also shown. We also detected collateral connections in conjunction with regions of exogenous monocyte infiltration. In this chapter we developed a novel means to effectively visualize and quantify monocyte distribution relative to the coronary network.

We used the methods described in **Chapter 5** to examine the transmural distribution of monocyte subpopulations in ischemic myocardium in **Chapter 6**. We showed selective sub-epicardial localization of both inflammatory and reparative monocyte subgroups. Angiogenic activity was also seen in the sub-epicardial and mid-myocardial regions, coinciding with regions consisting of the largest monocyte infiltration. These results suggest that myocardial tissue repair begins at the sub-epicardium. This was also the first study to examine heterogeneous monocyte populations in rabbits. Selective homing of each monocyte group in ischemic myocardium was also supported by different migratory properties for each monocyte subpopulation.

Genetic heterogeneity in collateral vessel growth

Progressive occlusion of coronary arteries due to atherosclerotic plaque development results in a pressure gradient in the downstream coronary network, and thereby intensifying blood flow through pre-existing collateral arteries. A large collateral network is important for preservation of myocardial function. Nonetheless, genetic heterogeneity predisposes some coronary artery disease patients to have less collateral artery development.

In **Chapter 7** we identified circulating microRNAs (miRNAs) that were associated with low collateral capacity. Chronic total occlusion (CTO) patients with low collateral capacity demonstrated significantly higher expression of miR423-5p, miR10b, miR30d and miR126 relative to high collateral capacity patients. Furthermore, these select miRNAs could be utilized as circulating biomarkers to distinguish between patients with insufficient or sufficient collateralization. We also found greater expression of miR30d and miR126 in CTO patients relative to healthy individuals. In this chapter we concluded the presence of differential circulating miRNA expression in patients with varying levels of collateralization, and that these miRNAs could be used as circulating biomarkers.

In **Chapter 8** we examined intracellular miRNAs associated with the degree of collateralization. MiRNA was isolated from various monocyte/macrophage phenotypes (freshly isolated monocytes, monocytes cultured without stimulant, or stimulation with lipopolysaccharide [LPS], interferon gamma [IFNγ], interleukin 4 [IL4], or transforming growth factor beta-1 [TGFβ1]). Next generation sequencing and subsequent validation by quantitative polymerase chain reaction revealed significantly decreased expression of miR339-5p in all stimulated monocyte/macrophage phenotypes (LPS, IFNγ, IL4 and TGFβ1) of patients with low collateral capacity. Comparative ingenuity pathway analysis of differential messenger RNA expression data between high and low collateral capacity patients with predicted gene targets of miR339-5p showed a significant association with the STAT3 pathway. Furthermore, a regulatory role for the STAT3 pathway was predicted.

In **chapter 9**, we discuss our findings and conclusions in the context of current developments in cardiovascular disease research and finally propose directions for future studies.

Samenvatting

Atherosclerose (aderverkalking) is een complexe aandoening. Deze aandoening kan worden beschreven door te kijken naar de atherosclerotische plaque zelf maar ook naar de gevolgen achter deze plaque, zoals myocardiale ischemie, monocyteninfiltratie, groei van collaterale vaten en remodellering van het vaatbed. Dit proefschrift zal zich richten op een piljers van ischemische hartziekten door het ontwikkelen van nieuwe diagnostische hulpmiddelen, het begrijpen van de inflammatoire infiltraten, samen met het identificeren van nieuwe therapeutische targets voor ischemische hartziekten. Hieronder zal ik onze belangrijkste bevindingen en vragen die zijn ontstaan uit ons werk bespreken.

Moleculaire beeldvorming van atherosclerotische plaques

De traditionele manier om de ernst van atherosclerotische plaques te bepalen is door het berekenen van het percentage stenose op een angiogram. Dit is echter een simplificatie van de werkelijkheid, waarbij alleen gekeken wordt naar de pathologische lipide afzetting binnen een slagader. De complexe moleculaire en cellulaire samenstelling van de plaque is een betere afspiegeling voor de scheur- gevoeligheid van een plaque. Moleculaire beeldvorming van matrixmetalloproteïnasen (MMPs), in het bijzonder MMP2 en MMP9 subtypes, maakt de detectie van instabiele laesies met actieve matrix remodelling mogelijk. Deze laesies zijn gevoeliger voor scheuren, iets wat mogelijk onopgemerkt zou blijven met traditionele beeldvormingstechnieken.

In **hoofdstuk 2** hebben we door ons te richten op MMP2 en 9, een nieuw contrastmiddel voor moleculaire beeldvorming ontwikkeld om atherosclerotische plaques met actieve matrix destabilisatie te visualiseren. We onderzochten een aantal MMP subtype-selectieve remmers die MMP2 en 9 subtypes kunnen binden. De samenstelling met het hoogste remmende vermogen werd radioactief gelabeld met Jodium-123. Vervolgens konden we de aanwezigheid van deze marker in atherosclerotische laesies van muizen aantonen, voornamelijk in gelatinase subtypes, MMP2 en 9. Er werd gekozen voor Jodium-123, aangezien deze radionuclide bruikbaar is voor het maken van een Single Positron Emission Tomography (SPECT) opname. SPECT-scanners hebben het voordeel dat deze aanwezig zijn in de meeste ziekenhuizen. Bovendien heeft Jodium-123 een gunstige halfwaardetijd van 13,2 uur, waardoor er geen noodzaak is voor een speciale eigen productie faciliteit.

In **hoofdstuk 3** hebben we de in hoofdstuk 2 ontwikkelde samenstelling nogmaals gesynthetiseerd en gelabeld met radioactief Fluor-18, een radionuclide geschikt voor Positron Emissie Tomografie (PET). Deze nucleaire beeldvorming modaliteit biedt het voordeel van een betere ruimtelijke resolutie. Ook deze nieuwe verbinding werd

succesvol opgenomen in atherosclerotische plaques van muis en mens, met behoud van specificiteit voor MMP2 en 9. De biologische verdeling van deze verbinding in wild-type muizen toonde echter een aanzienlijke opname van de radionuclide in het bot.

Infiltrerende monocyten in het ischemische myocardium

Distaal van een laesie kan geleidelijke vernauwing van een kransslagader leiden tot ischemische schade in het myocard. Monocyten zijn een van de eerste cellulaire infiltraten die bij het ontstaan van myocardiale ischemie aanwezig zijn en dragen bij aan de genezing en herstel van het myocard met negatieve remodelling. Het evenwicht tussen ontstekingsbevorderende en herstellende monocyten is belangrijk om therapeutische en pathologische resultaat te reguleren.

Bij ischemische hartschade als gevolg van een acute of progressieve vernauwing van een kransslagader vindt er een opregulatie van chemokinen in het myocard plaats, welke circulerende monocyten aantrekken en aldaar differentiëren in macrofagen. In **hoofdstuk 4** geven wij een overzicht van de factoren die betrokken zijn bij deze differentiatie. Talrijke studies, zowel preklinische als klinische, hebben aangetoond dat modulatie van een subset van macrofagen de genezing van het myocard kunnen beïnvloeden na een acuut myocard infarct (AMI). Klinische studies hebben ook een duidelijk spatiotemporeel patroon van infiltrerende monocyten in het infarct gebied van het myocardium aangetoond [4]. Bovendien kan langdurige aanwezigheid van pro-inflammatoire macrofagen leiden tot een uitbreiding van de ischemische schade [5, 6]. Daarom is het van belang om de bijdrage van een monocyt subset te begrijpen voor de ontwikkeling van therapeutische interventies.

Huidige cellulaire beeldvormingstechnieken hebben onvoldoende ruimtelijke resolutie voor een gedetailleerde driedimensionale (3D) afbeeldingen van infiltrerende monocyten / macrofagen ten opzichte van het vaatbed. In **hoofdstuk 5** hebben we een nieuwe werkwijze ontwikkeld voor het 3D en ex vivo weergeven van infiltrerende monocyten subsets in relatie tot het gehele coronaire vasculaire netwerk in het myocard. Met behulp van 3D episcopische fluorescerende cellulaire cyromicrotome beeldvorming konden we monocyten in een 3D vlak ten opzichte van het coronaire vaatbed met een micrometer resolutie weergeven. Wij ontwikkelden een methode om infiltrerende cellen te kwantificeren ten opzicht van het coronaire vaatbed. Verder toonde wij succesvol aan dat met behulp van meer kleurige fluorescente beeldvorming gelijktijdig meerdere populaties van cellen gedetecteerd kunnen worden. Verder hebben we enkele methoden voorgesteld om de transmurale verdeling van deze monocyten in een ischemische myocard na progressieve coronaire occlusie te kwantificeren. Een alternatieve methode van visualisatie van monocyten en macrofagen op orgaanniveau is mogelijke met behulp van magnetische resonantie beeldvorming (MRI). Moleculaire beeldvormingsmodaliteiten

maken gebruik van de fagocyterende eigenschap van monocyten om deze cellen te labelen. Hierna worden zij opnieuw in de bloedbaan van de patient gespoten voor de detectie van monocyt infiltratie in reumatoïde artritis, atherosclerose en myocardinfarct. Deze werkwijze heeft het voordeel van real-time beeldvorming, maar is ook beperkt door de beperkte ruimtelijke resolutie, signaalverval en het ontbreken van simultane reconstructie van de vasculaire structuur. De methode die we in **hoofdstuk 5** hebben beschreven, 3D episcopische fluorescerende cellulaire cyromicrotome beeldvorming, is een geschikte methode voor de validatie van klinische beeldvormende modaliteiten. Hoge-resolutie visualisatie van monocyt subgroepen op orgaanniveau en in relatie tot de vaatstructuur kan helpen om de bijdrage van deze cellen te bepalen ten opzichte van coronaire neovascularisatie en myocardiale ischemie.

In hoofdstuk 6 hebben we gebruik gemaakt van de in **hoofdstuk 5** beschreven methode om de transmurale verdeling van monocyt subpopulaties in reactie op progressieve kransslagader occlusie te onderzoeken. In deze studie toonden we selectieve sub-epicardiale lokalisatie van zowel inflammatoire en herstellende monocyt subgroepen aan. Ook bevestigden wij de aanwezigheid van angiogene activiteit in de sub-epicardiale en mid-myocardiale gebieden, wat ook de gebieden waren met de hoogste monocyt infiltratie van beide subpopulaties. Tezamen suggereerden deze bevindingen dat het myocardiale weefselherstel subepicardiaal begint. De transmurale heterogene verdeling van monocyt subgroepen zou kunnen worden veroorzaakt door verschil in doorbloeding, wat leidt tot een verschil in ischemische schade en verspreiding van chemokinen. Deze verschillen zouden uiteindelijk de verdeling van de infiltrerende monocyten kunnen beïnvloeden. Toekomstige onderzoek kan zich richten op het onderzoeken van de spatiotemporele patronen van ischemische herstel ten opzichte van de monocyt infiltratie, neovascularisatie en chemokine distributie. Dit zou kunnen leiden tot beter inzicht in de bijdrage van monocyt subgroepen aan bloedvat en het linker ventrikel herstel en remodellering.

Genetische heterogeniteit in collaterale groei

Geleidelijke afsluiting van kransslagaders door atherosclerose leidt tot een drukval in het arteriële vaatbed, stroomafwaarts van de afsluiting. Deze drukgradiënt verhoogt de bloedstroom door reeds bestaande collaterale arteriën, die zich daardoor een alternatieve route heeft weten te vinden. Een uitgebreid collateraal netwerk is geassocieerd met het behoud van de myocardiale functie en verminderd de gevoeligheid voor cardiale complicaties bij patiënten. Desalniettemin lijdt genetische heterogeniteit bij sommige patiënten tot een slechte prognose.

In **hoofdstuk 7** identificeerden we circulerende microRNAs (miRNAs) die verschillend tot expressie werden gebracht bij patiënten met een lage collaterale flow. Patiënten met een chronische totale occlusie (CTO) in combinatie met een lage collaterale flow toonde een significant verhoogde expressie van miR423-5p, miR10b, miR30d en miR126. Daarnaast toonde we aan dat deze miRNAs kunnen worden gebruikt als biomarkers om onderscheid te maken tussen patiënten met onvoldoende of voldoende collateralen. Dit is een waardevolle bevinding om patiënten die meer kans hebben op (ernstige) cardiale complicaties en kunnen leiden tot de dood, te identificeren. Vandaag de dag wordt het onderscheid tussen patiënten met een hoge en lage collaterale flow gemaakt met behulp van invasieve intracoronaire collaterale flow index (CFI) metingen, of angiografie gradering. De circulerende miRNAs, die geïdentificeerd zijn in **hoofdstuk 7,** zouden van waarde kunnen zijn bij het karakteriseren van een patiënt door middel van een eenvoudig bloedmonster.

Monocyten spelen een centrale rol in de groei en rijping van collaterale vaten. Het miRNA profiel van deze cellen is belangrijk voor de mate van collateraal vorming. In **hoofdstuk 8** onderzochten we het verband tussen intracellulaire miRNAs en de mate van collateraal vorming. Monocyten uit een perifeer bloedmonster van CTO patiënten werden verzameld en verdeeld in zes monocyt / macrofaag stimulatie groepen (vers geïsoleerde monocyten, monocyten kweek met of zonder lipopolysaccharide [LPS] stimulatie, interferon gamma [IFNy], interleukine 4 [IL4] of transforming groeifactor beta-1 [TGFB1]). Door middel van sequencing van alle tot uiting komende miRNAs hebben we verschillende miRNAs geïdentificeerd die tot differentiële expressie kwamen bij patiënten met een hoge versus een lage collaterale flow. Validatie van de geselecteerde miRNAs door middel van kwantitatieve polymerase-kettingreactie toonde een significante verminderde expressie van miR339-5p in alle gestimuleerde monocyten / macrofagen fenotypes (LPS, IFNy, IL4 en TGFB1) van patiënten met een lage collaterale doorbloeding. Dit differentiële expressiepatroon kwam niet tot expressie in de extracellulaire ruimte en is beperkt tot het intracellulaire milieu van de gestimuleerde monocyten / macrofagen. Dit suggereert een intrinsieke rol van miR339-5p en benadrukt het belang van macrofaag polarisatie. Om de mogelijke rol van miR339-5p te begrijpen, hebben we onderzocht welke signaalpaden worden geassocieerd met de voorspelde genen die gereguleerd worden door miR339-5p. We vonden een significant verband met de STAT3 pad. Omdat we niet weten welke type genen zijn op- of neergereguleerd door miR339-5p bij patiënten met verschillende mate van collateraal vorming, keken we ook naar signaalpaden die worden geassocieerd met de genexpressie data waarvan we weten welke genen op- en neergereguleerd zijn. In deze analyse vonden we een significant verband met het STAT3 pad. Dit suggereert een mogelijke rol voor het STAT3 pad in de ontwikkeling van collaterale vaten. Er werd bovendien een mogelijke regulerende rol voor STAT3 pathway werd geïdentificeerd.

Tot slot vatten wij in **hoofdstuk 9** onze bevindingen samen en bespreken de conclusies binnen het kader van de huidige ontwikkelingen in de cardiovasculaire onderzoek. Vervolgens geven we suggesties voor toekomstige onderzoeksrichtingen.

List of Publications

- **Hakimzadeh N**, Elias J, Wijntjens G, Theunissen R, van Weert A, Smulders MW, van den Akker N, Moerland PD, Verberne HJ, Hoebers LP, Henriques JPS, van der Laan AM, Ilhan M, Post M, Bekkers SC, Piek JJ. *Monocytic microRNA profile associated with coronary collateral artery function in chronic total occlusion patients.* Manuscript submitted.

- **Hakimzadeh N**, Molenaar G, de Bruin K, Kooijman E, Spaans A, Piek JJ, Booij J, Windhorst AD, Verberne HJ, van Eck-Smit BLF. *Fluorine-18 labelled molecular imaging ligands targeting matrix metalloproteinases 2 and 9 for visualization of vulnerable atherosclerotic plaques.* Manuscript submitted.

- **Hakimzadeh N***, Pinas VA*, Molenaar G, de Waard V, van Eck-Smit BLF, de Bruin K, Piek JJ, Eersels JLH, Booij J, Verberne HJ, Windhorst AD. *Novel molecular imaging ligands targeting matrix metalloproteinases 2 and 9 for imaging of unstable atherosclerotic plaques. *Authors contributed equally.* Manuscript submitted.

- Van der laan AM, ter Horst EN, **Hakimzadeh N**, Krijnen PA, Robbers LFHJ, Hirsch A, Nijveldt R, Lommerse I, Fontijn RD, Delewi R, Verberne HJ, van Royen N, Zijlstra F, van Rossum AC, van der Schoot CE, Niessen HWM, van der Pouw Kraan TCTM, Horrevoets AJ, Piek JJ. *Adverse left ventricular remodeling following acute myocardial infarction in patients is associated with attenuated type I interferon signaling.* Manuscript in preparation.

- **Hakimzadeh N**, Piek JJ. *MicroRNAs to take the place of collateral flow index measurements and Rentrop scoring? Reply to Papageorgiou et al.* Annals of Translational Medicine. 2016 Aug; 4(15):297.

- **Hakimzadeh N**, van Lier MG, van Horssen P, Daal M, Ha Ly D, Belterman C, Coronel R, Spaan JAE, Siebes M. *Selective subepicardial localization of monocyte subsets in response to progressive coronary artery constriction.* American Journal of Physiology - Heart & Circulatory Physiology. 2016 Jul 1; 311(1): H239-50.

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- **Hakimzadeh N**, Nossent AY, van der Laan AM, Schirmer SH, de Ronde MW, Pinto-Sietsma SJ, van Royen N, Quax PH, Hoefer IE, Piek JJ. *Circulating MicroRNAs Characterizing Patients with Insufficient Coronary Collateral Artery Function.* PLoS One. 2015 Sep 2; 10(9): e0137035.

- **Hakimzadeh N**, van Horssen P, van Lier MG, van den Wijngaard JP, Belterman C, Coronel R, Piek JJ, Verberne HJ, Spaan JA, Siebes M. *Detection and quantification methods of monocyte homing in coronary vasculature with an imaging cryomicrotome.* Journal of Molecular and Cellular Cardiology. 2014 Nov; 76: 196-204.

- **Hakimzadeh N**, Verberne HJ, Siebes M, Piek JJ. *The future of collateral artery research.* Current Cardiology Reviews. 2014 Feb; 10(1): 73-86.

- **Hakimzadeh N**, Piek JJ. *The coronary collateral circulation revisited.* Netherlands Heart Journal. 2013 Mar; 21(3): 144-5.

- **Hakimzadeh N**, Stewart DJ, Courtman DW. *The role of transglutaminase 2 and osteopontin in matrix protein supplemented microencapsulation of marrow stromal cells.* Biomaterials. 2010 Dec; 31(35): 9256-65.

Author Contributions

Chapter 1 **The future of collateral artery research.**

NH, JJP conception and design of manuscript outline; NH drafted manuscript. All authors edited and revised manuscript for important intellectual content; All authors approved final version of manuscript.

Chapter 2 **Novel molecular imaging ligands targeting matrix metalloproteinases 2 and 9 for imaging of unstable atherosclerotic plaques.**

NH, VAP, VdW, BLFvES, HJV and ADW conception and design of research; VAP, KdB, JLHE performed experiments; NH, VAP, KdB analyzed data; NH, VAP interpreted results of experiments; NH, VAP prepared figures; NH, VAP drafted manuscript; All authors edited and revised manuscript for important intellectual content; All authors approved final version of manuscript.

Chapter 3 **Fluorine-18 labelled molecular imaging ligands targeting matrix metalloproteinases 2 and 9 for visualization of vulnerable atherosclerotic plaques.**

NH, AS, KdB, ADW, BLFvES and HJV conception and design of research; NH, GM, KdB, EK, AS performed experiments; NH, GM, KdB, EK analyzed data; NH, GM interpreted results of experiments; NH prepared figures; NH drafted manuscript; All authors edited and revised manuscript for important intellectual content; All authors approved final version of manuscript.

Chapter 4 **Modulators of macrophage polarization influence healing of the infarcted myocardium.**

ENtH conception and design of manuscript outline; ENtH, NH drafted manuscript. All authors edited and revised manuscript for important intellectual content; All authors approved final version of manuscript.

Chapter 5 **Detection and quantification methods of monocyte homing in coronary vasculature with an imaging cryomicrotome.**

NH, RC, HJV, JJP, JAS, and MS conception and design of research; NH, MGvL, MD, and CB performed experiments; NH and PvH analyzed data; NH interpreted results of experiments; NH and PvH prepared figures; NH drafted manuscript; All authors edited and

revised manuscript for important intellectual content; All authors approved final version of manuscript.

Chapter 6 **Selective subepicardial localization of monocyte subsets in response to progressive coronary artery constriction.**

NH, RC, JAS, and MS conception and design of research; NH, MGvL, MD, and CB performed experiments; NH, PvH, MD, and DHL analyzed data; NH interpreted results of experiments; NH, MGvL, PvH, and MD prepared figures; NH drafted manuscript; All authors edited and revised manuscript for important intellectual content; All authors approved final version of manuscript.

Chapter 7 **Circulating microRNAs characterizing patients with insufficient coronary collateral artery function.**

NH, AYN, AMvDL, SHS, NvR, PHAQ, IEH and JJP conception and design of research; NH, AYN, SHS and JJP performed experiments; NH, AYN, SHS analyzed data; MWJdR, SJPS, AYN, PHAQ, IEH contributed reagents/materials/analysis tools; NH interpreted results of experiments; NH prepared figures; NH drafted manuscript; All authors edited and revised manuscript for important intellectual content; All authors approved final version of manuscript.

Chapter 8 **Monocytic microRNA profile associated with coronary collateral artery function in chronic total occlusion patients.**

NH, HJV, LPH, AMvdL, MP, JJP conception and design of research; NH, JE, GW, AvW, RT, MWS, JPSH, MI, SCB, JJP performed experiments; NH analyzed data; NH, PM interpreted results of experiments; NH prepared figures; NH drafted manuscript; All authors edited and revised manuscript for important intellectual content; All authors approved final version of manuscript.

Portfolio

PhD Period: January 2011 – March 2016
PhD Supervisor: Prof. dr. Jan J. Piek
PhD Co-supervisors: Dr. ir. Maria Siebes, Dr. Hein J. Verberne

PhD Training	**Year**	**Workload (ECTS)**
Courses		
AMC World of Science	2011	0.7
Laboratory Animals, Article 9	2011	3.9
Radiation Protection, Protection 5B	2011	1.7
Molecular Imaging, Dublin Ireland	2012	1.5
Cardiovascular Diseases - Vascular Biology	2012	1.5
CTMM Entrepreneurship	2013	0.6
CTMM Starting your own business	2013	0.6
CTMM Intellectual Property	2011	0.6
Seminars and workshops		
Department seminars	2011-2016	3
Journal club	2011-2016	3
Ruysch Lectures	2011-2016	1
(Inter)national Conference Presentations		
CTMM Annual Meeting, Utrecht, Netherlands (poster)	2011	0.5
FASEB, San diego, USA (poster)	2011	0.5
CTMM Annual Meeting, Utrecht (poster)	2012	0.5
CVC, Noordwijkerhout, Netherlands (poster)	2012	0.5
CTMM Annual Meeting, Utrecht, Netherlands (poster)	2013	0.5
IUPS Satellite Symposia, Amsterdam, Netherlands (poster)	2013	0.5
RICS, Noordwijkerhout, Netherlands (poster)	2013	0.5
CVC, Noordwijk Oude, Netherlands (poster)	2013	0.5
Shear Stress Symposium, Rotterdam, Netherlands (poster)	2013	1
Angiogenesis, Amsterdam, Netherlands (poster)	2014	1
FASEB, San diego, USA (poster)	2014	0.5
FASEB, Boston, USA (oral)	2015	1

Teaching

Medicine BSc Project	2013	1
Master Internship	2014	1
Biomedical Science specialization Cardiovascular Disease		
Practicum/Student demonstrations	2012-2015	1

Curriculum Vitae

Nazanin Hakimzadeh was born in Tehran, Iran on November 16, 1982. She and her family moved to Toronto, Canada in 1988. Nazanin was awarded the Western Scholarship of Distinction to attend the University of Western Ontario. In 2006, she completed a Bachelor of Engineering in Biochemical Engineering (cum laude) and a Bachelor of Science in Biology (cum laude). Her Bachelor of Engineering thesis was titled "*Diesel Fuel Desulfurization to Meet New Regulations*" and was awarded first place for Chemical/Biochemical Engineering thesis at the University of Western Ontario, and was awarded third place on the national level. In 2009, Nazanin completed a Master of Applied Science in Biomedical Engineering at the University of Toronto, in the group of Prof. dr. Duncan J. Stewart and Dr. David W. Courtman. Her master's thesis was entitled "*Matrix Supplemented Stem Cell Microencapsulation for Regenerative Medicine*" and was published in the Journal of Biomaterials. In 2009, Nazanin moved to the Netherlands to conduct an internship in the group of Prof. dr. ir. Jos Spaan for vascular and cellular imaging optimization using a 3D episcopic imaging cryomicrotome. In 2011, Nazanin began her Doctorate thesis under the supervision of Prof. dr. J.J. Piek, Prof. dr. ir. Jos Spaan, Dr.ir. M. Siebes and Dr. H.J. Verberne. This work comprises the thesis that is presented in this book.

Acknowledgments

"There, in the chords and melodies, is everything I want to say. The words just jolly it along. It's always been my way of expressing what for me is inexpressible by any other means."

David Bowie

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