Nederlandse samenvatting

Riassunto

List of abbreviations

List of publications

PhD portfolio

Curriculum vitae

Dankwoord

NEDERLANDSE SAMENVATTING

Gewrichtskraakbeenletsel vormt een grote klinische uitdaging in de orthopedie. De vooruitgang in de afgelopen decennia heeft de regeneratie van kraakbeen in de schijnwerpers gezet en de weg vrijgemaakt om de beperkingen van de huidige behandelingen te overwinnen. Om de klinische resultaten te verbeteren en nieuwe behandelstrategieën te ontwikkelen, beoogde dit proefschrift laboratoriummodellen te ontwikkelen die nodig zijn voor het testen van toekomstige behandelingen met biomaterialen zoals hydrogels, om kraakbeenletsel te hertellen. De geschiktheid van hydrogels als 3D-matrix om de ingroei van kraakbeenvoorlopercellen en hun differentiatie te ondersteunen en om biomoleculen te leveren om deze processen te verbeteren, werd geëvalueerd in onze modellen: een celmigratie-test in hydrogelen en een model van een kraakbeen-botexplantaat met een defect dat in kweek mechanisch belast werd in een bioreactor.

Celmigratie speelt een cruciale rol in het vroege proces van weefselherstel. Meerdere biochemische en biofysische factoren, afhankelijk van cel- en weefsel-eigenschappen, beïnvloeden migratie-efficiëntie. Cellen zijn dynamisch gevoelig voor de samenstelling, stijfheid en structuur van het biomateriaal wat gebruikt wordt om het defect te vullen, evenals voor bioactieve gradiënten, die celingroei kunnen versterken. In **Hoofdstuk 2** zijn verschillende formuleringen van hyaluronzuur (HA)-gebaseerde hydrogels in vitro en in vivo getest, met als doel het meest geschikte materiaal te selecteren voor regeneratieve therapieën die herstel door cellen in het lichaam stimuleren. We ontdekten dat veranderingen in mechanische eigenschappen de ingroei en differentiatie van cellen, en dus de productie van kraakbeenmatrix, beïnvloedden. Celingroei was omgekeerd-evenredig gecorreleerd met de stijfheid van de gel. Fibrine-hyaluronzuur (FB/HA)-gels lieten echter altijd de meest celingroei zien in vitro, zowel met als zonder migratiestimulerende factoren, en ook de beste chondrogene differentiatie. Onderhuidse implantatie van een kraakbeen-bot explantaat waarin het defect gevuld was met hydrogel bevestigde dit resultaat en liet endogene celrecrutering zien, zelfs in afwezigheid van migratie-stimulerende factor. Dit benadrukt het belang van de micro-omgeving en de hydrogelmatrix waarin cellen worden gerekruteerd, als een cruciale opstap naar het maken van functionele skeletale weefsels.

Aangezien letsels van kraakbeen en bot in het gewricht vaak optreden in het gewichtdragende gebied, lijkt het intuïtief dat er rekening moet worden gehouden met mechanische belasting als een essentiële factor die het weefselherstel beïnvloedt. Het succes van herstelprocedures die gebruik maken van hydrogels hangt af van het vermogen van cellen en hydrogels om druk- en schuifkrachten tijdens het belasten te weerstaan. In **Hoofdstuk 3** werd aangetoond dat complexe beweging bestaande uit druk-en schuifkrachten, met lage intensiteit uitgevoerd op FB/HA-gels met kraakbeencellen,

een positief effect had op het behoud van het kraakbeencel fenotype en de productie van kraakbeenmatrix. De reactie van cellen op deze krachten was effectiever wanneer de groeifactor FGF-18v werd toegevoegd aan ons in vitro kweekmodel. We toonden aan dat kraakbeencellen zich konden aanpassen aan een veranderende biochemische en mechanische micro-omgeving door de hoeveelheid en samenstelling van de kraakbeenmatrix aan te passen.

Conventionele bioreactorstudies voor kraakbeenregeneratie houden vaak geen rekening met het omliggende kraakbeen- en botweefsel. Met het doel om hydrogels geschikt te maken voor het herstel van kraakbeen- en botletsels in patienten, pasten we mechanische stimuli toe op hydrogels in een defect omgeven door kraakbeen en bot, om hun functioneren te evalueren. In **Hoofdstuk 4** hebben we eerst de ontwikkeling en validatie beschreven van een laboratorium model voor kraakbeen-botexplantaten onder mechanische druk- en schuifkrachten. Dit model biedt een representatieve fysiologische gewrichtachtige omgeving om reproduceerbare voorspellingen te doen van de prestatie van biomaterialen en van de effectiviteit van biomolecuulbehandeling. Dit model werd in **Hoofdstuk 5** gebruikt om de weefselherstelreacties na het inbrengen van een hydrogel in een kraakbeenbotdefect beter te begrijpen. Mechanische belasting werd geïdentificeerd als remmer van celingroei in de wondplaats, wat suggereert dat het aanbrengen van mechanische stimuli op een vroeg tijdstip niet nodig was. Interessant is dat het toevoegen van celmigratie-stimulerende factoren dit remmende effect niet tegenwerkte. Bovendien bood dit model de mogelijkheid om een potentiële celmigratieroute te ontdekken op de grenslaag tussen kraakbeen en bot; cellen die aanwezig zijn in het subchondrale bot of in het verkalkte kraakbeen namen in hoge mate deel aan het herstel van de defecten, wat het belang van de kraakbeen-bot eenheid benadrukt bij het evalueren van strategieën voor het herstel van gewrichtsletsel. De afwezigheid van een sterke celreactie op externe mechanische krachten op het vroege tijdstip gaf aan dat het afstemmen van signalen in de loop van de tijd nodig is om herstelprocessen te optimaliseren. Deze resultaten onderstrepen de essentie van het gebruik van representatieve modellen om inzicht te krijgen in de periode om dynamische belasting toe te passen na chirurgische ingrepen.

In dit proefschrift hebben we belangrijke aanwijzingen gegeven voor toekomstige verbetering van de behandeling van kraakbeenletsels met hydrogels. In **Hoofdstuk 6** wordt gepostuleerd dat een multifactoriële benadering cruciaal is om de huidige strategieën te verbeteren. Voor succesvol herstel van kraakbeenletsel het hydrogelontwerp nodig om georganiseerde endogene celingroei en differentiatie mogelijk te maken nauwkeurig worden afgestemd. Hierbij verdienen het nabootsen van de mechanische eigenschappen van het natuurlijke weefsel en een goed gecoördineerde mechanische belasting, bijzondere aandacht, derhalve de optimale omstandigheden te bieden voor kraakbeenregeneratie.

RIASSUNTO

La lesione della cartilagine articolare rappresenta una sfida significativa in clinica nel l'ambito ortopedico. I progressi degli ultimi decenni puntano i riflettori sulla rigenerazione della cartilagine, aprendo così la strada al superamento degli attuali limiti nei trattamenti odierni. Al fine di migliorare i risultati clinici e sviluppare nuove strategie di trattamento, questa tesi mira a fornire i modelli necessari per testare futuri approcci acellulari di riparazione assistita tramite l'utilizzo di biomateriali nell'articolazione del ginocchio. Gli idrogel, usati come modelli 3D, sono stati testati al fine di valutare la loro capacità di supportare l'infiltrazione di cellule condroprogenitrici e la loro differenziazione, nonché di rilasciare biomolecole per rafforzare questi processi valendosi di un saggio di migrazione basato sugli sferoidi e di un espianto di tessuto osteo-cartilagineo con difetto osteocondrale stimolato meccanicamente *ex vivo*.

La migrazione cellulare ha un ruolo critico nel processo iniziale di riparazione del tessuto a seguito dell'impianto di biomateriali, e la sua efficienza è influenzata dal microambiente attraverso una serie di fattori biochimici e biofisici che dipendono dalle proprietà cellulari e dalla matrice extracellulare utilizzata (ECM). Le cellule sono molto sensibili sia alla composizione del biomateriale che alla sua rigidità e struttura, nonché ai gradienti bioattivi, i quali possono potenziare la locomozione ed il movimento cellulare. Nel **Capitolo 2** sono state testate *in vitro* e *in vivo* diverse formulazioni di idrogel a base di acido ialuronico (HA) e diverse densità di reticolazione, con l'obiettivo di selezionare il gel più adatto a favorire terapie rigenerative che sfruttino le cellule endogene residenti. Abbiamo dimostrato che cambiamenti nelle proprietà meccaniche dell'idrogel influenzano la diffusione, la migrazione e la differenziazione cellulare. La coniugazione della fibrina con l'acido ialuronico era molto differente in termini di formulazione dell'idrogel e di concentrazione percentuale peso/volume rispetto al gel di HA-Tiramina ed alle sue diverse cinetiche di reticolazione (utilizzando 150, 300 o 600 µM di perossido di idrogeno, H_2O_2). Abbiamo osservato che la variazione della concentrazione di uno dei due agenti reticolanti il H_2O_2, mantenendo costante la concentrazione del gel di HA-Tiramina e dell'altro agente reticolante la perossidasi di rafano, è stato il principale fattore che ha influenzato sia la migrazione cellulare che la sintesi della matrice durante la condrogenesi delle cellule staminali mesenchimali. La migrazione era inversamente correlata con il modulo elastico del gel di HA-Tiramina in presenza del fattore di crescita derivato dalle piastrine BB. Ciò significa che il gel più morbido ha favorito una maggiore migrazione rispetto al gel più rigido. Tuttavia, gli idrogel di fibrina ed acido ialuronico (FB/HA) hanno sempre mostrato il più alto potenziale di migrazione cellulare, sia in presenza che in assenza dell'agente chemioattrattante *in vitro*, ed hanno anche favorito la differenziazione condrogenica. Il modello di espianto osteocondrale, impiantato sottocute *in vivo*, ha ulteriormente confermato il reclutamento di cellule endogene

nei gel, anche in assenza del fattore stimolante. Questo sottolinea l'importanza del microambiente e dell'idrogel usato come substrato nel quale vengono reclutate le cellule, rappresentando un cruciale trampolino di lancio verso l'ingegneria tissutale funzionale del tessuto musculare scheletrico.

Poiché i difetti dell'unità osteocondrale spesso si verificano nella regione che sostiente il peso, appare intuitivo che la riparazione delle lesioni focali dovrebbe tenere conto del carico meccanico come fattore essenziale influenzante la rigenerazione dei tessuti osteocondrali. Il successo dei trattamenti basati sull'utilizzo di biomateriali dipende dalla capacità delle cellule e degli idrogel di sostenere le forze di compressione e di taglio durante il carico. Nel **Capitolo 3** è stato dimostrato che l'applicazione meccanica del carico, ad orientamento biassiale ed a bassa intensità, sui gel di FB/HA miscelati con condrociti ha avuto un effetto positivo sul mantenimento del fenotipo condrogenico e sulla produzione della matrice cartilaginea. La meccanotrasduzione si è rivelata più efficace quando una variante esogena del fattore di crescita dei fibroblasti 18 (FGF-18v) è stata aggiunta al nostro modello nel mezzo di coltura *in vitro*, influenzando in modo interdipendente il metabolismo cellulare ed aumentando la qualità del tessuto costruito cartilagineo. Abbiamo dimostrato che i condrociti potrebbero sinergicamente adattarsi ad un microambiente biochimico e meccanico, modulando la quantità di ECM, down regolando enzimi degradanti la matrice e promuovendo una superficie articolare funzionale.

Studi convenzionali sui bioreattori che promuovono la rigenerazione della cartilagine spesso non tengono conto del tessuto osteocondrale. Con l'obiettivo di rendere clinicamente possibili le tecnologie di riparazione del difetto osteocondrale assistito da biomateriali, abbiamo applicato stimoli meccanici sui gel in un ambiente più confinato in modo da valutare la loro funzione all'interno del tessuto, proprio come accade in vivo. Nel **Capitolo 4** abbiamo descritto per la prima volta lo sviluppo e la validazione di un modello di difetto osteocondrale *ex vivo* sotto carico di compressione e taglio, che mima l'ambiente articolare del ginocchio al fine di consentire una riproducibile previsione delle prestazioni dei biomateriali e dell'efficacia del trattamento dei fattori di crescita utilizzati. Questo modello è stato utilizzato nel **Capitolo 5** per migliorare la nostra comprensione dei meccanismi che regolano la riparazione mediata da cellule endogene quando assistita da biomateriali in seguito a trauma. Il carico meccanico è stato identificato come un inibitore dell'infiltrazione cellulare nella zona della ferita, suggerendo che l'aggiunta di stimoli meccanici in questo sistema all'inizio del time point non era necessaria. È interessante notare che l'aggiunta di fattori chemiotattici non ha contrastato questo effetto inibitorio. Inoltre, il modello ha fornito l'opportunità di scoprire una potenziale via di migrazione cellulare nello strato di interfaccia dell'espianto osteocondrale; le cellule presenti nell'osso subcondrale o nella cartilagine calcificata partecipano fortemente al ripristino del difetto, evidenziando l'importanza

dell'unità osteocondrale nella valutazione delle strategie di riparazione dei tessuti articolari. L'assenza di una forte risposta cellulare in seguito all'applicazione di forze meccaniche esterne all'inizio del time point ha indicato che una fine regolazione dei segnali extracellulari nel tempo sarebbe necessaria per ottimizzare il loro utilizzo per modulare il processo decisionale cellulare. Questi risultati sottolineano l'essenza dell'uso di modelli rappresentativi per fornire un'idea sui tempi ottimali in cui applicare il carico dinamico dopo un intervento chirurgico.

In questa tesi abbiamo fornito importanti indizi per il miglioramento futuro di approcci acellulari di riparazione della cartilagine tramite l'utilizzo di biomateriali. Nel **Capitolo 6** si postula che un approccio multifattoriale è fondamentale per migliorare le strategie attuali. Per ottenere con successo la rigenerazione della cartilagine, la messa a punto del design di un idrogel richiede particolare attenzione al fine di consentire l'infiltrazione organizzata delle cellule endogene e la loro differenziazione, mentre riepiloga le proprietà meccaniche del tessuto nativo. In aggiunta il carico meccanico deve essere ben coordinato per fornire le condizioni ottimali per migliorare il rimodellamento della cartilagine.

LIST OF ABBREVIATIONS

AA-2-P	Ascorbic acid-2-phosphate
ACAN	Aggrecan
ACI	Autologous chondrocytes implantation
α-MEM	alpha-Minimum Essential Medium
AMIC	autologous matrix-induced chondrogenesis
BMSC	bone marrow mesenchymal stromal cell
CCM	Complete chondrogenic medium
CDFA-SE	Carboxyfluorescein Diacetate Succinimidyl Ester
CCL5	Chemokine ligand 5 or RANTES
CL	Cartilage layer
COL1A2	Collagen 1
COL2A1	Collagen 2
COL10	Collagen 10
COMP	Cartilage oligomeric matrix protein
DMEM-HG	Dulbecco's modified Eagle's medium
ECM	Extracellular Matrix
FBS	Fetal bovine serum
FGF-2	Fibroblast growth factor 2
FGF-18v	Fibroblast growth factor 18 variant
FGFR-3	Fibroblast growth factor receptor-3
FB/HA	Fibrin-Hyaluronan
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
G'	Storage modulus
G''	Loss modulus
HA	Hyaluronan
HA-Tyr	Hyaluronan-Tyramine
H_2O_2	Hydrogen peroxide
HRP	Horseradish peroxidase
ITS+	Insulin, transferrin and selenium
LDH	Lactate dehydrogenase
MACI	Matrix-induced/assisted ACI
MMPs	Matrix Metalloproteinases
MSCs	Mesenchymal stromal cells
OA	Osteoarthritis
PBS	Phosphate buffered saline
PDFG-BB	Platelet-derived growth factor BB
PEG	Polyethylene glycol

PGA	Polyglycolic acid
PLA	Polylactic acid
PRG4	Proteoglycan 4/Lubricin
P/S	Penicillin/Streptomycin
PU	Polyurethane
qRT-PCR	Quantitative real-time polymerase chain reaction
RPLP0	Ribosomal protein lateral stalk sub-unit P0
RGD	Arginin-Glycin-Aspartate
SB	Subchondral bone
SDF-1	Stromal cell-derived factor 1
SF	Serum free medium
sGAG	sulphated-Glycosaminoglycan
TGF-β1	Transforming growth factor beta 1
TKA	Total knee arthroplasty
VCAN	Versican

LIST OF PUBLICATIONS

[1] **M.L. Vainieri**, A.M. Blagborough, A.L. MacLean, M.L. Haltalli, N. Ruivo, H.A. Fletcher, M.P. Stumpf, R.E. Sinden, C.L. Celso, Systematic tracking of altered haematopoiesis during sporozoite-mediated malaria development reveals multiple response points, Open Biol 6(6) (2016).

[2] **M.L. Vainieri**, D. Wahl, M. Alini, G. van Osch, S. Grad, Mechanically stimulated osteochondral organ culture for evaluation of biomaterials in cartilage repair studies, Acta biomaterialia 81 (2018) 256-266.

[3] A. Lolli, K. Sivasubramaniyan, **M.L. Vainieri**, J. Oieni, N. Kops, A. Yayon, G. van Osch, Hydrogel-based delivery of antimiR-221 enhances cartilage regeneration by endogenous cells, Journal of controlled release : official journal of the Controlled Release Society 309 (2019) 220-230.

[4] **M.L. Vainieri**, A. Lolli, N. Kops, D. D'Atri, D. Eglin, A. Yayon, M. Alini, S. Grad, K. Sivasubramaniyan, G. van Osch, Evaluation of biomimetic hyaluronic-based hydrogels with enhanced endogenous cell recruitment and cartilage matrix formation, Acta biomaterialia 101 (2020) 293-303.

[5] B.P. Antunes, **M.L. Vainieri**, M. Alini, E. Monsonego-Ornan, S. Grad, A. Yayon, Enhanced chondrogenic phenotype of primary bovine articular chondrocytes in Fibrin-Hyaluronan hydrogel by multi-axial mechanical loading and FGF18, Acta biomaterialia 105 (2020) 170-179.

[6] F. Colella, J.P. Garcia, M. Sorbona, A. Lolli, B. Antunes, D. D'Atri, F.P.Y. Barré, J. Oieni, **M.L. Vainieri**, L. Zerrillo, S. Capar, S. Häckel, Y. Cai, L.B. Creemers, Drug delivery in intervertebral disc degeneration and osteoarthritis: Selecting the optimal platform for the delivery of disease-modifying agents, Journal of controlled release : official journal of the Controlled Release Society (2020).

[7] S.W. Myriam L.R. Haltalli, Nicola K. Wilson, Kira Eilers, Alexander Lipien, Heather Ang, Flora Birch, Sara Gonzalez Anton, Chiara Pirillo, Nicola Ruivo, **Maria L. Vainieri**, Constandina Pospori, Robert E. Sinden, Tiago C. Luis, Jean Langhorne, Ken R. Duffy, Berthold Göttgens, Andrew M. Blagborough, Cristina Lo Celso Manipulating niche composition limits damage to haematopoietic stem cells during Plasmodium infection, Nature Cell Biology (2020).

[8] **M.L. Vainieri**, M. Alini, A. Yayon, G. van Osch, S. Grad, Mechanical Stress Inhibits Early Stages of Endogenous Cell Migration: A Pilot Study in an Ex Vivo Osteochondral Model, Polymers (Basel) 12(8) (2020).

This thesis is based on the following publications: 2, 4, 5 and 8.

PHD PORTFOLIO

Summary of PhD training and teaching

Name PhD student: Maria Letizia Vainieri	**PhD period:** September 2015 – December 2019
Erasmus MC Department: Orthopaedics	**Promotor:** Prof. Gerjo J.V.M. van Osch, PhD
Research School: MolMed	**Co-promotor:** Sibylle Grad, PhD

1. PhD training

		Year	Workload (ECTS)
Courses			
-	ESR Nanomedicine Training Session (Imperial College London, UK)	10-2015	0.1
-	Scientific Presentation (Academia Retica, Davos, CH)	12-2015	0.5
-	ESRs RNA interference Training Session (iNANO, Aarhus University, DK)	05-2016	0.1
-	Statistics for Biology and Medical Research (SIAF Institute, Davos, CH)	06-2016	0.6
-	Personalised Therapy Symposium at TERMIS-EU Conference (Davos, CH)	07-2016	0.1
-	Enabling Technologies (Utrecht University, NL)	10-2016	1.5
-	Image J Analysis Workshop (Utrecht University, NL)	10-2016	0.1
-	ESRs Stem Cells and Organoids Training Session (Utrecht University, NL)	12-2016	0.1
-	German Course (ARI, Davos, CH)	2016	4
-	Proposal Writing & Funding (Graduate School Graubunden, Davos, CH)	12-2017	0.3
-	Summer School: Complex Cell Systems (6th SBMS, Interlaken, CH)	05-2017	0.5
-	ESRs Training on Patents and IP strategies (Technion University, Haifa, IL)	01-2018	0.1
-	Summer School: Complex Cell Systems (6th SBMS, Interlaken, CH)	05-2018	0.5
-	Data Processing Advanced Course with Graph Pad Prism (ARI, Davos, CH)	07-2018	0.6
-	ESRs Preclinical Imaging Training Session (University of Leiden, NL)	07-2018	0.1
-	Research Integrity (Erasmus MC, Rotterdam, NL)	02-2019	0.3
-	Summer School: The Osteochondral Interface (7th SBMS, Interlaken, CH)	05-2019	0.5
Conferences – Podium Presentations			
-	5th Graubunden Forscht Conference, Davos, CH:	09-2016	1
	Investigating the homing behavior of endogenous stem cells in a joint bioreactor to regenerate articular cartilage.		
-	SSB+RM Conference, Fribourg, CH:	05-2018	1
	Novel hyaluronan-based hydrogels to support endogenous cartilage repair.		
-	6th Graubunden Forscht Conference, Davos, CH:	09-2018	1
	HA-based hydrogels for cartilage tissue engineering		
-	TERMIS-EU Conference, Rodhes, GR :	05-2019	1
	Biomimetic hyaluronic acid-hydrogel enhances endogenous cell recruitment and healing process of cartilage lesions		
Conferences – Poster Presentations			
-	Gordon Research Conference Cartilage Biology, Lucca, IT:	04-2017	1
	Novel ex-vivo osteochondral defect model in a joint bioreactor system for articular cartilage repair studies		

-	TERMIS-EU, Davos, CH: *Novel ex-vivo osteochondral defect model in a joint bioreactor system for articular cartilage repair studies*	06/2017	1
-	ICRS 14th World Congress, Macau, China: *Hyaluronic acid-based hydrogels promote mesenchymal stem cell ingrowth and cartilage production in vitroand in vivo.*	04/2018	1
-	eCM XVIII Conference Cartilage and Disc Repair, Davos, CH: *Novel ex-vivo osteochondral model for cartilage repair in mechanical stimulated bioreactor*	06/2018	1
-	ORS Annual Meeting, Austin, TX, USA: *Effect of mechanical stimulation combined with FGF-18 on bovine chondrocytes embedded in a novel Fibrin:Hyaluronan hydrogel*	02/2019	1
Presentations and meetings			
-	Lab Meetings IVD/Cartilage (weekly)	2015-2019	2
-	Muskuloskeletal Regeneration Programme Meetings (monthly)	2015-2019	1
-	Journal club (monthly)	2015-2018	1
-	WP4 TargetCare Meetings (monthly)	2016	0.2
-	Lab Meetings dept. Orthopaedics (weekly)	05-09 '18	0.1
-	Research Meetings dept. Internal Medicine/Skeleton Meetings (weekly)	05-09 '18	0.1
-	ESRs TargetCare Consortium Meetings (biannually)	2015-2019	2
2. Teaching			
		Year	**Workload (ECTS)**
-	Workshop "Synovial Joint and Articular Cartilage" for ETH and Winterthur Master Students, ARI Davos, CH (annually)	2016-2019	2
Supervising and Tutoring			
-	Partial Supervision Master Student, Dal Fabbro Lea Tiziana, Winterthur, CH, (3 months)	2017	1
-	Co-Supervision PhD Student, Bernardo Antunes, Hebrew University, IL (8 months)	2019	3
Miscellaneous			
-	Active Board Member of Young Scientists (YS) of Swiss Society of Biomaterial and Regenerative Medicine (SSB+RM)	2016-2020	
-	Organization YS Lab-Networking event at RMS Foundation, Bettlach, CH	01-2017	0.5
-	Organization Pre-Conference SSB+RM YS Workshop "Training for scientific writing: Tips and Tricks"	05-2017	0.5
-	Co-Chair Session TargetCare Symposium at TERMIS-EU, Davos, CH	06-2017	
-	Organization YS Symposium Universitäts Spital Zürich	11-2017	5
-	Review of scientific papers (2x)	2017	0.3
-	Organization YS Lab-Networking event at AO Foundation, Davos, CH	04-2018	0.5
-	Visiting PhD Student at the Connective Tissue Regeneration Lab, Erasmus MC Rotterdam, NL (5 months)	05-09 '18	5
-	Co-chair Session Delivery and Imaging System at eCM XVIII Conference, Davos Congress, CH	06-2018	

-	Organization Pre-Conference SSB+RM YS Workshop “You and Your career”	06-2018	0.5
-	Visiting PhD Student at Procore Ltd, Weissman Park, Ness Ziona, IL	01-2019	1
-	Organization YS Lab-Networking event at Geistlich Pharma, Wolhusen, CH	01-2019	0.5
-	Organization Pre-Conference Workshop “Patient-Specific Implants produced By Additive Manufacturing”	05-2019	0.5
Other			
-	Die Südostschweiz Newspaper Interview on PhD life and challenges	03-2016	
-	Organization Group Activity Muskuloskeletal Program (5 days)	2016	5
-	Organization Group Activity Muskuloskeletal Program (5 days)	2019	5
Grant and Award			
-	Best Oral Presentation Award, 5th Conference Graubunden Forscht “Young Scientist in Contest”, Davos, CH	2016	
-	Swiss Bone Mineral Society Travel Fellowship, Bern, CH	2019	

CURRICULUM VITAE

Maria Letizia Vainieri was born on the 22nd of December 1983 in Melfi (PZ), Italy. After graduating from Scientific Lyceum "Federico II di Svevia", she started Biotechnology at La Sapienza University of Rome. For her bachelor assignment, she gained her first experience with scientific research during the 8 months internship at Biochemistry Department of La Sapienza University. This resulted in the bachelor thesis "ERp57 and the oxidative stress" in November 2008. In 2009 she started the Master in Molecular, Cellular and Medical Biotechnology at La Sapienza University of Rome. In 2011 she was given the opportunity to conduct a year study on the establishment of bone marrow and hematopoietic niche *in vivo* by reversion of chondrocytes differentiation of human bone marrow stromal cells. This research was carried out under supervision of Prof. Paolo Bianco in the bone tissue regeneration group at University Hospital Policlinico Umberto I in Rome. Her interest in cartilage, bone and bone marrow led to the graduation research entitled "New heterotopic transplantation protocol of human mesenchymal stem cells" obtaining a master's degree in October 2012. In 2013 she won two scholarships and moved to Imperial College London to investigate how Malaria infection affects hematopoietic stem cells and bone marrow. Then in September 2015 her PhD training began as a Marie Curie ITN Fellow (TargetCare Consortium) at Musculoskeletal Regeneration group at AO Foundation in Davos (CH) under supervision of dr. Sybille Grad and at Connective Tissue Repair group at the Erasmus MC, University Medical Center in Rotterdam (NL) under supervision of prof. dr. Gerjo J.V.M. van Osch. As of January 2020, she is working as education project coordinator at AO Foundation in Davos (CH).

DANKWOORD

And here it comes….after 5 intense years this day finally arrived: the last touch of this thesis, the acknowledgements. It has been an epic journey of deep learning both at scientific and personal level, which resulted in a strong impact on my personality. Doing a PhD in a such remote place in the middle of Alpes push your boundaries, an unforgettable experience which I would certainly repeat. This thesis, far from being the final goal of my PhD, is the expression of a fruitful human and scientific experience disseminated from meeting many people of different cultures and special places. While writing, I realized the PhD is about people you had fortune to meet and I should say I had the chance to encounter so many pioneers in the field from cartilage matrix development to stem cells and tissue engineering, each of them inspired me and helped me to find solutions to my questions, belief and ideas.

My deepest and sincere acknowledgements go to my promotor **Prof. dr. Gerjo van Osch** who transmitted me her passion and dedication in this creative but challenging job. You are a true inspiration and totally on top of this game, who certainly had a fundamental role in my growth: step by step on my side guiding me and clarifying any doubts whenever needed. Despite most of the time has been a distance learning, I am very thankful for your critical thinking and your empathy, because every single moment I needed (even when hidden between lines) you never lacked to support and encouraged me. I am enormously grateful for your precious teachings and also glad to have had the unique opportunity to work with such a beautiful and brilliant mind.

I would like also to express my gratitude to my supervisor **dr. Sibylle Grad** for believing in my capacities and supporting my enthusiasm in any experiment. You gave me the chance to travel around the world and gain theorical and practical experiences, which helped me to build and shape my project. Thank you very much for always being there when though times came and for giving me this beautiful opportunity I would have never imagined to live, I guess a very important one. I grew up here.

Dieter, I was the lucky one who still have had the pleasure to work with before your retirement. You are the most creative, explicative and handyman engineer I ever met, without you half of the project will still be on hold. A boundless thanks for your guidance in the model development under the bioreactor.

Andrea thanks for your great cooperation and all the practical suggestions that have sometimes made my life in the lab a little easier. You have been not only my colleague

but also, despite the distance, the Post-Doc I missed on my side during this journey. I enjoyed the time spent in Rotterdam, thanks also for hosting and "standing" me.

Kavitha I adored the time we spent together in the lab, you are a great scientist. Thanks for teaching me your fancy assay and picking up my calls when uncertain on fast decision making, I learnt a lot from you and I am extremely thankful. I remember you with a smile and always laugh when I think at your typical head booble and voice while saying to me :"You are crazy, you are crazy"!!

A profound gratitude to my Paranymphs: **Angela** and **Arturo**. I would have never survived in this journey without you. **Angela**, my working partner and also partner in crime. Some friendships start with pains (we started with a fight), but in the end I managed to touch your heart. Because without you, my psychological supporter, I would not know on whom to vent my anger. Angi immense thanks for your support, moments of complicity, trips, laughs, funny times, Italian spices and more. Non c'è storia, mi manchi al lavoro. **Arturo**, my flatmate and the gentle man every woman wish to live with. You reflect my private life side and the safe place. During these long years we had time to know each other and surprisingly get well along despite having opposite characters, I've always felt close to home thanks to our countless dinners, home-made pasta and pizza and much more. I am very thankful for your support, for your strong pan-South Italianess, for being a good listener and advisor. Mi mancherai.

Keith thanks for letting your wife being late several nights, for standing our endless chitchat and for opening your house without ceremonies. I took sometimes to understand your personality, but finally I learned the real British humor.

A special thought goes to all **TargetCare PhD colleagues**: I always felt a part of a bigger picture. **Joao, Jacopo, Lucia, Marco, Florian, Yunpeng, Serdar** and **Bradley**, thanks for this wonderful adventure, I guess a beautiful example of inspiring collaboration among young scientists, not to mention our unmissable dinners together, so much fun I could never forget. Particular thanks to **Bernardo**, we have worked side-by-side for all mine and your secondment, thank you very much for your support. **Domenico** one of my favourite TargetCare guy, big thanks of sorting out last minute the way to count migrated cells and for always supporting me and Lulu' during this journey, best of luck at NIH. **Luana** was love at first sight, thank you very much for sharing calls when we needed to cheer us up during though times, for the parties, aperitives and tons of laughs, I will never forget our intense week in Utrecht. Your friendships (Lulu' and Dome) are one of those nice things that came out of the PhD. And, of course, huge thanks to all **TargetCare Professors and Project Managers** to make this experience possible. Especially to **Dr. Avner Yayon,** your

contribution was important for me to succeed in one of the main points of the project. Thanks for hosting me in Israel.

I would like to acknowledge all the lab technicians I had the chance to work in Davos and Rotterdam. **Robert**, at the beginning you were yelling at me because of my Italianess, likely time has given us a chance to go beyond apparences and became more evident how much alike we are. I am very thankful for everything. **Nora** thanks for being so though with me :-). Despite everyone is scared of getting downstairs (I said it!), in the end I enjoyed making mess and playing around! **Mauro** thanks also to you for troubleshooting with my MMA sections and for your patience. **Flavio**, thanks for your willingness of pick up the joints in Zurich. **Nicole**, you always smile, thank you so much for taking care of animals experiments and helping me out with histological stainings in Rotterdam. **Wendy** and **Janneke** thanks for being there when I had a doubt and for carrying over some experiments while I was travelling.

Thanks, **Sandra**, for your reminders and for guiding me through all the administration, because without you the transition between Hora Est and Hora Finita system could not have been so smooth as it did.

I would like to thank you the **Committee** for accepting the invitation and taking the time to read my dissertation despite your busy schedule.

To all **Musculoskeletal Regeneration Program** in Davos, carrying out this thesis without you wouldn't have been the same. To the old ones, **Marianna**, **David, Zhen** and **Matteo** each one of you thought me something and I thank you for help me out. **Martin** special thanks to you, despite guiding another group your door was always open for me. Thank you very much for bridging the gaps and for giving me the chance to follow your lab meetings. **Sebastian** thanks for making more pleasant my stay in ARI, for sharing laughs and advices. Good luck with your career and don't dare give up on playing music. **Reihane**, thanks for sharing the pain. **Sonia**, thanks for our endless chat about life. **Federico**, **Catarina**, **Jessie** and **Mitko** thank you because your presence during the first odd year in Davos gave me a great sense of relief and pleasure.

To all **PhD students, Post Doc and Professors of the 16th floor** at Erasmus MC, thanks for your conviviality and for the cake of the week! In particular, thanks to **Andrea, Panithi**, **Sohrab**, **Diego**, **Shorouk** for making me feel part of the group, for the dinners and Friday drinks after work. **Lizette**, you were in Italy, but thanks for the nice chats. **Tim** so short were the moments we shared the office, but I liked you since the beginning. Big thanks to you, because after 5 years I managed to go down the slopes skiing, you are a great teacher!

To my flatmate **Kirstin**, thanks for your pleasant company, all the nice discussions and for often being the bridge across all research institutes in Davos. I enjoyed our social nights and surprisingly also the game nights you always organized and for which you are so found of.

Ringrazio **Giuseppe** per gli innumerevoli weekend trascorsi insieme, stare con te ha allegerito notevolmente le mie giornate davosiane. Sei uno spasso, decisamente una piacevole scoperta. **Ilenia**, tipico esempio di donna del sud, in soli tre mesi sei riuscita a catturarci e farci sentire a casa. Mi hai ospitato senza conoscermi, ti ringrazio per quella semplicità e spontaneità che qui ho fatto gran fatica a trovare, e che dopotutto credo siano le uniche vere sorgenti di bellezza. **Marchino**, the only Swiss guy I definitely got well along with, thanks for our chats, trips and music sessions.

Ai miei amici storici, grazie per essere sempre presenti nonostante le mie lunghe assenze, rincontrarvi è sempre così rigenerante. Grazie in particolare a **Francesco**, **Francesca e Federica** sempre sulla stessa lunghezza d'onda sin da piccolissimi. **Sabi,** grazie per ricordarmi ogni volta che sei sempre tu quella a chiamarmi, perché io non ho mai tempo. Ad **Antonio**, grazie per sollecitarmi costantemente proponendomi svariate opportunità di lavoro, lo so è arrivato il momento di tornare a casa. **Laura**, a te il ringraziamento più speciale, la mia grande amica, quella delle grasse risate, ma anche dei lunghi silenzi a cui poter confidare «senza riserve» i miei pensieri, grazie per essere sempre al mio fianco.

E qui arriva **«la famigghia»**, la mia roccia sempre. Non basterebbe una vita per ringraziarvi, i vostri opportuni consigli ed il vostro supporto sono fondamentali. Grazie per avermi permesso di seguire sempre il mio cuore e per aver sopportato i miei continui sbalzi di umore. Prima o poi tutte le ansie e le preoccupazioni che vi ho gentilmente donato dovevano essere ripagate! Durante questi lunghi anni la felicità è una sola: tornare a casa. Preparatevi perché ritornerò presto ad impepare le vostre giornate…

Last but never least, my hearty thanks go to **Prof. dr. Mauro Alini**. Grazie infinite per avermi accolto, per avermi messo in riga e per avermi gentilmente donato milioni di opportunità di crescita professionale e personale. Ho imparato molte cose e ne esco certamente arricchita. La tua positività e tranquillità ha sempre reso piacevole e divertente questo lungo e duro periodo di prova, che aggiunta alla tua speciale empatia ha fatto sempre sentire tutti parte di una grande famiglia. GRAZIE.
P.S. Non credo ti libererai facilmente di me. :-)