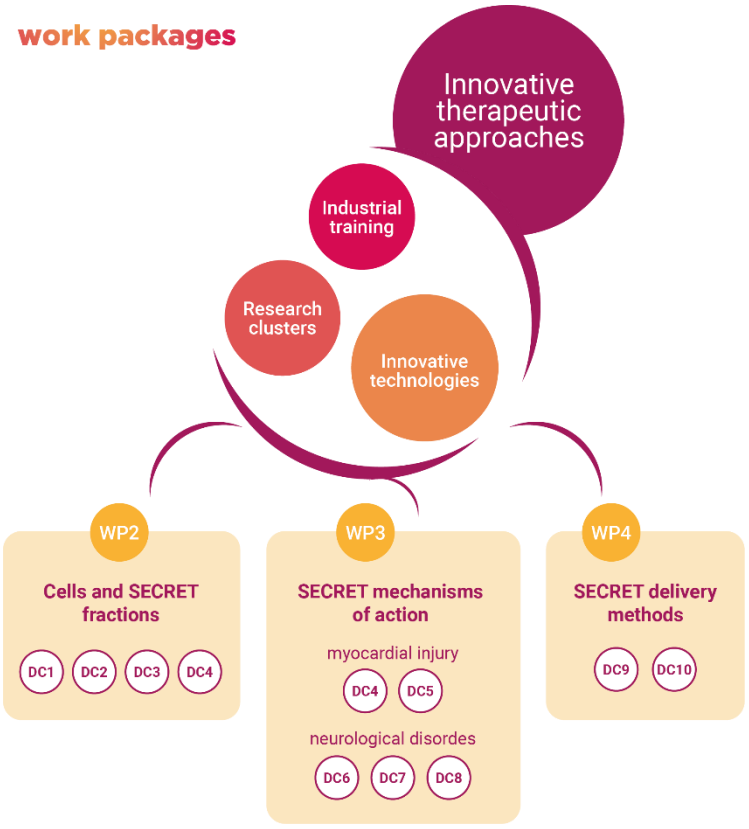
Marie Sklodowska-Curie Action Doctoral Network (MSCA-DN)

SECRET: "Exploring the therapeutic potential of perinatal cell SECRETomes"

SECRET Call for applications for 10 Doctoral (PhD) Training Positions in stem cell biology, paracrine signalling mechanisms, advanced delivery methods and pre-clinical models for regenerative medicine applications.

Offer Description



SECRET is a Doctoral Network funded by the European Union Horizon Europe Programme. The SECRET Consortium is a research network of leading European scientists from academia and industry, experts in human perinatal cells, extracellular vesicle biology, tissue engineering and biocompatible materials, to address key questions on SECRET through a coordinated, interdisciplinary effort in regenerative medicine. **SECRET proposes 10 independent doctoral research projects with the ambition of providing its trainees with a comprehensive understanding of paracrine signalling mechanisms, cardiovascular repair, neuro-degenerative diseases, sophisticated modelling of disease and screening platforms, and biomaterials/tissue engineering**. Through its research and training activities, the SECRET project will contribute to scientific advancement and innovation in Europe, ultimately leading to societal and economic benefits.

Graduates of SECRET will be well-prepared to enter the workplace with an innovative and beyond state-of-the-art view on different aspects of pre-clinical research and the process of transitioning cellular products into the clinic.

Participating in SECRET offers doctoral candidates many unique opportunities, including:

- A project as Marie Skłodowska Curie trainee in one of the participating institutions with the objective of receiving a doctoral degree (PhD).

- State-of-the art, exciting research in an international consortium with highly integrated research projects.
- Expert training in basic and applied research, along with a thorough understanding of the various phases involved in transitioning from pre-clinical to clinical research.
- A research training period in another consortium member's lab lasting from a few weeks up to six months, performed for the most part in a different EU country than the country where most of the project will take place.
- Training in both academic and commercial research environments.
- Salary according to EU guidelines for Marie Skłodowska Curie trainees, including mobility payments and family allowances where applicable.

Application Process

SECRET will select Doctoral Candidates through a 2-step recruitment process.
The selection procedure will be open, transparent, and merit-based, fully aligned with the Code of Conduct for the Recruitment of Researchers. Although the selection will be based on the quality of applications, gender balance will also be considered.
Candidates can apply for maximum three PhD projects and the applications need to be submitted separately.
Applications (in English) must include:

- a **cover letter** which will also include the motivation for the position, emphasizing the candidate's strength regarding the project and the requirements (max 3 pages);
- a **CV** (max 2 pages);
- a **scanned copy of all relevant diplomas or certificates** that formally entitle the candidate to embark on a doctorate. Typically, these documents will include Bachelor's and Master's Degree certificates. In case the Master's Degree has not been obtained yet at the closing date for application, the candidate has to submit a declaration signed by their supervisor or University official stating that the degree will be obtained by the time of PhD enrolment;
- **Letter of Recommendation** from two appropriate referees or contact details of two referees.

Application documents in a **single pdf file** should be sent by email to relevant project supervisors (see email address in individual project descriptions). **The subject line of the email must be in the following format: "SECRET: application for Project#_Title of PhD project".**

The closing date for applications is **30 September, 2024.**
Applicants are advised to familiarise themselves thoroughly with the projects, for which they apply and be ready to answer questions on their chosen topics. After reviewing all project applications, supervisors of individual projects will contact selected applicants to organise an initial screening interview by telephone or videoconferencing. The most promising candidates may then be invited to a personal interview at the host institution or a further videoconference, potentially with several other project supervisors.

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Research projects offered by SECRET

DoC	Project Title	Primary Supervisor	Institution	EU State
1	Isolation and in vitro characterization of hAMSC and hUC/WJ-MSC-derived secretomes	PAROLINI, Ornella ornella.parolini@unicatt.it sezione.biologiaapplicata@unicatt.it	Università Cattolica del Sacro Cuore (UCSC)	IT
2	Isolation and in vitro characterization of III trimester hAFSC-derived secretome	PAROLINI, Ornella ornella.parolini@unicatt.it sezione.biologiaapplicata@unicatt.it	Università Cattolica del Sacro Cuore (UCSC)	IT
3	Establishment and optimization of the scaled production of therapeutic EVs	GIEBEL, Bernd Bernd.Giebel@uk-essen.de	Universitatsklinikum Essen (UKEssen)	GE
4	Identify the most cardio-active secretome from PCs to enhance cardiac repair	BOLLINI, Sveva sveva.bollini@unige.it	Università degli Studi di Genova (UniGe)	IT
5	Development of an optimized human 3D organoid-on-plate microfluidic platform for disease modelling and screening	LANZ, Henriëtte https://mimetas.easycruit.com/	MIMETAS B.V (MIM)	NL
6	Evaluate and unravel the neuroprotective features of perinatal cell-derived secretomes using hiPSC-derived brain organoids.	PONSAERTS, Peter peter.ponsaerts@uantwerpen.be	Universiteit Antwerpen (UA)	BE
7	Evaluate and unravel the neuroprotective features of perinatal cell-derived secretomes in a mouse model of neuro-inflammation, -degeneration and -regeneration.	PONSAERTS, Peter peter.ponsaerts@uantwerpen.be	Universiteit Antwerpen (UA)	BE
8	In vivo validation of three selected secretomes in a stroke mouse model	HERMANN, Dirk Dirk.Hermann@uk-essen.de	Universitatsklinikum Essen (UKEssen)	GE
9	Injectable supramolecular hydrogels and microfabricated patches for the controlled release of secretome/EV fractions for cardiac regeneration	PIRES, Ricardo A. info@i3bs.uminho.pt	Universidade do Minho (UMINHO)	PT
10	Development of biomaterial-based vehicles for the delivery of secretome fractions to the CNS regions affected by MS and IS	REIS, Rui L. info@i3bs.uminho.pt	Universidade do Minho (UMINHO)	PT

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DoC1	**Isolation and in vitro characterization of hAMSC and hUC/WJ-MSC-derived secretomes**
Host Institution	Università Cattolica del Sacro Cuore (UCSC)
Primary Supervisor	PAROLINI Ornella
Email address	ornella.parolini@unicatt.it ; sezione.biologiaapplicata@unicatt.it
Planned duration	36 months
Subject Area	Immunology, cell-based secretomes, in vitro testing, molecular profiling

Introduction: Cellular therapies are an important focus in current regenerative medicine research due to their broad-ranging clinical potential for many diseases that cannot be effectively treated, yet. However, the last decade has also witnessed that stem cell therapeutics mainly act via their secretome, either as a whole or via extracellular vesicles (EVs) present within it. Secretome-based drugs are a novel type of biologics that do not fall into the Advanced Therapy Medicinal Product (ATMP) category since not cells, but cell-free derivatives are the actual therapeutic agent. They may provide many advantages over conventional cellular therapeutics, especially when perinatal tissues/(stem) cells over adult stem cells are employed as a source for the production of secretome-based therapeutics. As compared to adult mesenchymal stromal cells (MSC), those derived from perinatal tissues are developmentally much younger and have not been exposed to a lifetime of environmental stimuli (lifestyle, aging, infections, trauma, etc.) that may negatively affect their therapeutic potential. In addition, perinatal tissue-derived MSC have been recently demonstrated to possess remarkable immunomodulatory and trophic paracrine potential. The most extensively characterized perinatal derivatives in regenerative medicine are MSC derived from the amniotic membrane (hAMSC), umbilical cord (hUC/WJ-MSC), and amniotic fluid (hAFSC). The candidates within this program will thus be triggered to identify the most potent perinatal tissue-derived secretome or EVs for anti-inflammatory and/or pro-regenerative properties, that will subsequently be used to develop a highly innovative treatment schedule to achieve therapeutic success over multiple pathologies, including myocardial ischemia, multiple sclerosis and ischemic stroke.

Objective: Perform a detailed characterization of the secretome (CM) of hAMSC and hUC/WJ-MSC. More specifically, the candidate will be involved in the following activities: define the optimal stimulatory pre-conditioning stimuli such as low oxygen tension versus control normoxia, as well as different culture conditions, to obtain the most bioactive secretome from hAMSC and hUC/WJ-MSC, and obtaining an understanding of the procedures for CM fractionations for EV isolation, and EV characterization. The candidate will compare the paracrine potential of cell secretomes (conditioned medium CM as a whole, or EVs concentrated thereof) by molecular profiling and in vitro functional assays to characterize immunomodulatory properties and epigenetic mutations in immune cells.

Expected Results: R1: Isolation of MSC from the amniotic membrane (hAMSC) and the umbilical cord (hUC-MSC)/Wharton's jelly (hWJMSC) of human term placenta and immune phenotype characterization. **R2:** Generation and collection of cell-specific secretome (CM). **R3:** Characterization of secretome based on molecular profiling and functionality. **R4:** Identification of differences and similarities between hAMSC, hUC/WJ-MSC and hAFSC (DoC 2) secretomes (conditioned medium CM as a whole, or EVs concentrated thereof) in terms of their ability to modulate immune cells, and comparison with gold standard secretome from bone marrow, both at molecular and proteomic level. Our results will lead to the identification of the most efficient perinatal cell secretome in terms of immune modulation.

Secondments: (1) 6 months at CREM (M8) to train on hAMSC and hUC/WJ-MSC isolation, immune

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phenotyping, culturing and preparation of secretome; **(2)** 2 months at UKEssen (Bernd Giebel) to train on isolation of EV preparations (M30).

Enrolment in Doctoral degree(s): Università Cattolica del Sacro Cuore - Department of Life Science and Public Health

Project-specific selection criteria: Candidates should have a Master's degree or similar education level in Immunology, Biology, Biotechnology or Biomedical sciences. Knowledge of cell biology and basic immunology, and a proven track record are required. Additionally, practical experience in flow cytometry will be a plus. The candidate should be familiar with basic techniques (e.g., cell culture techniques, molecular and proteomic assays). Candidates should be familiar with perinatal cell biology (mesenchymal stromal cells isolated from the amniotic membrane and umbilical cord of human term placenta), cell secretome-based therapy, and regenerative medicine. Candidates should be a team player with good communication skills.

Recommended reading:

- ***Perinatal Derivatives: Where Do We Stand? A Roadmap of the Human Placenta and Consensus for Tissue and Cell Nomenclature.*** Silini AR, Di Pietro R, Lang-Olip I, Alviano F, Banerjee A, Basile M, Borutinskaite V, Eissner G, Gellhaus A, Giebel B, Huang YC, Janev A, Kreft ME, Kupper N, Abadía-Molina AC, Olivares EG, Pandolfi A, Papait A, Pozzobon M, Ruiz-Ruiz C, Soritau O, Susman S, Szukiewicz D, Weidinger A, Wolbank S, Huppertz B, Parolini O. Front Bioeng Biotechnol. 2020 Dec 17;8:610544. doi: 10.3389/fbioe.2020.610544. Review.
- ***Comparison of EV-free fraction, EVs, and total secretome of amniotic mesenchymal stromal cells for their immunomodulatory potential: a translational perspective*** Papait A, Ragni E, Cargnoni A, Vertua E, Romele P, Masserdotti A, Perucca Orfei C, Signoroni PB, Magatti M, Silini AR, De Girolamo L, Parolini O. Front Immunol. 2022 Aug 16;13:960909. doi: 10.3389/fimmu.2022.960909. eCollection 2022.
- ***Human amnion favours tissue repair by inducing the M1-to-M2 switch and enhancing M2 macrophage features***. Magatti M, Vertua E, De Munari S, Caro M, Caruso M, Silini A, Delgado M, Parolini O. J Tissue Eng Regen Med. 2017 Oct;11(10):2895-2911. doi: 10.1002/term.2193.
- ***Perinatal derivatives: How to best validate their immunomodulatory functions***. Papait A, Silini AR, Gazouli M, Malvicini R, Muraca M, O'Driscoll L, Pacienza N, Toh WS, Yannarelli G, Ponsaerts P, Parolini O, Eissner G, Pozzobon M, Lim SK, Giebel B. Front Bioeng Biotechnol. 2022 Sep 14;10:981061. doi: 10.3389/fbioe.2022.981061.

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DoC2	**Isolation and in vitro characterization of III trimester hAFSC-derived secretome**
Host Institution	Università Cattolica del Sacro Cuore (UCSC)
Primary Supervisor	PAROLINI Ornella
Email address	ornella.parolini@unicatt.it ; sezione.biologiaapplicata@unicatt.it
Planned duration	36 months
Subject Area	Immunology, cell-based secretomes, in vitro testing, molecular profiling.

Introduction: Cellular therapies are an important focus in current regenerative medicine research due to their broad-ranging clinical potential for many diseases that cannot be effectively treated, yet. However, the last decade has also witnessed that stem cell therapeutics mainly act via their secretome, either as a whole or via extracellular vesicles (EVs) present within it. Secretome-based drugs are a novel type of biologics that do not fall into the Advanced Therapy Medicinal Product (ATMP) category since not cells, but cell-free derivatives are the actual therapeutic agent. They may provide many advantages over conventional cellular therapeutics, especially when perinatal tissues/(stem) cells over adult stem cells are employed as a source for the production of secretome-based therapeutics. As compared to adult mesenchymal stromal cells (MSC), those derived from perinatal tissues are developmentally much younger and have not been exposed to a lifetime of environmental stimuli (lifestyle, aging, infections, trauma, etc.) that may negatively affect their therapeutic potential. In addition, perinatal tissue-derived MSC have been recently demonstrated to possess remarkable immunomodulatory and trophic paracrine potential. The most extensively characterized perinatal derivatives in regenerative medicine are MSC derived from the amniotic membrane (hAMSC), umbilical cord (hUC/WJ-MSC), and amniotic fluid (hAFSC). The candidates within this program will thus be triggered to identify the most potent perinatal tissue-derived secretome or EVs for anti-inflammatory and/or pro-regenerative properties, that will subsequently be used to develop a highly innovative treatment schedule to achieve therapeutic success over multiple pathologies, including myocardial ischemia, multiple sclerosis and ischemic stroke.

Objective: Perform a detailed characterization of the secretome (CM) of III trimester human amniotic fluid stem cells (term hAFSC). More specifically, the candidate will be involved in the following activities: define the optimal stimulatory pre-conditioning stimuli such as low oxygen tension versus control normoxia, as well as different culture conditions, to obtain the most bioactive secretome from and hAFSC, and obtaining an understanding of the procedures for CM fractionations for EV isolation, and EV characterization. The candidate will compare the paracrine potential of cell secretomes (conditioned medium CM as a whole, or EVs concentrated thereof) by molecular profiling and in vitro functional assays to characterize immunomodulatory properties and epigenetic mutations in immune cells.

Expected Results: **R1:** Isolation of stem cells from term hAFSC and immune phenotype characterization. **R2:** Generation and collection of term hAFSC secretome. **R3:** Characterization of secretome based on molecular profiling and functionality. **R4:** Identification of differences and similarities with secretome (conditioned medium CM as a whole, or EVs concentrated thereof) from hAMSC and hUC/WJ-MSC (DoC 1), and comparison with gold standard secretome from bone marrow, both at molecular and proteomic level. Our results will lead to the identification of the most efficient perinatal cell secretome in terms of immune modulation.

Secondments: **(1)** 6 months at UniGe (M8) to learn about hAFSC isolation, immune phenotyping, culturing and preparation of secretome; **(2)** 2 months at UKEssen to train on isolation of EV preparations (M30).

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Enrolment in Doctoral degree(s): Università Cattolica del Sacro Cuore- Department of Life Science and Public Health

Project-specific selection criteria: Candidates should have a Master's degree or similar education level in Immunology, Biology, Biotechnology or Biomedical sciences. Knowledge of cell biology and basic immunology, and a proven track record are required. Additionally, practical experience in flow cytometry will be a plus. The candidate should be familiar with basic techniques (e.g., cell culture techniques, molecular and proteomic assays). Candidates should be familiar with perinatal cell biology (stem cells isolated from amniotic fluid), cell secretome-based therapy, and regenerative medicine. Candidates should be a team player with good communication skills.

Recommended reading:

- ***Perinatal Derivatives: Where Do We Stand? A Roadmap of the Human Placenta and Consensus for Tissue and Cell Nomenclature***. Silini AR, Di Pietro R, Lang-Olip I, Alviano F, Banerjee A, Basile M, Borutinskaite V, Eissner G, Gellhaus A, Giebel B, Huang YC, Janev A, Kreft ME, Kupper N, Abadía-Molina AC, Olivares EG, Pandolfi A, Papait A, Pozzobon M, Ruiz-Ruiz C, Soritau O, Susman S, Szukiewicz D, Weidinger A, Wolbank S, Huppertz B, Parolini O. Front Bioeng Biotechnol. 2020 Dec 17;8:610544. doi: 10.3389/fbioe.2020.610544. Review.
- ***Comparison of EV-free fraction, EVs, and total secretome of amniotic mesenchymal stromal cells for their immunomodulatory potential: a translational perspective.*** Papait A, Ragni E, Cargnoni A, Vertua E, Romele P, Masserdotti A, Perucca Orfei C, Signoroni PB, Magatti M, Silini AR, De Girolamo L, Parolini O. Front Immunol. 2022 Aug 16;13:960909. doi: 10.3389/fimmu.2022.960909. eCollection 2022.
- ***Cardiac Restoration Stemming From the Placenta Tree: Insights From Fetal and Perinatal Cell Biology.*** Bollini S, Silini A, Banerjee A, Wolbank S, Balbi C, Parolini P. Front Physiol. 2018 Apr 11:9:385. doi: 10.3389/fphys.2018.00385

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DoC3	**Establishment and optimization of the scaled production of therapeutic EVs**
Host Institution	Universitätsklinikum Essen (UKEssen)
Primary Supervisor	GIEBEL, Bernd
Email address	Bernd.Giebel@uk-essen.de
Planned duration	36 months
Subject Area	Extracellular vesicles (EVs) research, cell biology, immunology, and regenerative medicine

Introduction: The University Hospital Essen (UKEssen) is the largest university hospital in the Ruhr region, renowned for its top-tier medical services, integrating patient care, research, and education. The Institute for Transfusion Medicine, which handles blood product manufacturing and transplantation diagnostics, houses four research groups. One group, led by Prof. Dr. Bernd Giebel, a leading expert in early human hematopoiesis and EV research, is internationally recognized for therapeutic EV research. Following the first global clinical application of MSC-EVs, his team is refining large-scale MSC-EV production for clinical translation and advancing EV characterization.

Objectives: **1)** Identify PCs secreting EVs with the most potent immunomodulatory and pro-angiogenic activities. **2)** Establishment of clonally expanded immortalized cells lines for optimizing the scaled EV production. **3)** Optimization of upstream and downstream processes for the scaled and optimized EV production. **4)** Evaluation of the therapeutic potential of EVs produced under the optimized conditions.

Expected Results: **R1:** Cell lines will be established and protocols will be optimized for the scaled EV production. **R2:** The EVs produced under optimal conditions will exert immunomodulatory and pro-angiogenic activities in vitro and provide therapeutic activities in all animal models of the consortium.

Secondments: **(1)** 3 months at CREM learning handling of placental tissues and initial raising of different PC derivatives (M7-M9); **(2)** 3 months at UniGe training on hAFSC isolation, immune phenotyping, culturing and preparation of secretome (M10-M12); **(3)** 3 months at UCSC training on advanced proteomic profiling of PC derivatives (M13-M15).

Enrolment in Doctoral degree(s): Universitätsklinikum Essen, University of Duisburg-Essen, Biome graduate school

Project-specific selection criteria: Candidates should have a Master's degree or similar education level in Immunology, Biology, Biotechnology or Biomedical sciences. Combined knowledge of cell biology, basic immunology and the biology of extracellular vesicles (EVs) is mandatory, a proven track record required. The candidate should be familiar with basic techniques in cell culture and EV preparation and characterization. Elaborated and practical experience in flow cytometry is essential. Expertise in filtration and chromatography-based methodologies is a plus. The candidate should be highly motivated to work in an international research team and provide excellent communication and writing skills. Furthermore, the candidate should have the passion to work with a collection of modern machines including imaging flow cytometry, flow cytometric cell sorting and bioreactors.

Recommended reading:

- ***Extracellular vesicles from immortalized mesenchymal stromal cells protect against neonatal hypoxic-ischemic brain injury.*** Labusek N, Mouloud Y, Köster C, Diesterbeck E, Tertel T, Wiek C,

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Hanenberg H, Horn PA, Felderhoff-Müser U, Bendix I, Giebel B, and Herz J. Inflamm Regen. 2023; 43(1): 24. DOI: 10.1186/s41232-023-00274-6

- ***Independent human mesenchymal stromal cell–derived extracellular vesicle preparations differentially attenuate symptoms in an advanced murine graft-versus-host disease model.*** Madel RJ, Börger V, Dittrich R, Bremer M, Tertel T, Phuong NNT, Baba HA, Kordelas L, Staubach S, Stein F, Haberkant P, Hackl M, Grillari R, Grillari J, Buer J, Horn PA, Westendorf AM, Brandau S, Kirschning CJ, and Giebel B. Cytotherapy. 2023. DOI: 10.1016/j.jcyt.2023.03.008

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DoC4	**Identify the most cardio-active secretome from PCs to enhance cardiac repair**
Host Institution	Università degli Studi di Genova (UniGe)
Primary Supervisor	BOLLINI, Sveva
Email address	sveva.bollini@unige.it; sveva.bollini@hsanmartino.it
Planned duration	36 months
Subject Area	Cardiac repair and regeneration, Stem cell research, Paracrine biology and regenerative medicine

Introduction: The team led by prof. Bollini at the Department of Experimental Medicine at University of Genova, Genova, Italy, has broad experience in stimulating endogenous mechanisms of cardiac repair and regeneration by means of the human perinatal progenitor cell secretome. Prof. Bollini's team has extensive know-how on human amniotic fluid stem cell (hAFSC) paracrine biology. hAFSC represents a very appealing source of developmentally juvenile MSC with an appealing cardio-active secretory profile, which can be easily obtained either from left over samples of II trimester amniotic fluid obtained from prenatal screening from amniocentesis procedure (fetal hAFSC) and/or as clinical waste from III trimester samples from scheduled C-section delivery at term (perinatal hAFSC). Prof. Bollini has broad expertise in the optimization of hAFSC secretome formulations as future candidate therapeutics against myocardial injury, anthracycline-derived cardiomyopathy and premature ageing and cardiac fibrosis, as from several national and international fundings. Specific focus is dedicated to the role of hAFSC-secreted extracellular vesicles (EVs) in preserving cardiomyocyte functional profile in different cardiovascular disease models both in vitro and in vivo and in the implementation of improved delivery systems for secretome formulations.

Objectives: The selected Candidate will be actively involved in: **1)** Isolation of human stem cells from left over samples of II trimester amniotic fluid from routine prenatal screening (fetal hAFSC) and the optimization of in vitro culture to implement the collection and concentration of fetal hAFSC secretome formulations under different pre-conditioning strategies.;**2)** In vitro analysis of the cardio-protective potential of PC secretome formulations (CM/EVs) on relevant preclinical model of myocardial injury/disease; **3)** Validation of the most promising secretome formulation on 3D cardiac organ-on-plate system (CM or EVs) and investigation of the MoA. **4)** In vivo definition of the most cardio-active PC secretome in a MI mouse model by optimized in situ delivery. The main goals of this project will be achieved via synergic collaboration and interaction with partners from UKEssen (supervising DoC3), Istituto CardioCentro Ticino, MIMETAS and Amsterdam University Medical Centres (supervising DoC5).

Expected Results: **R1:** Identification of in vitro fetal hAFSC secretome formulations endowed with relevant cardio-protective and immunomodulatory potential in vitro. **R2:** Definition of the most cardio-active PC secretome formulation (CM vs EVs) with insights on the MoA by 3D modelling. **R3:** Definition of paracrine factor formulation within the selected PC secretome with therapeutic relevance for enhancing cardiac repair in vivo.

Secondments: **(1)** 2 months at UKEssen to receive ad hoc training on EV separation advanced techniques (M13-M15); **(2)** 1 month at ICCT to learn how to differentiate hiPSC into cardiomyocytes and design in vitro functional tests (M24-M25); **(3)** 1 month at MIM to learn complex co-culture design and 3D disease modelling (M30-M31); **(4)** 6 months at UMC to perform scRNAseq analyses on cardiac samples from the preclinical mouse model of MI (M34-M41).

Enrolment in Doctoral degree(s): Universita' di Genova – Department of Experimental Medicine (DIMES)

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Project-specific selection criteria: Candidates should have a Master's degree in Biology, Biotechnology, Biomedical Engineering or similar Biomedical sciences. Knowledge of stem cell biology, including paracrine communication and extracellular vesicle modulation of target cells and a proven track record are required. Candidates must not have resided in Italy for more than 12 months in the 3 years immediately before the recruitment date, nor have carried out their main activity (work, studies, etc.) in Italy during that period. Candidates should have a good knowledge and proven expertise of in vitro cell culture procedures and standards and be familiar with basic cell and molecular biology techniques (e.g. primary cell culture, cell viability assay, in vitro preclinical models of disease, standard biochemical assay, molecular biology and microscopy techniques). Candidates should be familiar with mechanisms of cardiac repair and regeneration. Relevant knowledge of preclinical models of myocardial injury/disease and practical experience in cardiovascular cell culture and of separation and concentration of cell secretome formulations and extracellular vesicles will be a plus. Candidates should be highly motivated, flexible, with great team-work and problem-solving attitude and be fluent in English communication, reading and writing.

Recommended reading:

- ***First Characterization of Human Amniotic Fluid Stem Cell Extracellular Vesicles as a Powerful Paracrine Tool Endowed with Regenerative Potential.*** Balbi C. et al. Stem Cells Transl Med 2017 May; 6(5):1340-1355. doi: 10.1002/sctm.16-0297;
- ***Reactivating endogenous mechanisms of cardiac regeneration via paracrine boosting using the human amniotic fluid stem cell secretome*** Balbi C. et al., Int J Cardiol. 2019 Jul 15:287:87-95. doi: 10.1016/j.ijcard.2019.04.011;
- ***The Human Fetal and Adult Stem Cell Secretome Can Exert Cardioprotective Paracrine Effects against Cardiotoxicity and Oxidative Stress from Cancer Treatment.*** Villa F. et al. Cancers (Basel). 2021 Jul 24;13(15):3729. doi: 10.3390/cancers13153729;
- ***Investigating the Paracrine Role of Perinatal Derivatives: Human Amniotic Fluid Stem Cell-Extracellular Vesicles Show Promising Transient Potential for Cardiomyocyte Renewal.*** Costa A. et al. Front Bioeng Biotechnol. 2022 Jun 8:10:902038. doi: 10.3389/fbioe.2022.902038. eCollection 2022.

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DoC5	**Development of an optimized human 3D organoid-on-plate microfluidic platform for disease modelling and screening**
Host Institution	MIMETAS B.V (MIM), Netherlands
Primary Supervisor	LANZ, Henriëtte
Applications address	https://mimetas.easycruit.com/
Planned duration	36 months
Subject Area	*In vitro* disease modelling, drug discovery and development, bioengineering

Introduction: MIMETAS is a rapidly growing company that specializes in human disease modelling using organ-on-a-chip technology. We are on a mission to pioneer the first generation of medicines grounded entirely in human data, using our unique disease modelling platform that is human, comprehensive, and scalable. Our disease models are designed to discover new therapeutic approaches and bring more drugs to patients by reducing attrition, while lowering development costs. MIMETAS has an open culture, where achievements go hand in hand with a fun place to work. Our close-knit team stays successful by maintaining a good atmosphere while working in a highly competitive field. MIMETAS' headquarters are based in Leiden, The Netherlands and our manufacturing facility is based in Enschede, The Netherlands. We have subsidiaries in Gaithersburg, MD, USA, and Tokyo, Japan.

The biology team lead by Dr. Lanz focusses on developing novel human tissue and disease models which can be leveraged for drug discovery and development.

Objectives: **1)** Design/adapt the OrganoPlate to allow development of read-outs for immune cell migration/function, fibroblasts and fibrosis, including for myocardial ischemia. **2)** First validation of the perinatal EVs using our established liver fibrosis model. **3)** Apply selected targets to the cardiac ischemia model to test cardio-protective and anti-inflammatory potential.

Expected Results: **R1:** New organ-on-a-chip model that mimics the injured infarcted myocardium thereby allow the investigation of cardioprotective and anti-inflammatory secretomes for injured infarcted myocardium. **R2:** Insights in the potential of the secretome to modulate liver fibrosis and cardiac ischemia.

Secondments: **(1)** 3 months at ICCT to learn how to differentiate hiPSC into cardiomyocytes and design in vitro functional tests (M9-M12); **(2)** 3 months at UA to test the performing secretome identified in their models in our cardiac ischemic model, and this in comparison to the gold standard secretome from bone marrow (M33-M36).

Enrolment in Doctoral degree(s): Academic Medical Center of the University of Amsterdam

Project-specific selection criteria: Affinity with bioengineering (organ-on-a-chip), liver and heart biology, extracellular vesicle biology, and/or iPS technology. Experience with (confocal) microscopy and standard molecular analysis techniques.

Recommended reading:

- ***In vitro grafting of hepatic spheroids and organoids on a microfluidic vascular bed*** Bonanini *et al* (2022) *Angiogenesis* https://doi.org/10.1007/s10456-022-09842-9
- ***An immunocompetent human kidney on a chip model to study renal inflammation and immune-mediated injury*** Gijzen *et al* (2024) *bioRxiv* https://www.biorxiv.org/content/10.1101/2024.06.11.598417v1
- ***Phenotypic screening in Organ on a Chip systems: a 1537 kinase inhibitor library screen on a 3D angiogenesis assay*** Soragni *et al* (2023) *Angiogenesis* https://doi.org/10.1007/s10456-023-09888-3

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DoC6	**Evaluate and unravel the neuroprotective features of perinatal cell-derived secretomes using immune-competent human iPSC-derived neurospheroids.**
Host Institution	University of Antwerp (UA), Belgium
Primary Supervisor	PONSAERTS, Peter
Email address	peter.ponsaerts@uantwerpen.be
Planned duration	36 months (MSCA-DN) + 12 months (UA)* * A PhD trajectory at UA requires a minimum of 4-years
Subject Area	Stem Cell Biology, Tissue Engineering, Cellular and Molecular Immunology; Regenerative Medicine, Neuroscience, Transcriptomics, Proteomics, Metabolomics

Introduction: Neuroinflammation is a common feature of many neurological disorders, whose main players are activated microglia, astrocytes and infiltrating peripheral immune cells. From an experimental point of view, the study of neuro-inflammation, as well as the pre-clinical validation of novel immune-modulatory and regeneration-inducing therapies, is commonly performed in rodent animal models. Given the world-wide recognition that efforts should be made to reduce animal experiments for ethical, scientific and practical reasons, there is an unmet need for the development, validation and application of novel pre-clinical screening tools before moving towards animal studies and/or human clinical trials. In this context, immune-competent 3D brain models, such as organoids and spheroids, are emerging in vitro tools that offer a more complex and realistic representation of the in vivo neural environment as compared to classical 2D cell co-cultures. At current, there is compelling evidence that stem cell-derived neurospheroids (NSPHs) outperform 2D cell co-cultures in terms of 3D architecture, cell heterogeneity, cell-cell interactions, gene expression, phenotypic profile, response to inflammatory stress and drug response. As such, it is clear that deep phenotypic and functional profiling of NSPHs using a complementary toolbox consisting of live cell imaging, electrophysiology, transcriptomics, proteomics and metabolomics is essential within the SECRET consortium to perform in-depth studies with regard to the regenerative properties of perinatal cell-derived secretomes.

Objective: In this PhD project, you will apply human iPSC-derived tri-partite NSPHs, containing neurons, astrocytes and microglia, that are subjected to cellular stress (by means of pro-inflammatory stimuli or oxygen/glucose deprivation) to evaluate the therapeutic potential of different perinatal cell-derived secretomes in terms of immunomodulatory and/or neuro-protective/regenerative capacity. To investigate this topic, you will apply our continuously expanding NSPH interrogation toolbox, including live-cell Ca-imaging and multi-electrode array recordings (to determine electrophysiological network behaviour), multi-omics analyses (including transcriptomics, proteomics and metabolomics), as well as intensive microscopic imaging (to validate multi-omics findings).

Expected Results: These studies should **R1:** allow the candidate to perform in-depth studies with regard to the therapeutic mode-of-action of perinatal cell-derived secretome(s); **R2:** allow the SECRET consortium to identify the most potent perinatal cell-derived secretome(s) to be applied in subsequent in vivo studies.

Secondments: **(1)** 3 months at the Center for Advanced Studies and Technology (CAST) at the University of G. d'Annunzio Chieti (Italy) to perform proteomic and metabolomic analyses of hiPSC-derived NSPHs (M19-M21) under supervision of prof. Damiana Pieragostino and prof. Piero Del Boccio. **(2)** 2 months at the Academic Medical Centre (AMC) of the University of Amsterdam (The Netherlands) to perform single cell

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RNA-sequencing of hiPSC-derived NSPHs(M32-M33), under supervision of Dr. Monika Gladka.

Enrolment in Doctoral degree(s): University of Antwerp – Faculty of Medicine and Health Sciences

Project-specific selection criteria: The candidate has obtained – or will obtain before start of the position – a master degree in Biotechnology, Biochemistry, Biology, Pharmacy, (Bio)Medicine, Bio-engineering, or an equivalent discipline. The candidate has resided in Belgium for more than 12 months in the 3 years immediately prior to the recruitment date (and not have carried out main activity - work, studies, etc. - in Belgium). The candidate can present outstanding study results during the Bachelor and Master studies (minimum top-20 of the study year) and is fluent in academic English (speaking and writing). The candidate has expertise with cell culture (hiPSC-derived neuronal models) and/or electrophysiology (live cell Ca-imaging and/or multi-electrode arrays) and/or multi-omics analyses (transcriptomics, proteomics and/or metabolomics) will be highly appreciated.

Recommended reading:

- ***Transcriptomic and proteomic profiling of bi-partite and tri-partite murine iPSC-derived neurospheroids under steady-state and inflammatory condition.*** Di Stefano J, Garcia-Pupo L, Di Marco F, Motaln H, Govaerts J, Van Breedam E, Mateiu LM, Van Calster S, Ricciardi L, Quarta A, Verstraelen P, De Vos W, Rogelj B, Cicalini I, Di Laurenzi V, Del Boccio P, FitzGerald U, Vanden Berghe W, Verhoye M, Pieragostino D, Ponsaerts P. Brain Behav Immun. 2024 Jul 11:S0889-1591(24)00475-6. doi: 10.1016/j.bbi.2024.07.008. Online ahead of print.
- ***Promising Strategies for the Development of Advanced In Vitro Models with High Predictive Power in Ischaemic Stroke Research.*** Van Breedam E, Ponsaerts P. Int J Mol Sci. 2022 Jun 27;23(13):7140. doi: 10.3390/ijms23137140.
- ***Luminescent Human iPSC-Derived Neurospheroids Enable Modeling of Neurotoxicity After Oxygen-glucose Deprivation.*** Van Breedam E, Nijak A, Buyle-Huybrecht T, Di Stefano J, Boeren M, Govaerts J, Quarta A, Swartenbroekx T, Jacobs EZ, Menten B, Gijsbers R, Delputte P, Alaerts M, Hassannia B, Loeys B, Berneman Z, Timmermans JP, Jorens PG, Vanden Berghe T, Fransen E, Wouters A, De Vos WH, Ponsaerts P. Neurotherapeutics. 2022 Mar;19(2):550-569. doi: 10.1007/s13311-022-01212-z.

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DoC7	**Evaluate and unravel the neuroprotective features of perinatal cell-derived secretomes in a mouse model of neuro-inflammation, -degeneration and -regeneration.**
Host Institution	Universiteit Antwerpen (UA)
Primary Supervisor	PONSAERTS, Peter
Email address	peter.ponsaerts@uantwerpen.be
Planned duration	36 months (MSCA-DN) + 12 months (UA)* * A PhD trajectory at UA requires a minimum of 4-years
Subject Area	Animal Models, Drug Delivery, Cellular and Molecular Immunology; Regenerative Medicine, Neuroscience, Magnetic Resonance Imaging, Transcriptomics, Proteomics, Metabolomics

Introduction: Neuroinflammation is a common feature of many neurological disorders, whose main players are activated microglia, astrocytes and infiltrating peripheral immune cells. From an experimental point of view, the study of neuro-inflammation, as well as the pre-clinical validation of novel immune-modulatory and regeneration-inducing therapies, is commonly performed in rodent animal models. As such, our laboratory has a long-lasting experience with the cuprizone (CPZ) mouse model of neuro-inflammation, -degeneration and -regeneration. Hereto, using a multidisciplinary approach we now rely on an extended analysis toolbox, including magnetic resonance imaging, bioluminescence imaging, gene delivery, histological analyses, transcriptomics, metabolomics and proteomics, to study – dependent on the research question – several cellular and molecular processes related to neuro-inflammation, -degeneration and -regeneration in an in vivo context. As such, it is clear that deep phenotypic, molecular and functional profiling of the rodent brain using a complementary toolbox of state-of-the-art techniques is essential within the SECRET consortium to perform in-depth studies with regard to the regenerative properties of perinatal cell-derived secretomes in pre-clinical in vivo studies.

Objective: In this PhD project, you will apply the cuprizone mouse model to evaluate the therapeutic potential of different perinatal cell-derived secretomes in terms of immunomodulatory and/or neuro-protective/regenerative capacity. To investigate this topic, you will contribute to the development of a novel drug delivery system for perinatal cell-derived secretomes and apply our continuously expanding CPZ mouse model interrogation toolbox, including magnetic resonance imaging, histological analyses, transcriptomics and proteomics, to study in vivo therapeutic benefit. Additionally, you will extend our analysis with profound metabolomics (incl. lipidomics) studies.

Expected Results: These studies should **R1:** allow the candidate to perform a unique integrative characterisation study of the CPZ mouse models using complementary magnetic resonance imaging, histological analyses, transcriptomics, proteomics and metabolomics (incl. lipidomics); **R2:** allow the candidate to perform in-depth studies with regard to the therapeutic mode-of-action of perinatal cell-derived secretome(s) upon delivery using a newly developed drug delivery system; **R3:** allow the SECRET consortium to identify the most potent perinatal cell-derived secretome(s) to be applied in future human clinical trials.

Secondments: **(1)** 3 months at the Center for Advanced Studies and Technology (CAST) at the University of G. d'Annunzio Chieti (Italy), to perform proteomic and metabolomic (incl. lipidomic) analyses on murine brain tissue (M24-M26), under supervision of prof. Piero Del Boccio and prof. Damiana Pieragostino. **(2)** 3 months at the 3B's Research Group from the University of Minho (Portugal) to contribute to the development of a drug-delivery system for perinatal cell-derived secretomes (M15-M17), under supervision of prof. Rui

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Reis and dra. Ana Rita Araújo.

Enrolment in Doctoral degree(s): University of Antwerp – Faculty of Medicine and Health Sciences

Project-specific selection criteria: The candidate has obtained - or will obtain before start of the position – a master degree in Biotechnology, Biochemistry, Biology, Pharmacy, (Bio)Medicine, Bio-engineering, or an equivalent discipline. The candidate has obtained has not resided in Belgium for more than 12 months in the 3 years immediately prior to the recruitment date (and not have carried out their main activity - work, studies, etc. - in Belgium). The candidate can present outstanding study results during Bachelor and Master studies (minimum top-20 of study year) and is fluent in academic English (speaking and writing). The candidate has expertise with small animal care/surgery and/or magnetic resonance imaging and/or multi-omics analyses (transcriptomics, proteomics and/or metabolomics) will be highly appreciated.

Recommended reading:

- ***Intracerebral transplantation of interleukin 13-producing mesenchymal stem cells limits microgliosis, oligodendrocyte loss and demyelination in the cuprizone mouse model.*** Le Blon D, Guglielmetti C, Hoornaert C, Quarta A, Daans J, Dooley D, Lemmens E, Praet J, De Vocht N, Reekmans K, Santermans E, Hens N, Goossens H, Verhoye M, Van der Linden A, Berneman Z, Hendrix S, Ponsaerts P. J Neuroinflammation. 2016 Nov 9;13(1):288. doi: 10.1186/s12974-016-0756-7.
- ***Interleukin-13 immune gene therapy prevents CNS inflammation and demyelination via alternative activation of microglia and macrophages.*** Guglielmetti C, Le Blon D, Santermans E, Salas-Perdomo A, Daans J, De Vocht N, Shah D, Hoornaert C, Praet J, Peerlings J, Kara F, Bigot C, Mai Z, Goossens H, Hens N, Hendrix S, Verhoye M, Planas AM, Berneman Z, van der Linden A, Ponsaerts P. Glia. 2016 Dec;64(12):2181-2200. doi: 10.1002/glia.23053.
- ***Diffusion kurtosis imaging probes cortical alterations and white matter pathology following cuprizone induced demyelination and spontaneous remyelination.*** Guglielmetti C, Veraart J, Roelant E, Mai Z, Daans J, Van Audekerke J, Naeyaert M, Vanhoutte G, Delgado Y Palacios R, Praet J, Fieremans E, Ponsaerts P, Sijbers J, Van der Linden A, Verhoye M. Neuroimage. 2016 Jan 15;125:363-377. doi: 10.1016/j.neuroimage.2015.10.052.
- ***Longitudinal monitoring of metabolic alterations in cuprizone mouse model of multiple sclerosis using 1H-magnetic resonance spectroscopy.*** Orije J, Kara F, Guglielmetti C, Praet J, Van der Linden A, Ponsaerts P, Verhoye M. Neuroimage. 2015 Jul 1;114:128-35. doi: 10.1016/j.neuroimage.2015.04.012.
- ***Cuprizone-induced demyelination and demyelination-associated inflammation result in different proton magnetic resonance metabolite spectra.*** Praet J, Orije J, Kara F, Guglielmetti C, Santermans E, Daans J, Hens N, Verhoye M, Berneman Z, Ponsaerts P, Van der Linden A. NMR Biomed. 2015 Apr;28(4):505-13. doi: 10.1002/nbm.3277.
- ***Cellular and molecular neuropathology of the cuprizone mouse model: clinical relevance for multiple sclerosis.*** Praet J, Guglielmetti C, Berneman Z, Van der Linden A, Ponsaerts P. Neurosci Biobehav Rev. 2014 Nov;47:485-505. doi: 10.1016/j.neubiorev.2014.10.004.

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DoC8	In vivo validation of three selected secretomes in a stroke mouse model
Host Institution	Universitatsklinikum Essen (UKEssen)
Primary Supervisor	HERMANN, Dirk
Email address	Dirk.Hermann@uk-essen.de
Planned duration	36 months
Subject Area	regenerative medicine, neuroscience, neuro-protection

Introduction: EVs have enormous clinical potential in the treatment of neurological diseases, most notably under conditions of ischemic stroke. Our group has previously shown that the efficacy of MSC-EVs is tightly linked to their immunomodulatory actions. We would like to explore them further in this study, figuring out how the activity of EVs may be enhanced, allowing for the optimal protection of ischemic brain tissue.

Objectives: **1**) Identify the most potent secretome conferring structural neuro-protection and functional neurological recovery in an in vivo mouse model of IS. **2)** Compare neuro-protective efficacy of supernatants and EVs of promising secretomes in the in vivo mouse model of IS. **3)** Evaluate the effect of hypoxic pre-conditioning on neuro-protective efficacy of supernatants and EVs in the mouse model of IS. **4)** Characterize the immunomodulatory actions of supernatants and EVs in the peripheral blood and brain of mice with IS.

Expected Results: **R1:** Identify to which degree the neuro-protective and recovery-promoting effects of secretomes are associated with soluble supernatant contents or EVs and whether and how they are influenced by hypoxic pre-conditioning. **R2:** compare the immunomodulatory effects of supernatants and EVs. **R3:** evaluate if and how the neuro-protective efficacy is associated with immunomodulatory actions in the peripheral blood and brain.

Secondments: **(1)** 3 months at UKEssen in Giebel group (internal secondment) to learn about advanced EV isolation, purification and characterization techniques (M8-M10)**; (2)** 2 months at MIM for training on organ-on-a-chip models (M15-M16); **(3)** 3 months at UA for training on organoid models (M19-M21); **(4)** 1 month at UniGe for training on in vitro models (M24).

Enrolment in Doctoral degree(s): Universitätsklinikum Essen, University of Duisburg-Essen

Project-specific selection criteria: The candidate should have a Master's degree or similar education level in Biology, Immunology, or Biomedical sciences. Knowledge of in vivo animal handling are required. A proven track record of experimental studies is desirable. Profound knowledge of cell biology, immunology, neuroscience, and disease biology is mandatory. The candidate should be familiar with standard histochemistry, immunohistochemistry and microscopy techniques. Experience in animal disease models is a plus. Candidates should be a strongly dedicated, highly motivated, and flexible team player with innovative thinking and excellent (English) communication skills. The candidate should have a passion to support new therapy discovery and development and have an affinity to work in an international, fast-growing high-tech environment at the edge of clinical application.

Recommended reading:

- ***Extracellular vesicles set the stage for brain plasticity and recovery by multimodal signalling.*** Hermann DM, Peruzzotti-Jametti L, Giebel B, Pluchino S. Brain. 2024 Feb 1;147(2):372-389. doi: 10.1093/brain/awad332. PMID: 37768167; PMCID: PMC10834259.
- ***Emerging roles of extracellular vesicle-associated non-coding RNAs in hypoxia: Insights from cancer, myocardial infarction and ischemic stroke.*** Hermann DM, Xin W, Bähr M, Giebel B, Doeppner

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TR. Theranostics. 2022 Jul 18;12(13):5776-5802. doi: 10.7150/thno.73931. PMID: 35966580; PMCID: PMC9373807.

- ***Small extracellular vesicles obtained from hypoxic mesenchymal stromal cells have unique characteristics that promote cerebral angiogenesis, brain remodeling and neurological recovery after focal cerebral ischemia in mice*** Gregorius J, Wang C, Stambouli O, Hussner T, Qi Y, Tertel T, Börger V, Mohamud Yusuf A, Hagemann N, Yin D, Dittrich R, Mouloud Y, Mairinger FD, Magraoui FE, Popa-Wagner A, Kleinschnitz C, Doeppner TR, Gunzer M, Meyer HE, Giebel B, Hermann DM. Basic Res Cardiol. 2021 Jun 8;116(1):40. doi: 10.1007/s00395-021-00881-9. PMID: 34105014; PMCID: PMC8187185.
- ***Mesenchymal Stromal Cell-Derived Small Extracellular Vesicles Induce Ischemic Neuroprotection by Modulating Leukocytes and Specifically Neutrophils.*** Wang C, Börger V, Sardari M, Murke F, Skuljec J, Pul R, Hagemann N, Dzyubenko E, Dittrich R, Gregorius J, Hasenberg M, Kleinschnitz C, Popa-Wagner A, Doeppner TR, Gunzer M, Giebel B, Hermann DM. Stroke. 2020 Jun;51(6):1825-1834. doi: 10.1161/STROKEAHA.119.028012. Epub 2020 Apr 21. PMID: 32312217.
- ***Extracellular Vesicles Improve Post-Stroke Neuroregeneration and Prevent Postischemic Immunosuppression.*** Doeppner TR, Herz J, Görgens A, Schlechter J, Ludwig AK, Radtke S, de Miroschedji K, Horn PA, Giebel B, Hermann DM. Stem Cells Transl Med. 2015 Oct;4(10):1131-43. doi: 10.5966/sctm.2015-0078. Epub 2015 Sep 3. PMID: 26339036; PMCID: PMC4572905.

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DoC9	**Injectable supramolecular hydrogels and microfabricated patches for the controlled release of secretome/EV fractions for cardiac regeneration**
Host Institution	Universidade do Minho (UMINHO), Portugal
Primary Supervisor	PIRES, Ricardo A and REIS, Rui L.
Email address	info@i3bs.uminho.pt
Planned duration	36 months
Subject Area	biomaterials, micropatterning, controlled release and regenerative medicine

Introduction: Cardiac tissue is characterized by its anisotropic structure (*i.e.* cellular alignment), which, associated with the myocardium mechanics, ensures the proper environment for muscle contraction. Biomaterials designed for cardiac regeneration are usually processed to present chemical (*e.g.* bioactive sites) and morphological (*e.g.* nano or micro-topography) cues, that promote cellular attachment and alignment. A particular emphasis has been given to biomaterials that mimic the composition of the extracellular matrix (ECM), such as decellularized ECM, hyaluronan, collagen, as well as proteoglycans/glycoproteins. The PhD candidate will develop a glycopeptide-based supramolecular hydrogel that gathers key biochemical, physical and nanomorphological features of the heart's ECM. The hydrogels will be prepared using building blocks that combine a peptide and a saccharide component to mimic the sulphated glycans present in the ECM, *i.e.* chondroitin sulphate (CS), benefiting from their known capacity to load and release (under a controlled manner) proteins and growth factors (as we have previously shown, *Brito et al.*, 2019). Secretome fractions (proteins/extracellular vesicles) that are known to present cardiac regeneration potential will be loaded and entrapped during the assembly of these hydrogels. Furthermore, the proposed hydrogel system will be processed into a patch (with lines and grooves at the microscale) to guide cardiomyocytes' alignment (as observed in the native cardiac tissue) as an approach to induce cardiac regeneration.

Objectives: **1)** Development of injectable hydrogels and hydrogel-based cardiac patches loaded with secretome fractions obtained from perinatal cells. **2)** In situ entrapment of the secretome fractions and their sustained delivery to cardiomyocytes. **3)** Validation of the efficacy of the engineered systems in vitro and in vivo using myocardial infraction models (collaboration with DoC4).

Expected Results: **R1**: Injectable peptide amphiphile formulations able to: gel under physiological conditions; load secretome/extracellular vesicles fractions and release them to the surrounding environment. **R2**: Peptide amphiphile formulations able to produce micropatterned surfaces and aligned structures for the generation of cardiac patches. **R3:** Inclusion of conductive nanoparticles able to improve the stability of loaded secretome proteins and induce cardiac contractility. **R4:** Micropatterned patches loaded with secretome/extracellular vesicle-rich fractions able to modulate cardiac cells' behaviour.

Secondments: **(1)** 3 months at CREM for perinatal cells' isolation (hAMSC and hUC/WJ-MSC) and preparation of secretome fractions (M19-M21); **(2)** 2 months at UniGe to learn on the in vivo administration of the developed patches/hydrogels in a pre-clinical murine model of myocardial infraction (M32-M33).

Enrolment in Doctoral degree(s): Universidade Do Minho

Project-specific selection criteria: The candidate must have a Master's degree in Chemistry, Biochemistry, Biotechnology, Biomedical Sciences, Biomedical Engineering or related areas. The candidate must not have resided in Portugal for more than 12 months in the 3 years immediately before the recruitment date, nor have carried out their main activity (work, studies, etc.) in Portugal during that period. The candidate must have expertise in biomaterials processing, cell culture (e.g. primary cells and cell lines), imaging techniques

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(e.g. fluorescence microscopy) and standard molecular analysis techniques (e.g. DNA assay, western blotting) will be valorized. The candidate must have good communication skills (fluent in English - speaking and writing).

Recommended reading:

- ***Glycopeptide-Based Supramolecular Hydrogels Induce Differentiation of Adipose Stem Cells into Neural Lineages.*** Vânia I. B. Castro, Ana R. Araújo, Filipa Duarte, António Sousa-Franco, Rui L. Reis, Iva Pashkuleva and Ricardo A. Pires, ACS Appl. Mater. Interfaces 2023, 15, 25, 29998–30007. doi: 10.1021/acsami.3c05309
- ***Carbohydrate amphiphiles for supramolecular biomaterials: Design, self-assembly, and applications.*** Alexandra Brito, Salma Kassem, Rui L. Reis, Rein V. Ulijn, Ricardo A. Pires and Iva Pashkuleva, Chem 2021, 7, 11, 2943-2964. doi: 10.1016/j.chempr.2021.04.011
- ***Minimalistic supramolecular proteoglycan mimics by co-assembly of aromatic peptide and carbohydrate amphiphiles.*** Alexandra Brito, Yousef M. Abul-Haija, Diana S. Costa, Ramon Novoa-Carballal, Rui L. Reis, Rein V. Ulijn, Ricardo A. Pires and Iva Pashkuleva, Chemical Science 2019, 10, 2385-2390. doi: 10.1039/C8SC04361B

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DoC10	**Development of biomaterial-based vehicles for the delivery of secretome fractions to the CNS regions affected by MS and IS**
Host Institution	Universidade Do Minho (UMINHO)
Primary Supervisor	REIS, Rui L. and ARAÚJO, Ana R.
Email address	info@i3bs.uminho.pt;
Planned duration	36 months
Subject Area	biomedical engineering, biomaterials and drug delivery systems

Introduction: Ischemic stroke (IS) and multiple sclerosis (MS) are both neurological conditions that result in significant damage to neural tissue. IS occurs due to an interruption in the brain's blood supply, leading to tissue damage, loss of neurological function, neuroinflammation, and neurodegeneration. In contrast, MS is an autoimmune disorder characterized by the progressive destruction of myelin sheaths around neurons, which impairs neural signalling and also causes neuroinflammation. Despite their different underlying mechanisms, both conditions share common outcomes, including tissue damage, loss of neurological function, and neurodegeneration. This highlights the need for effective neural regeneration strategies. Importantly, biomaterials, mimicking the brain's extracellular matrix (ECM), offer a promising solution for supporting neural recovery post-stroke. Moreover, there are also evidence that perinatal cells' secretome can be used as a regenerative strategy under this context. This project focuses on developing glycopeptide-based micelles and hydrogels that mimic neural ECM components like mimetics of hyaluronan and collagens. These biomaterials will be designed to cross the blood-brain barrier and release the secretome's therapeutic proteins. Using micelles of short peptide/saccharide amphiphiles as a starting point, we will enhance their properties for targeting damaged tissue and releasing the secretome formulations. These systems will be validated in vitro and in vivo using models of MS and IS. This project aims to develop glycopeptide-based micelles with tuneable properties to target the damaged tissue and release secretome-based proteins inducing a functional recovery following neurological impairments.

Objectives: **1)** Preparation of biomaterials (micelles and hydrogels) for the loading of secretome/extracellular vesicles fractions and their local delivery to the CNS regions affected by MS and IS. **2)** Optimization of the loading conditions of the secretome/extracellular vesicles fractions obtained from perinatal cells and their local delivery. **3)** Validation of the efficacy of the engineered systems, in vitro and in vivo (using MS and IS models) (collaboration with DoC7).

Expected Results: **R1:** Micelle formulations with adequate size to load secretomes (obtained from DoC1 and DoC2). **R2:** Secretome loaded micelles that can cross the BBB and reach the affected regions of the CNS. **R3:** Hydrogel formulations that are able to load secretome fractions (obtained from DoC1 and DoC2) and maintain their bioactivity of long periods of time, as well as to release them to the surrounding environment over time. **R4:** Micellar and/or hydrogel formulations that are able to promote the regeneration of the CNS tissues affected by MS and IS.

Secondments: **(1)** 1 month at UniGe for hAFSC secretome preparation (M25); **(2)** 3 months at CREM for perinatal cell isolation (hAMSC and hUC/WJ-MSC) and preparation of secretome fractions (M31-33); **(3)** 6 months at UA for in vivo evaluation of the developed secretome/extracellular vesicles-loaded systems (M35-M40).

Enrolment in Doctoral degree(s): Universidade Do Minho

Project-specific selection criteria: The candidate must have a master's degree in biotechnology, Biochemistry, Biology, Pharmacy, Biomedical engineering, or equivalent. The candidates must not have resided in Portugal for more than 12 months in the 3 years immediately before the recruitment date, nor have carried out their main activity (work, studies, etc.) in Portugal during that period. The candidate must

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have expertise in peptide synthesis, biomaterials processing, cell culture (e.g. primary cells and cell lines), imaging techniques (e.g. fluorescence microscopy) and standard molecular analysis techniques (e.g. DNA assay, western blotting) will be valorized. The candidate must have good communication skills (fluent in English - speaking and writing).

Recommended reading:

- ***Glycopeptide-Based Supramolecular Hydrogels Induce Differentiation of Adipose Stem Cells into Neural Lineages.*** Vânia I. B. Castro, Ana R. Araújo, Filipa Duarte, António Sousa-Franco, Rui L. Reis, Iva Pashkuleva and Ricardo A. Pires, ACS Appl. Mater. Interfaces 2023, 15, 25, 29998–30007. doi: 10.1021/acsami.3c05309
- ***Carbohydrate amphiphiles for supramolecular biomaterials: Design, self-assembly, and applications.*** Alexandra Brito, Salma Kassem, Rui L. Reis, Rein V. Ulijn, Ricardo A. Pires and Iva Pashkuleva, Chem 2021, 7, 11, 2943-2964. doi: 10.1016/j.chempr.2021.04.011
- ***Redox-Responsive Micellar Nanoparticles from Glycosaminoglycans for CD44 Targeted Drug Delivery.*** Ana M. Carvalho, Raquel Teixeira, Ramón Novoa-Carballal, Ricardo A. Pires, Rui L. Reis and Iva Pashkuleva, Biomacromolecules, 2018, 19, 7, 2991-2999. doi: 10.1021/acs.biomac.8b00561

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