HIGHLIGHT
DIVISION VESSELS

ARMAND JAMINON

ReGen – the next level

Some years ago, CARIM wanted to create a platform to connect the PI groups working in the various CARIM divisions. To this end, each division was partly supported by a postdoc, in order to horizontally connect the different PI groups. In June 2023, I was appointed as a CARIM postdoc for the Division Vessels. In this highlight article, I present my research plans.

SCIENTIFIC BACKGROUND

Vascular regenerative medicine aims to repair damage and enhance vessel functionality, thereby improving cardiovascular health. Traditional clinical interventions, often involving surgery, are demanding and expensive. Transitioning towards the reuse or regeneration of vascular specimens offers promising alternatives that could reduce the need for major surgeries, ultimately lowering the morbidity and mortality associated with cardiovascular disease (CVD). While coronary artery bypass graft surgery is commonly performed to treat coronary artery disease, its long-term effectiveness is often impeded by occlusion (restenosis), despite ongoing advancements in surgical techniques and medical management.

Restoring the functionality of a diseased vessel requires targeted intervention aiming at multiple cell types, including endothelial cells (ECs; intima), vascular smooth muscle cells (VSMCs; media), and various cells within the adventitial layer (adipocytes, fibroblasts, macrophages, and lymphocytes). Our research at CARIM has achieved significant progress in this field by developing innovative pluripotent stem cell lines in collaboration with Stem Cell Research University Maastricht (SCRUM) and the Heart+Vascular Center (HVC), creating CRISPR/Cas9 mediated knock-out pluripotent stem cell lines. Furthermore, we established a comprehensive framework for generating diverse populations of vascular cells, including ECs, cardiac fibroblasts, epicardium (smooth muscle and fibroblasts), VSMCs (specific to aortic root, aortic arch, and descending aorta), as well as mesenchyme-like stromal cells.

Mechanisms such as oxidative stress, senescence, apoptosis, and mechano-transduction are at the forefront of our targets in vascular regeneration. In addition, the use of stem cell technology and pathological specimens obtained from surgery at the HVC provides a unique complementary tool to support the clinical relevance and robustness of our findings. Furthermore surgical biopsies, combined with relevant clinical data, aid in identifying personalised mechanisms of arterial diseases that affect CVD outcome. Modulation of these molecular pathways will help us design and develop vascular regeneration strategies.

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This postdoc research project of the Division Vessels will focus on the regenerative development of all vessels within the cardiovascular system and will study pathological disease processes affecting them. To this end, this research proposal comprises three main research pillars:

RESEARCH STRATEGY

Research pillar 1: Biobank management

To study the pathology of vessels, we receive vascular specimens from HVC and from the Departments of Cardiothoracic and Vascular Surgery. Specimens obtained cover a wide variety of CVDs, such as thoracic and abdominal aneurysms, coronary artery disease (CAD), peripheral artery disease (PAD) and aortic valve calcification (AVC).

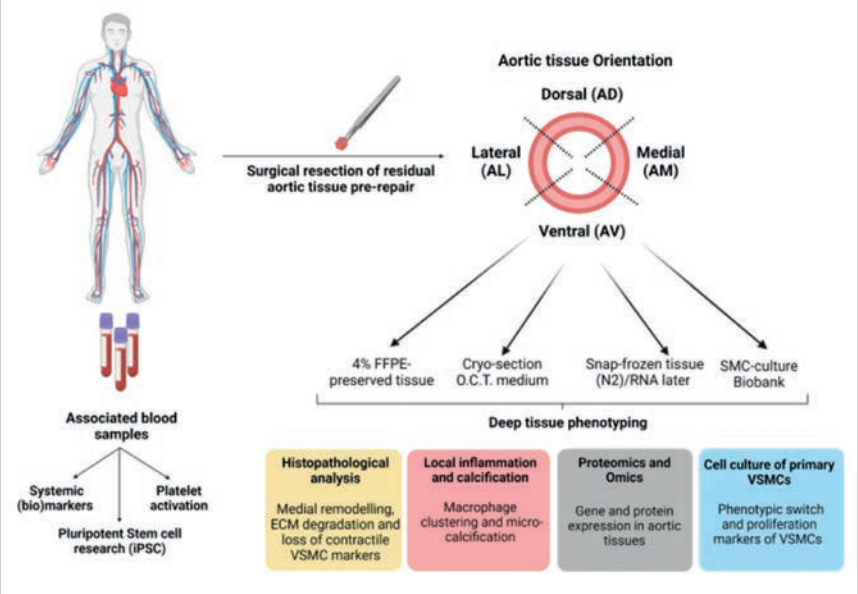

FIGURE 1 Tissue processing; obtained from Ganizada et al. Biomedicines 11, no. 8: 2095. https://doi.org/10.3390/biomedicines11082095. Location-specific characterisation of aortic aneurysm tissue as well as systemic arterial blood, enabling correlation with local differences in mechanical stresses.

Within this research pillar, we continue to support the management of vascular tissue and blood collected during routine corrective surgery. This involves coordinating the logistics and sample preparation to ensure that additions to the biobank meet standardised quality levels. Post-surgery, blood is processed for biomarker assessment and stored as serum and plasma for subsequent use. Vascular tissue is categorised by the surgical team according to pathology and anatomical classification; a section of the tissue is preserved in paraformaldehyde for histological analysis and in RNAlater for transcriptomic studies. An overview of the aortic tissue processing is displayed in **Figure 1**. If there is excess tissue, it will be utilised for primary cell isolation, decellularisation, or biophysical phenotyping using the Flexcell system, all conducted at the the Department of Biochemistry. An overview of the workflow of the HVC biobank is displayed in **Figure 2**.

In collaboration with the Department of Biomedical Engineering, we are developing and acquiring advanced platforms to characterise cellular mechanobiological signalling and responses related to tensile, compressive, and shear stress, in conjunction with biochemical markers of cell phenotype.

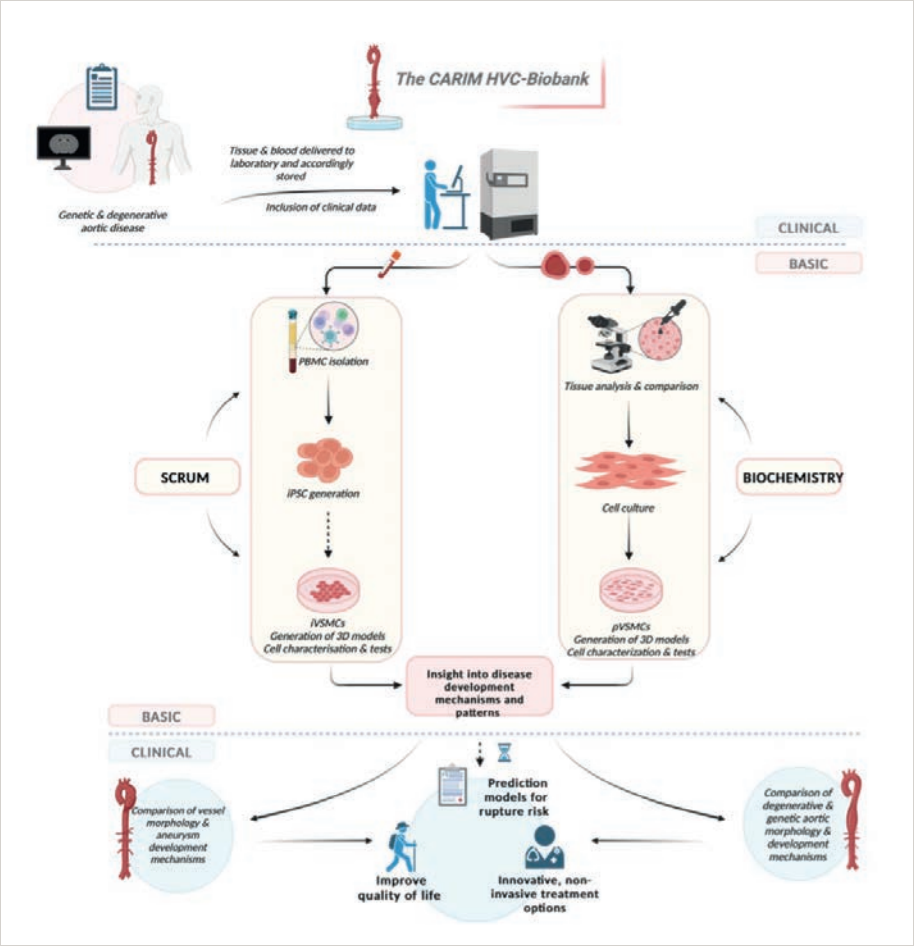

FIGURE 2 The CARIM+HVC Biobank.
Cardiothoracic and vascular surgery will deliver tissues upon inclusion of genetic and degenerative vasculopathy. Depending on the available tissue, different processing routes will be followed. All tissues will be extensively phenotyped for morphology (chemical and histochemical staining), stiffness (nano-indentation) and expression (cytochemistry/live imaging/omics). If abundant material is available, primary cells will be isolated from vascular specimens obtained, which can be applied in the cellular models. In certain cases (monogenetic disorders), PBMCs will be isolated and stored for later iPSC generation with subsequent differentiation of iVascular cells. These reprogrammed cells can then be directly compared with primary (exposed/diseased) cells, or assessment of differences between healthy and diseased cells can be conducted. All measurements can be correlated with clinical data from these patients, ultimately leading to improved quality of life or innovative treatment options.

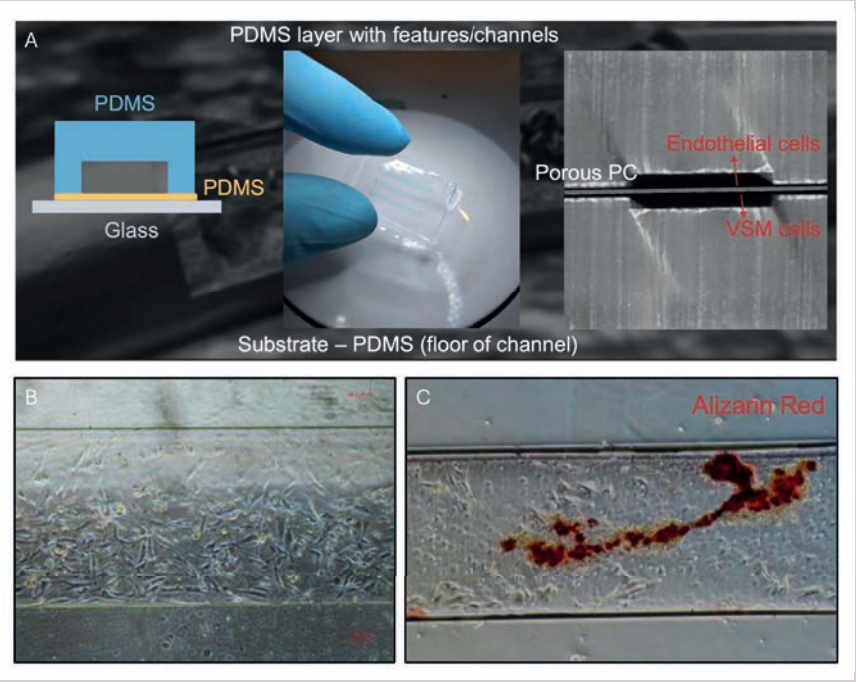

FIGURE 3 Vascular graft.
A. Schematic design of the graft, which is made from thermoplastic (PDMS) and can simultaneously culture multiple cell types. B. Attached VSMCs inside the channel cultured for multiple days, forming a confluent layer. C. After calcification induction (high calcium conditions), there is Alizarin Red positivity, indicative of the presence of calcification.

To this end, we have acquired a nano-indentation device (Optics11) which is coupled with a confocal microscope to follow fluorescent markers (Confocal.nl). With this platform we can measure tissue and/or cellular stiffness while simultaneously analysing fluorescent reporter expression.

Patients presenting with congenital aortic pathologies are another highly interesting patient population within the HVC, and will be identified by medical staff. These congenital patients will be invited to donate blood for pluripotent stem cell research. Supported by the existing ethical research framework at Maastricht UMC+, consent and blood tissue donation will be obtained in conjunction with standard patient care procedures. Peripheral blood mononuclear cells (PBMCs) will be isolated from whole blood samples and stored along with clinical and genetic information for the generation of induced pluripotent stem cell (iPSC) lines. These iPSC lines will be incorporated into the CARIM+HVC BioBank. Within SCRUM, we will facilitate the generation of novel iPSC lines for the purpose of studying congenital aorto-pathies, including Marfan syndrome, Loeys-Dietz syndrome, Ehlers-Danlos syndrome, and other connective tissue disorders.

Research pillar 2: Differentiation of vascular cells

In this research pillar we aim to develop vascular grafts that can be used to study 'vascular disease in a dish'. For this purpose, we collaborate with the MERLN Institute for Technology-Inspired Regenerative Medicine and have developed a device that can culture cells in a tubular structure and under flow. The first ideas centred around the development of such a model, after which diseases such as vascular calcification could be induced. **Figure 3** shows some pilot results, where we successfully produced and cultured the VSMCs in a tubular structure and were able to perfuse the system, which was performed in the context of the Regenerative Medicine crossing border consortium (RegMed XB) and in collaboration with MERLN. VSMCs were cultured under flow and were stimulated to calcify. Staining with Alizarin Red (calcification) showed positivity located in proximity to the VSMCs, which is indicative of pathological calcification build-up.

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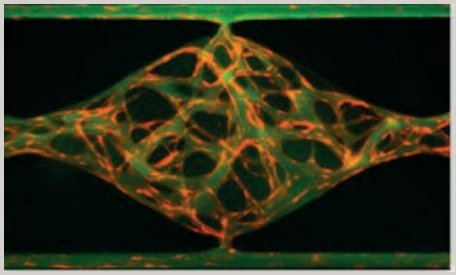

FIGURE 4
Vascularised micro-organism
A representation of spontaneous vessel formation in the VMO.

Additionally, smaller vessels, such as the 'vessel on a chip model', have been developed in collaboration with Prof. Hughes (visiting professor at CARIM; UC Irvine). The vascularised micro-organ (VMO) requires the use of stem cells which develop into spontaneously arranged microvessels (**Figure 4**). Our iPSC biobank can be used to differentiate towards the development of vascular cells (iVascular cells). iVascular cells will be applied in the VMO system to study disease development. iVascular cells that we aim to develop include iECs from different vascular beds (i.e arterial, venous and capillaries) as well as iVSMCs of different embryonic origins to investigate characteristics in relation to coagulation (in collaboration with the Division Blood), stiffness, functionality, and remodelling of the scaffold.

The 'vascular grafts and VMO' can be directly used to study molecular or pathological pathways affecting vascular disease. Furthermore, this research will connect researchers studying small vessel disease linked to pathologies such as chronic kidney disease (Dr Ed Eringa), The Maastricht Study (Dr Marleen van Greevenbroek) and type 2 diabetes (Prof. Casper Schalkwijk).

Research pillar 3: Improving functionality of regenerative vessels

In this final work package, we will initiate work on CRISPR/Cas9 technology to enhance its use in studying vascular biology and disease mechanisms. CRISPR/Cas9 genetic engineering will enable the creation of isogenic controls, providing a more thorough understanding of the role of mutations and molecular mechanisms in monogenic aortopathy. Additionally, CRISPR/Cas9 gene editing will be employed to disrupt functional pathways or modify specific genetic targets involved in vascular disease.

Using this technology, we will be able to assess the functionality of the scaffolds developed (from RP2), using techniques that evaluate vessel compliance and integrity, including myograph measurements, nano-indentation and two-photon microscopy (in collaboration with Dr Koen Reesink, Biomedical Engineering). This research will investigate how modulation of molecular pathways impacts vascular graft viability. Larger scaffolds will be used to explore their properties concerning vessel, tissue, and cellular stiffness. A recently developed mechanosensor (YAP/TAZ sensor) incorporated into iVSMCS will facilitate the study of interactions between cells and the extracellular matrix.

Ultimately, diseased vascular specimens obtained from vascular surgery will be used to study regenerative processes. Removing diseased cells or decalcifying vascular tissue is crucial for reimplantation of host tissue or graft development. Reusing patient vascular tissue is advantageous because it eliminates the need for donor tissue and avoids immune system activation. Radiobiochemical cardiovascular imaging will be applied, using various ex vivo techniques, such as tonometry (to measure vessel compliance), development of novel PET/CT tracers (^{18}F-NaF), and imaging with fluorescently labelled tracers (such as annexin A5, fetuin-A, and CCL5) to assess apoptosis, calcification, and inflammation, respectively.