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REVIEW

Novel therapeutic concepts

Coronary microcirculatory pathophysiology: can we afford it to remain a black box?

Axel R. Pries[1,2]* and Bettina Reglin[1]

[1]Department of Physiology, Center for Cardiovascular Research, Charité, Charitéplatz 1, D-10117 Berlin, Germany; and [2]Deutsches Herzzentrum Berlin, Augustenburger Platz 1, D-13353 Berlin, Germany

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Coronary microvascular networks play the key role in determining blood flow distribution in the heart. Matching local blood supply to tissue metabolic demand entails continuous adaptation of coronary vessels via regulation of smooth muscle tone and structural dilated vessel diameter. The importance of coronary microcirculation for relevant pathological conditions including angina in patients with normal or near-normal coronary angiograms [microvascular angina (MVA)] and heart failure with preserved ejection fraction (HFpEF) is increasingly recognized. For MVA, clinical studies have shown a prevalence of up to 40% in patients with suspected coronary artery disease and a relevant impact on adverse cardiovascular events including cardiac death, stroke, and heart failure. Despite a continuously increasing number of corresponding clinical studies, the knowledge on pathophysiological cause–effect relations involving coronary microcirculation is, however, still very limited. A number of pathophysiological hypotheses for MVA and HFpEF have been suggested but are not established to a degree, which would allow definition of nosological entities, stratification of affected patients, or development of effective therapeutic strategies. This may be related to a steep decline in experimental (animal) pathophysiological studies in this area during the last 15 years. Since technology to experimentally investigate microvascular pathophysiology in the beating heart is increasingly, in principle, available, a concerted effort to build 'coronary microcirculatory observatories' to close this gap and to accelerate clinical progress in this area is suggested.

Keywords Microvascular heterogeneity • Endothelial surface layer • Glycocalyx • Conduction • Metabolic regulation • Mathematical modelling • CMVD • MVA • HFpEF

Introduction

About 50 years ago, Likoff *et al.* described the 'paradox of normal selective coronary arteriograms in patients considered to have unmistakable coronary heart disease' and first suggested coronary microvascular disorder to be the underlying cause.[1] Angina attacks in the absence of apparent cardiac or systemic disease are described as 'microvascular angina' (MVA)[2] or 'primary MVA'.[3] (It has to be kept in mind that an apparently normal angiogram does not exclude epicardial coronary pathology and that modern imaging techniques identify epicardial coronary pathology also in most instances of MVA.) These conditions are observed in up to 40% of patients with suspected coronary artery disease.[4,5] The prognosis of these conditions is associated with increased risk of major adverse cardiovascular events including cardiac death, stroke, and heart failure, especially if impaired endothelial function is diagnosed.[6–9]

Numerous studies have confirmed that abnormalities in structure and function of the coronary microcirculation occur in many clinical conditions as important markers of risk and as contributors to cardiac pathophysiology, a condition referred to as 'coronary microvascular dysfunction' (CMVD, see ref.[10]). Current hypotheses with respect to the aetiology and diagnostic assessment strategies of MVA/CMVD as well as pharmacological approaches are summarized in many recent reviews (e.g. refs.[3,11–14]). However, these reviews document significant deficits in knowledge and consensus on detailed pathophysiological mechanisms involving coronary microcirculation, their relation to different manifestations of these diseases, and, most importantly, effective treatment strategies.

Recently, CMVD was suggested to be a relevant factor in the development of heart failure with preserved ejection fraction (HFpEF),[15] relevant for nearly half of all patients with a clinical

* Corresponding author. Tel: +49 30 450 570 521, Fax: +49 30 450 570 952, Email: axel.pries@charite.de

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diagnosis of heart failure.[16] A new conceptual paradigm was suggested,[17] which shifted emphasis from left ventricular overload excess to microvascular pathology as a cause for HFpEF. This hypothesis was supported by findings on the association of microvascular density with left ventricular fibrosis in autopsy specimen from subjects with ante-mortem diagnosis of HFpEF.[18]

The present article based on the 2015 Harvey lecture by A.R.P., one of the authors, at the European Society of Cardiology (ESC) Congress in London aims to address the lack of proven pathophysiological insights and consequently rational therapeutic strategies for the mentioned conditions from a (coronary) microvascular perspective. As a possible approach for improvement, the establishment of 'coronary microcirculatory observatories' is proposed, which would allow clinical and experimental researchers to collectively address these clinically relevant diseases.

Coronary microcirculation: an adaptive system

The coronary microcirculation includes vessels with diameters below ~300 μm ranging from small arteries/arterioles to similar sized venules, and is the site of regulation of flow resistance and thus perfusion. It represents the 'business end' of the circulation supplying oxygen and nutrients to the tissue, removing waste products, and controlling inflammation and repair as well as fluid exchange with the tissue. Coronary microcirculation and regulation of coronary blood flow are summarized in recent reviews.[19–21]

Vessel arrangement and morphology in microvascular networks do not follow a fixed 'blue print' but rather emerge from vascular adaptation in response to changing conditions and functional demands (angioadaptation).[22,23] Vascular adaptation needs to establish low diffusion distances from vessel lumen to all tissue and acceptable perfusion heterogeneity within the network.[24] This requires a multitude of parallel flow pathways with well-coordinated vessel diameter distributions while minimizing overall energy demand for perfusion. Normal vascular adaptation guarantees adequate perfusion during rest and sufficient perfusion reserve. However, microvascular network mal-adaptation may cause inadequate blood flow distribution and/or insufficient blood flow increase and consequential dysfunction of the tissue.

For vessel diameter and wall thickness adaptation in microvascular networks, a minimal set of adaption rules was derived, combining *in vivo* experiments in the rat mesentery with theoretical modelling, postulating vascular responses to haemodynamic signals (wall shear stress, transmural pressure/vessel wall stress) and oxygenation-based metabolic signals acting locally and being transferred throughout the microvascular network[22,23] (*Figure 1*). These adaptation rules were validated for mesentery vascular networks but can be assumed to also govern microvascular adaptation in the heart since coronary microvessels exhibit the assumed vascular reactions, including dilation to increased wall shear stress, myogenic responses, and metabolic reactions.[19,25]

A hallmark of microvascular networks including the heart is heterogeneity (*Figure 2*).[20,24,26,27] Real microvascular networks need to supply all tissue regions including those adjacent to main feeding and draining vessels and are generated by stochastic angiogenetic processes. As a consequence of this topological and morphological heterogeneity,[28] a pronounced heterogeneity of all functional vessel parameters emerges.[29] In the heart, both regional myocardial blood flows and local oxidative metabolism are spatially heterogeneous, and correlate to one another[30] with remarkable temporal stability.[31] The effects of the variation of perfusion during the cardiac cycle will add a temporal component to perfusion heterogeneity in the heart.

Myocardial ischaemia: large coronary vessels vs. microvascular networks

Myocardial ischaemia may be caused by irregularities of both epicardial arteries and downstream microvascular networks. However, respective consequences differ substantially (see *Figure 3*). Epicardial arteries determine total blood supply (bulk perfusion) to a larger myocardial region. A flow-limiting stenosis of these vessels will therefore cause global or regional myocardial supply-to-demand mismatch, and thus an increased extraction and arteriovenous difference of oxygen content (AVD-O_2) (*Figure 3*, left). In contrast, the microcirculation controls blood flow distribution within terminal vascular networks safeguarding a functionally adequate supply distribution. Microvascular dysfunction typically causes increased heterogeneity of flow and oxygen (with or without a parallel increase in global flow resistance), which will lead to functional shunting, local hypoxia, and to a reduced mean AVD-O_2 (*Figure 3*, right).[28,32]

A bulk reduction of oxygenation may not be typical for microvascular dysfunction but rather a local mosaic of high and low oxygenated areas. However, clinical imaging approaches do not provide the resolution necessary to detect heterogeneity caused by microvascular mal-distribution. Also, the patchy impairment of perfusion can make the detection of contractile abnormalities and ischaemic metabolite release difficult.[33] The high basal oxygen extraction capacity of the normal heart compared with other organs is a strong evidence for the fact that myocardial microvascular perfusion heterogeneity under physiological conditions is well controlled.[20] The even more efficient oxygen extraction at high myocardial blood flow depends on a concomitant reduction of capillary transit time (CTT) heterogeneity and thus perfusion heterogeneity. In turn, the heart is especially vulnerable to all mechanisms that increase heterogeneity, leading to functional shunting and thus lower oxygen extraction.

Coronary microvascular dysfunction

In the literature, a substantial number of hypotheses for the pathophysiology of CMVD have been proposed (*Table 1*).[3,11–14] Some findings on the most common hypotheses are briefly addressed in what follows.

Pathophysiological hypotheses

Endothelial dysfunction

A normally functioning vascular endothelium is needed for appropriate dilatation of arterial vessels since endothelial cells produce several mediators with vasorelaxing, anti-proliferative, and antithrombotic effects [nitric oxide (NO), prostacyclin, endothelium-derived

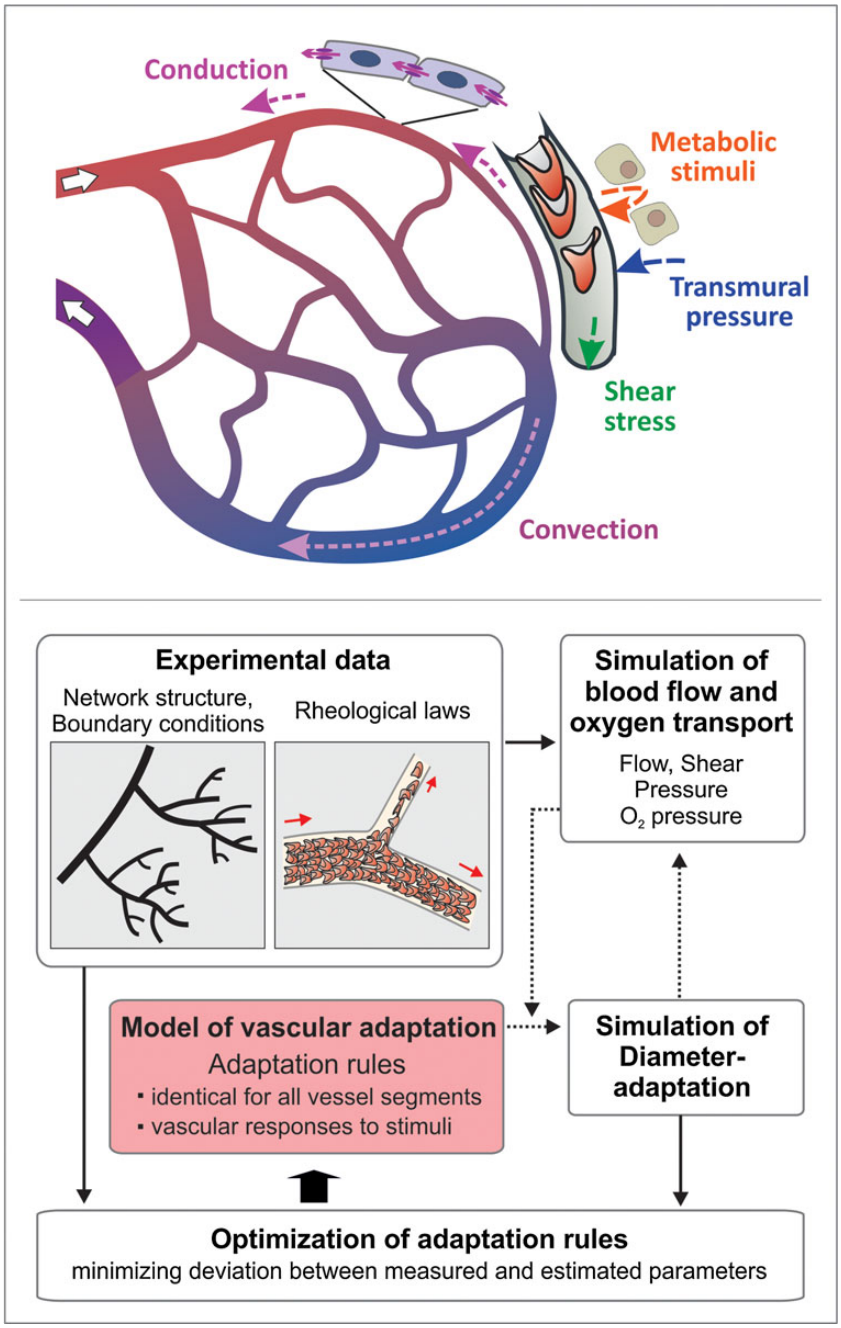

Figure 1 Model for vascular diameter adaptation in microvascular networks. Top: adaptation concept. Every vessel responds to local haemodynamic signals (wall shear stress, transmural pressure/circumferential wall stress), to metabolic signals, and to signals transmitted via upstream signal conduction through gap junctions in the vessel wall and downstream via convection of signal substances with the flowing blood. Bottom: modelling approach. *In vivo* experimental data from large microvascular networks are combined with theoretical simulations to derive a minimal set of adaptation rules. Network haemodynamics and oxygen distribution are calculated for all vessel segments. Segment adaptation of diameter and wall thickness in response to local conditions is determined according to an assumed set of adaptation rules. Haemodynamics and metabolics are re-calculated evoking further adaptation iteratively until convergence is achieved. Adaptation rules are optimized by comparing *in vivo* and calculated flow velocity for all vessels.[22,23]

hyperpolarizing factor]. Endothelial cells in the heart also regulate the contractile state of cardiomyocytes via autocrine and paracrine signalling involving NO and endothelin-1. In addition, they exert anti-apoptotic effects and promote cardiomyocyte-synchronized contraction via influencing the expression of principal gap junction protein connexin-43 expression.[17]

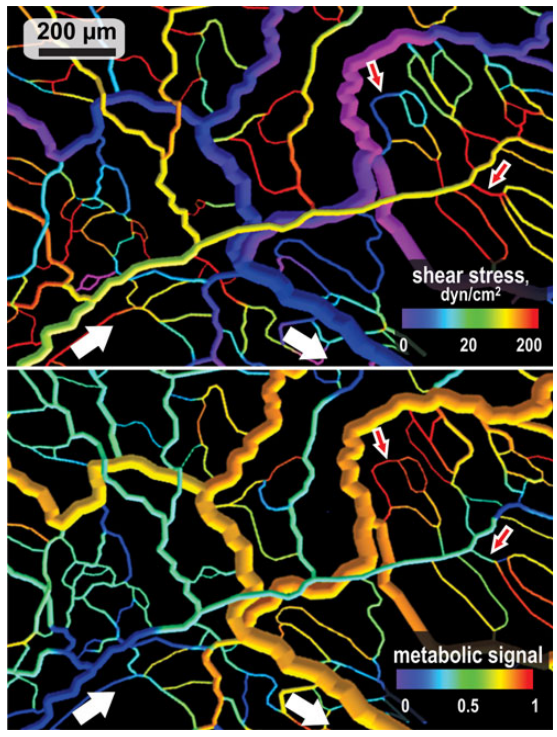

Figure 2 Heterogeneity of microvascular networks. Computer reconstruction of a mesenteric microvascular network. White arrows indicate the main feeding (draining) arteriole (venule). The vessels are colour coded for wall shear stress (upper panel) and a metabolic signal (lower panel), which increases with decreasing pO_2 as calculated by a computer model of vascular adaptation (see above).[22,23] Red arrows indicate two closely spaced capillaries with very different haemodynamic and metabolic conditions.

In 'endothelial dysfunction' due to increased oxidative stress, the endothelium may release substances with opposing effects, such as endothelin, thromboxane A_2, prostaglandin H_2, and superoxide. Consequently, a net dilator response to a variety of stimuli is shifted to a net constrictor response. Endothelial dysfunction also involves a switch from a quiescent to an activated state promoting inflammatory responses, chemokine and adhesion molecule expression, and consecutive interaction with platelets and leukocytes (see ref.[25]). Reduced bioavailability of NO causes migratory and angiogenic incompetence of endothelial cells shifting the balance between vessel generation and pruning towards vessel destruction, evoking microvascular rarefaction and decreased microvascular density.[34] Endothelial cell function is usually tested by intravascular application of acetylcholine evoking release of vasodilating mediators. In several studies, a reduced flow increase upon intracoronary infusion of acetylcholine was observed despite normal diameter increase of epicardial vessels.[35]

Smooth muscle cell dysfunction

Evidence of a primary impairment of smooth muscle cell relaxation is usually derived from a reduced coronary blood flow response to endothelium-independent vasodilators (e.g. adenosine, dipyridamole, papaverine) as has been reported repeatedly in patients with MVA[36] but was not observed in other studies[35] and was not related to dilatation during atrial pacing.[37]

Microvascular spasm/sympathetic dysfunction

In patients with normal coronary angiograms and atypical chest pain, there is only negligible α-adrenergic coronary constrictor tone at rest,[38] but α_2-adrenergic activation reduces cardiac perfusion[38] by microvascular constriction.[39] In the presence of coronary endothelial dysfunction and atherosclerosis, responses to α-adrenoceptor activation as observed during exercise and coronary interventions are augmented. Mediated via both α_1- and α_2-adrenoceptors in epicardial conduit arteries and microvessels, vasoconstriction is powerful enough to induce myocardial ischaemia. However, usefulness of therapeutic application of α-antagonists is still under debate.[21]

Altered microvascular remodelling

Some studies on myocardial biopsies from patients with MVA have found evidence of sclerosis of small arteries and arterioles with perivascular fibrosis, swollen endothelial nuclei in capillaries, and irregular lumina of small arteries.[40] Another study, however, reported no evidence of significant alteration of small vessels.[41]

Additional pathophysiological hypotheses include vascular rarefaction, extramural compression, vascular wall infiltration, and luminal obstruction. In the following, we propose three further hypotheses for the pathophysiology of MVA based on experimental and modelling research on microvascular networks.

New hypotheses

Degraded endothelial surface layer/glycocalyx

The luminal surface of the endothelium is lined with an 'endothelial surface layer' (ESL, also called glycocalyx, estimated thickness 0.5 to over 1 µm), consisting of a thin layer composed of membrane-bound macromolecules (the glycocalyx in a strict sense) and an additional relatively thick layer of adsorbed plasma components.[42] The ESL is critically involved in the regulation of blood flow (transmission of shear stress).[42] Structural ESL degradation results in a decrease of shear-dependent NO-mediated arteriolar vasodilation.[43] The ESL is very labile and can easily be degraded, e.g. by oxygen radicals,[42] ischaemia,[44] inflammation, or altered plasma composition.[45] These findings suggest that a degradation of the coronary ESL could be involved in CMVD.

Compromised conduction

In addition to local metabolic and haemodynamic vascular growth signals, upstream conduction of signals along the vessel wall via gap junctions (connexons composed of connexins) is needed to maintain adequate flow and oxygen distribution in microvascular networks[22] (*Figures 1* and *4*, upper panels). If conduction is compromised (*Figure 4*, lower panels), functional shunting occurs and the distribution of flow and oxygen becomes very patchy. It has been hypothesized that a compromised conduction contributes to the very heterogeneous oxygen distribution in tumour tissue.[32] While the regulation of vascular connexins in the heart is not known in detail, there are reports showing the relation of connexin expression and function to ageing,[46] to hypertension,[47] and to ischaemia/reperfusion,[48] again

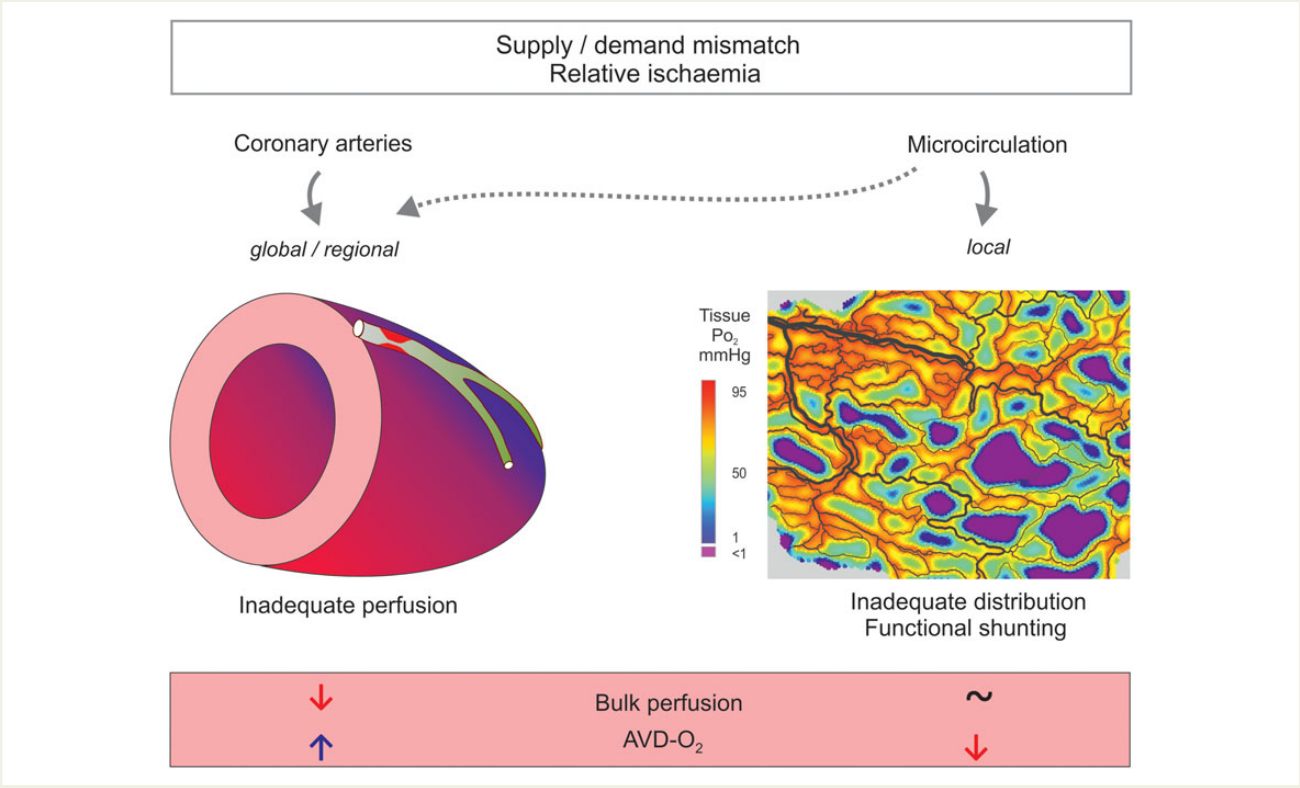

Figure 3 Consequences of compromised macro- and microvascular function. Coronary artery stenosis (left) and consequential increase of flow resistance lead to decreased bulk perfusion, global or regional supply-to-demand mismatch, and increased oxygen extraction (increased arteriovenous difference of oxygen content, AVD-O_2). In the microcirculation, a generalized dysfunction on the arteriolar level may also lead to global tissue ischaemia. Typically, however, microvascular disturbance leads to an increased heterogeneity of flow and oxygen distribution (right). Underperfused tissue regions with low flow and high extraction may border to overperfused regions with high flow and low extraction through which blood is functionally shunted resulting in reduced mean arteriovenous difference of oxygen content.[28,32]

Table 1 Pathophysiological mechanisms proposed for coronary microvascular dysfunction

Proposed mechanism	Evidence level			
	Hypothesis	Correlation	Causality	Pathophysiological chain
Endothelial dysfunction	Yes	yes	yes	no
Smooth muscle dysfunction	yes	yes	?	no
Microvascular spasm	yes	yes	yes / ?	no
Sympathetic Dysfunction	yes	yes	?	no
Altered microvascular remodeling	yes	yes	?	no
Vascular rarefaction	yes	yes	?	no
Extramural compression	yes	yes	?	no
Vascular wall infiltration	yes	yes	?	no
Luminal obstruction	yes	yes	yes / ?	no
Degraded ESL / glycocalyx	yes	no	no	no
Compromised conduction	yes	no	no	no
Impaired metabolic feedback	yes	no	no	no

The level of evidence for the respective mechanism is indicated. Correlation: evidence for the respective alteration has been found in the heart or in other tissues. Causality: direct tests have shown the contribution of the mechanism to the condition. Pathophysiological chain: the events leading from the proposed mechanism and to angina symptoms have been demonstrated. The last three mechanisms (orange letters) are suggested in the present article.
ESL, endothelial surface layer.

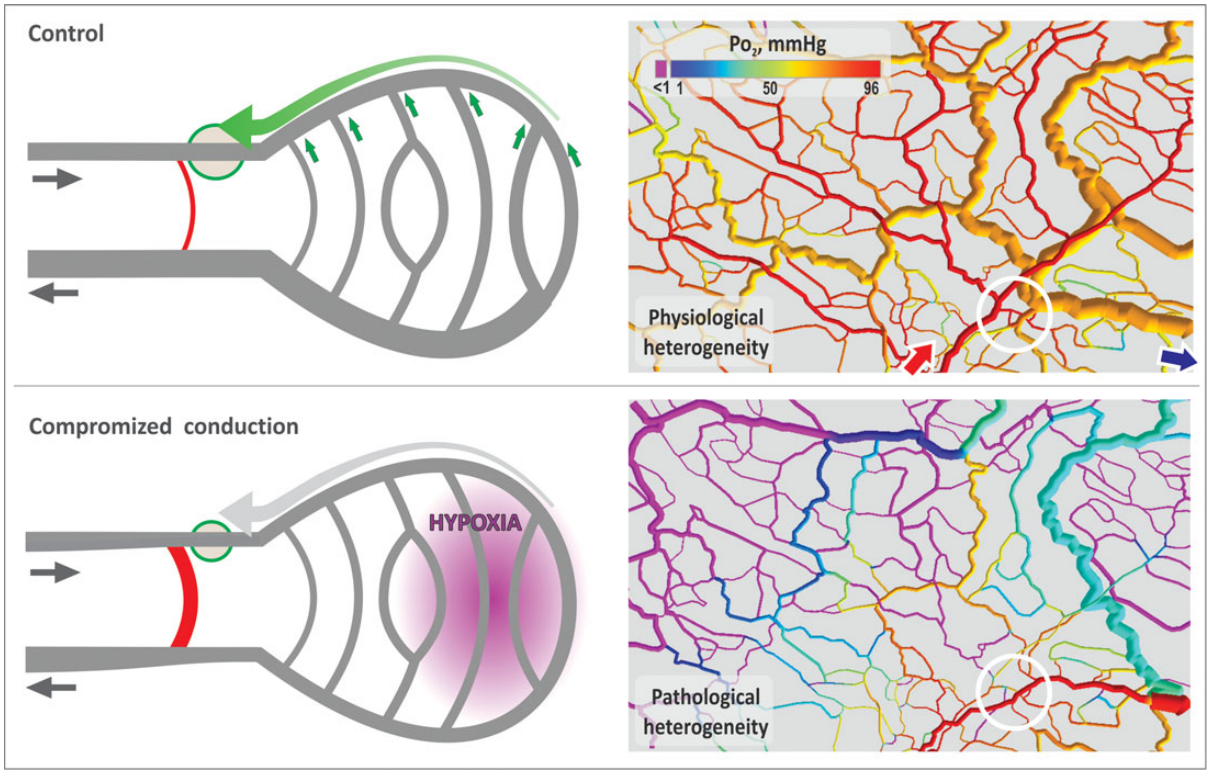

Figure 4 Conducted signals in vascular adaptation and effect of compromised conduction. Top: real vascular networks exhibit long and short arteriovenous flow pathways. Left: a short pathway (red) branches from an arteriole (green circle) that feeds a number of more distal capillaries. To prevent functional shunting, the feeding arteriole must exhibit a high diameter and low flow resistance relative to the short pathway. However, both vessels experience the same metabolic conditions, and the short connection exhibits high wall shear stress, which acts as growth signal. To prevent shunting, a strong compensating growth signal is required exclusively for the feeding arteriole.[32] This signal is generated in response to metabolic conditions in the supported capillaries (small green arrows) and transmitted via connexins along the vessel wall to the feeding vessel (large green arrow). Thus, the flow in the feeding arteriole is maintained high and heterogeneity of oxygen distribution remains on an acceptable level. Right: a similar situation is shown for a network in the rat mesentery. The main arteriole (red arrow) is connected to the main draining venule of the network (blue arrow) via a short but narrow flow pathway (white circle). Bottom: if conduction is low or absent, the diameter of the feeding vessel relative to that of the short arteriovenous connection decreases (left panel and right panel, white circle). Thus, blood flow to distal regions is low, leading to a very heterogeneous oxygen distribution and large hypoxic areas.

rendering it plausible that a compromised conductive function may contribute to CMVD.

Impaired metabolic feedback

Vascular responses to metabolic signal substances released from different sources (*Figure 5A*) are needed to match local blood flow to tissue demand. Under resting steady-state conditions, metabolic signalling evoking negative feedback regulation is required to limit spatial heterogeneity of supply. In conditions of increased demand, metabolic signalling is needed to boost blood flow increase.[49,50] The main effects of impaired metabolic signalling would thus be an increase in spatial perfusion heterogeneity in resting state (*Figure 5B*, see also *Figure 3*) and a reduced perfusion increase in conditions of increased demand, e.g. during physical exercise. In principle, metabolic feedback signalling could be compromised at any step in the complex signalling chain, including oxygen sensing, production of signal substance, or activity of respective receptors. Currently, there is little direct evidence for such a mechanism to be involved in coronary MVA. However, an impairment of red blood cell (RBC) capacity to produce NO has been reported[51] in patients with MVA, possibly reflecting an impaired RBC metabolic signalling.

The vascular adaptation of the coronary microcirculation allows adequate responses to physiological stimuli—but under pathophysiological conditions, mal-adaptation may cause and aggravate problems of supply and tissue function. For some of the referenced pathophysiological mechanisms, especially for the ones introduced here, *Figure 6* indicates possible relations to steps of the vascular adaptation cascade. Also, possible pathophysiological chains connecting these mechanisms with the two conditions (MVA, HFpEF) are considered.

The research situation

As discussed, the definitive pathophysiological causes of MVA and HFpEF remain poorly known and the respective evidence seems to be restricted to a correlative level. *Figure 7* shows the results of

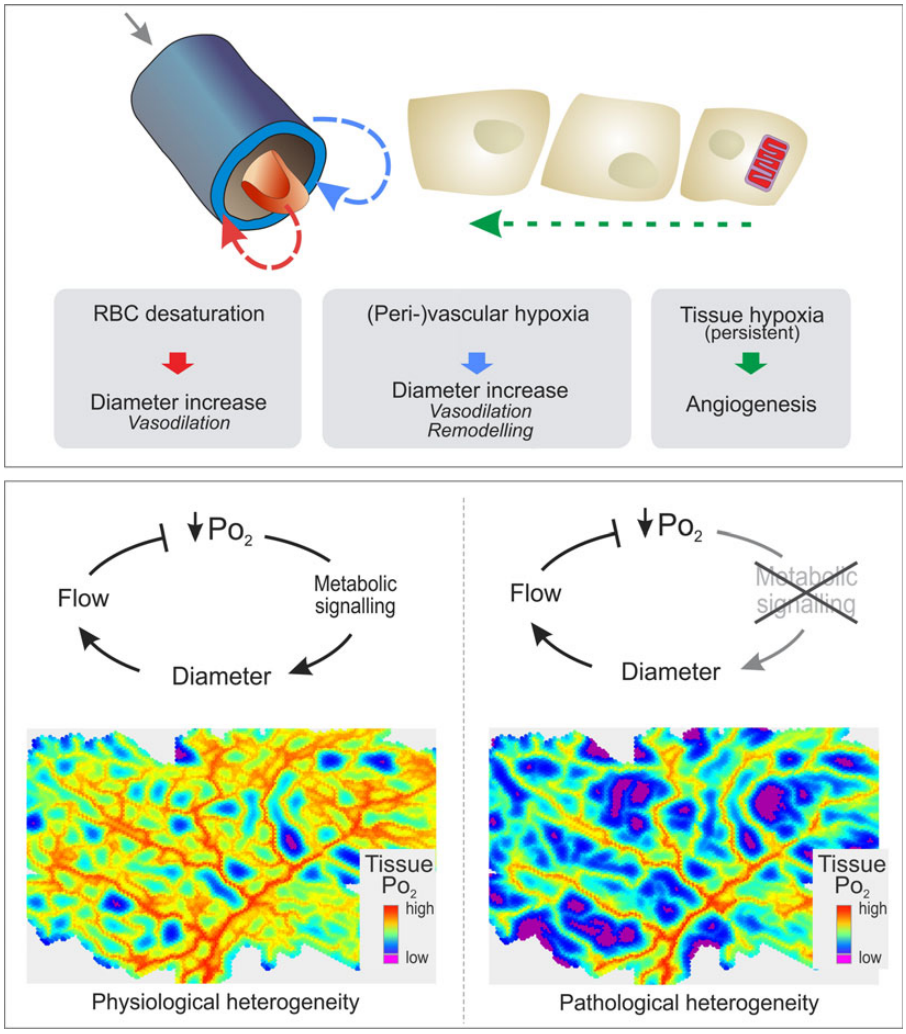

Figure 5 Possible effects of compromised metabolic signalling in vascular adaptation. Top: metabolic signals for vascular diameter adaptation may be generated by red blood cells (red arrows), by the vessel wall and the perivascular region (blue arrows), and by parenchymal tissue cells (green arrows).[49,50] Bottom: tissue oxygen distribution in the presence of functional metabolic signalling (left) exhibiting a physiological level of heterogeneity and upon compromised metabolic signalling (right) with strongly increased spatial heterogeneity not compatible with normal tissue function.

a PubMed research with respect to publication frequency in the area of coronary microcirculation. Numbers are given for clinical studies including humans (blue) and for experimental studies including animals (red) while studies combining both categories were excluded. The number of publications for clinical and experimental research exhibit three phases. From about 1970 to mid-1980, the number remains stable on a relatively low level. The remaining two to three decades of the 20th century saw a substantial increase in publication frequency both for clinical and experimental work. The last phase, however, starting about the turn of the century, shows a very different picture. While the number of clinical studies continued to increase steadily, the frequency of experimental studies declined sharply. In 2014, experimental publications amounted to less than one-third of that of studies including human research.

In the clinic, the development of new modalities for invasive (e.g. pressure/flow wire) and non-invasive (e.g. PET, functional MRI) investigation of the coronary microcirculation has been very dynamic in the last three decades (see refs.[25,52]). This has stimulated an increase in respective investigations, which were crucial in establishing the prevalence and relevance of coronary microvascular disturbances as described earlier. Since direct visualization of the human cardiac microcirculation *in vivo* is not possible at present, assessment of CMVD is usually based on evaluating coronary reactivity to acetylcholine and of coronary flow reserve (CFR) in response to metabolic or pharmacological stimuli using both non-invasive and invasive methods for measuring cardiac perfusion, CFR, intracoronary pressure, and transit time (see refs.[52,53]). The low spatial resolution of these approaches and limitations with respect to specific determination of microvascular function pose a significant hurdle for the clinical investigation of pathophysiology.

The observed parallel decline in the experimental domain may have been influenced by two factors. On one hand, cellular and

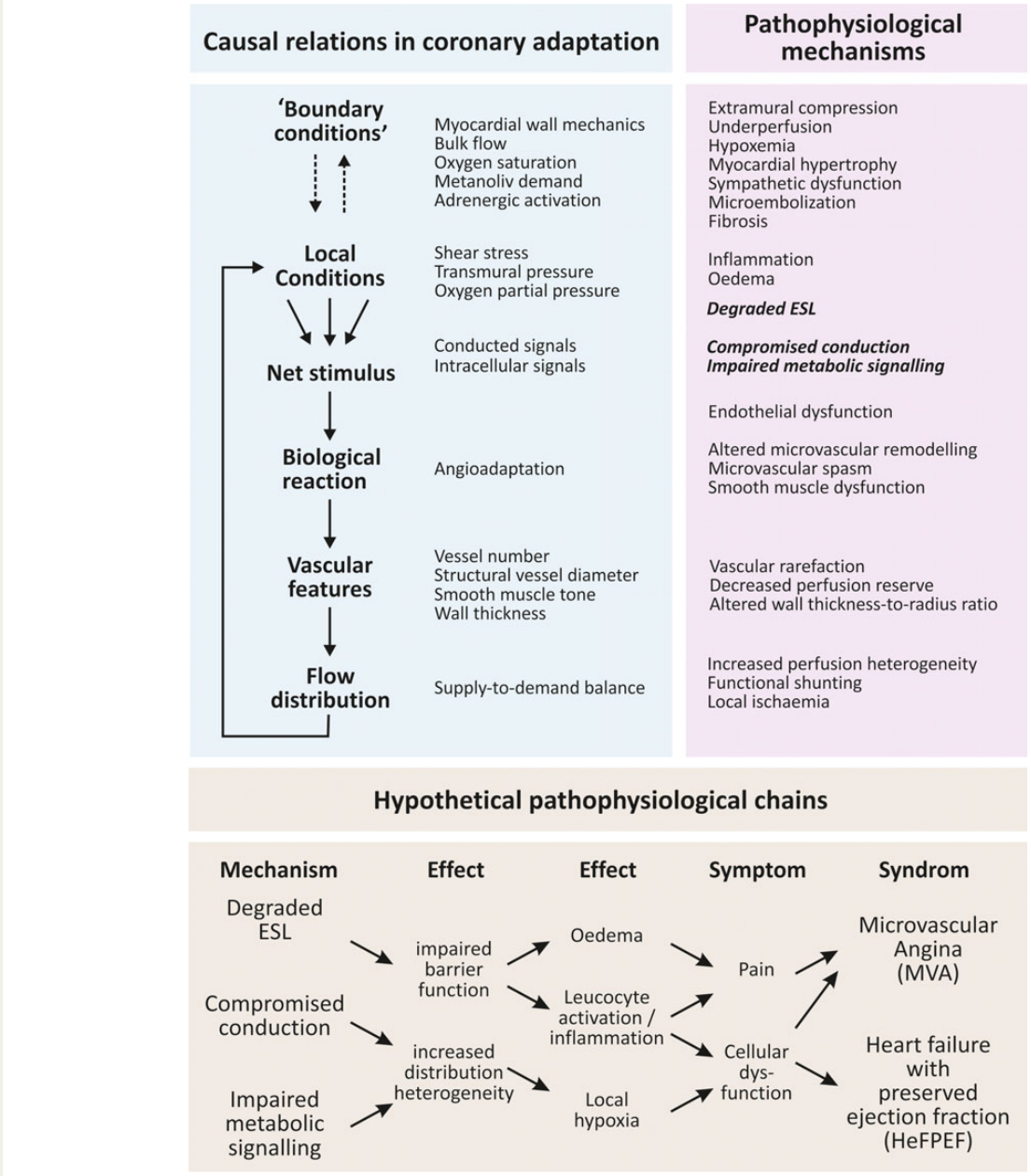

Figure 6 Pathophysiological relations. Top: each coronary vessel, especially in the microcirculation, is subject to continuous adaptation to local conditions leading to changes in vessel phenotype and perfusion pattern (left). Deficits on the steps of this cascade correspond to different pathophysiological mechanisms (right). Bottom: for the pathophysiological mechanisms proposed here, hypothetical causal chains can be considered, which would link these mechanisms to the main conditions considered here, i.e. microvascular angina and heart failure with preserved ejection fraction.

molecular techniques evolved dramatically as a main focus of life sciences. In the cardiovascular field, they were used to target a number of very prominent basic topics (e.g. endothelial function). On the other hand, myocardial microcirculation is notoriously hard to visualize due to the low transparency of the tissue (*Figure 8*, upper panel) and the difficult mechanical environment of a beating heart *in situ* even in experimental settings. Thus, it was not necessarily very attractive for researchers to move into this area. However, newly developed microscopic techniques allow a much better access even to tissues with difficult optical properties like the heart *in vivo* (*Figure 8*, lower panel).

As a consequence of these developments, the 'middle level' of *in vivo* (or *ex vivo*) studies of the coronary microvascular pathophysiology became underpopulated in the last decades despite the growing evidence for the need of respective studies. This type of studies is critically required to link mechanistic hypotheses generated in

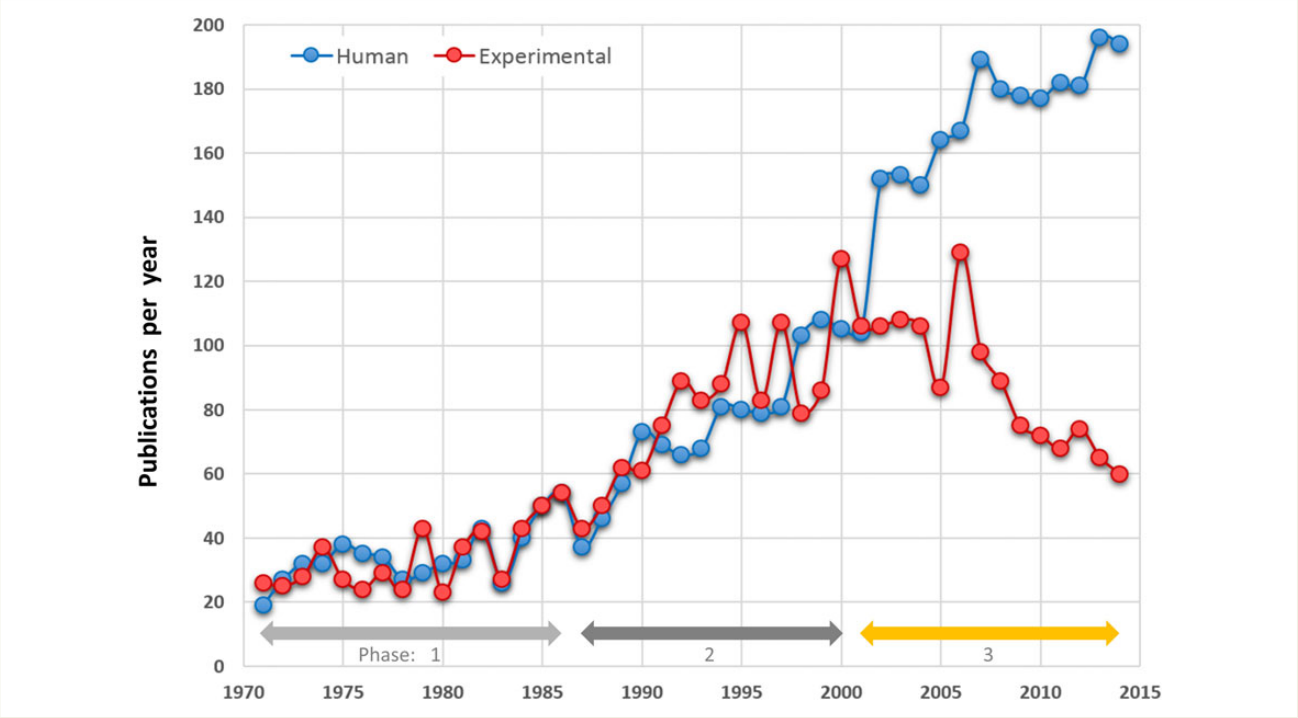

Figure 7 Development of publication frequency in the area of coronary microcirculation. PubMed search: (((coronary microcirculation) OR (cardiac microcirculation) OR (myocardial microcirculation)) AND 'humans'[MeSH Terms:noexp] NOT 'animals'[MeSH Terms:noexp]); (((coronary microcirculation) OR (cardiac microcirculation) OR (myocardial microcirculation)) AND 'animals'[MeSH Terms:noexp] NOT 'humans'[MeSH Terms:noexp]) on 5 September 2015.

strongly reductionist *in vitro* approaches with observations of clinical phenomena obtained in patient studies.

In vivo studies preserve many of the known and unknown interactions of the constituents of a given tissue and thus allow estimating the relevance of a given mechanisms for a specific condition. Compared with clinical studies, the number of measurable parameters and the imaging resolution are much higher. Options to evoke or rescue a certain condition are available, which allow critical testing of pathophysiological hypotheses.

In the present situation without tested and accepted pathophysiological hypotheses, it is very difficult to establish

- a generally accepted stratification of the disease or classification of distinct disease entities,
- a related diagnosis algorithm, and
- suitable targets for focussed therapeutic approaches.

Unfortunately, incentives for researchers in the current scientific environment are coupled to their output within a relatively short time window and not so much to the societal requirements posed by a specific disease or condition.[54] It is not very attractive to move into a field where testing a given hypothesis might prove very difficult and would take much longer than generating several mechanistic concepts in an *in vitro* model. Thus, it is a responsibility of the cardiovascular scientific community and its stakeholders to consider alternative approaches. One such approach may be to establish 'coronary microcirculatory observatories' following a concept suggested earlier by Wayland.[55]

Coronary microcirculatory observatories

A coronary microcirculatory observatory (CMO) would be an 'international core facility' based on concepts and expertise in clinical microvascular research, microvessels, lymphatics, intravital microscopy, animal models, and molecular biology. Support for such an initiative should be obtained from major funding bodies, including the EU. A respective application, supported by scientific backing from associations including the ESC, may come from leading laboratories, working groups, or consortia covering the field of clinical and experimental coronary microvascular pathophysiology. The application would have to cover the establishment of the observatory as well as training and research stipends for early-stage researchers. In a sustained phase, projects should mainly be based on individual grants.

To provide the necessary infrastructural support, a CMO should be built as an extension to an existing laboratory of one of the participating groups. An independent advisory board would select the location in a competitive procedure and oversee scientific and technological approaches. The governance concepts could borrow expertise from similar and mostly much larger European initiatives in other scientific areas. A user group would establish rules concerning access and fees for the use of the infrastructure and support by expert staff. Expertise on animal models, advanced intravital microscopy, functional imaging, interventional approaches, data analysis and modelling with cutting-edge image analysis, and molecular

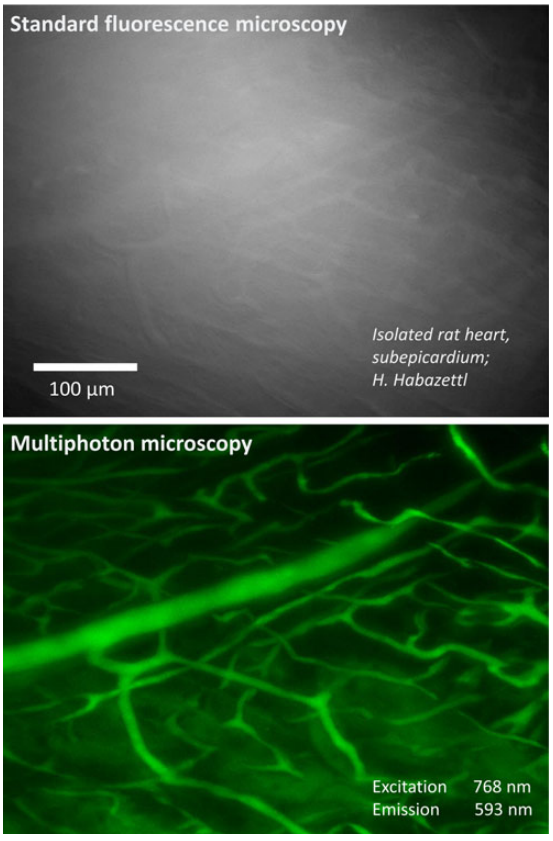

Figure 8 Intravital microscopy of the subepicardial microcirculation with standard and multi-photon microscopy (Langendorff preparation, rat heart; courtesy of H. Habazettl).

approaches would have to be provided by a coordinator and skilled scientific staff.

While the establishment of a CMO may require in the range of €10 million, the translational and clinical benefit would certainly outweigh this effort. Coronary microcirculatory observatories would allow researchers to test their hypotheses on CMVD, MVA, HFpEF, or other coronary microvascular disturbances and develop pharmaceutical targets or therapeutic options without the need to establish the respective cutting-edge technologies in their own labs.

In order to fulfil this role, a CMO would have to

- generate and provide animal models for coronary microvascular disturbances including MVA,
- optimize and provide experimental approaches (e.g. microscopy, stabilization, manipulation, histology, data analysis), and
- stimulate scientific meetings and symposia.

Given the difficult optical and mechanical properties of the heart as an object for microvascular investigations, it is justified to question the technical feasibility of this approach. However, during the last decades, a substantial number of new and advanced modalities have been developed (*Table 2*).

Scientific areas including neurosciences have successfully adapted these technique.[56] This provided relevant information, e.g. on the relation of neuronal activity and capillary perfusion and oxygen supply (neurovascular coupling).[57] Obtaining such insights required the analysis of the respective mechanisms in the complex *in vivo* environment. Multimodal optical techniques combined with genetic approaches and functional dyes proved very potent for this purpose. The cardiovascular field still has to catch up with respect to these techniques, and consequently also with respect to the corresponding scientific discoveries and related clinical benefits.

Table 2 Experimental approaches for visualizing the coronary microcirculation

Method	Challenges	Developments/characteristics
Intravital microscopy	Difficult optical properties	Fluorescence microscopy – Functional parameters (e.g. NO production) – Visualization of targeted cells (e.g. green fluorescent protein) Confocal microscopy – High resolution, but low penetration depth Multi-photon microscopy – High resolution, high penetration depth – Additional modalities [e.g. fluorescence-lifetime imaging microscopy, optical parametric oscillator (OPO): infra-red imaging] Optical coherence tomography (OTC) Photoacoustic (optoacoustic) tomography
	Myocardial movement	Electromechanical uncoupling Movement compensation – Hardware (passive/active stabilization) – Software
Ex vivo imaging	Lack of transparency	Light sheet microscopy – High penetration depth, large area, complete networks
	Lack of functional information	Micro-CT – High penetration depth, large area, complete networks, but low resolution

Summary

Coronary MVA (angina symptoms in patients without haemodynamically relevant stenosis in epicardial arteries) and HFpEF are clinical conditions with significant prevalence and a relevant prognostic impact. Despite an increasing number of clinical studies, the pathophysiology of these conditions, the proper definition of included nosological entities, and the development of respective treatment options are not progressing at a satisfactory pace. Both conditions have been linked to CMVD. A substantial list of potential pathophysiological mechanisms for CMVD, including endothelial dysfunction, smooth muscle dysfunction, microvascular spasm, sympathetic dysfunction, altered vascular remodelling, and, suggested here, degradation of the ESL, compromised conduction, and impaired metabolic feedback, have been put forward. However, these mechanisms could not be critically tested establishing accepted pathophysiological concepts. There is no adequate information on the relevance of individual mechanisms in general, let alone in individual patients. More severely, the stratification of individual patients and the rational development of targeted strategies is underdeveloped.

One cause for this state is the declining number of experimental functional studies in the coronary microvascular bed, which are technically demanding and not necessarily attractive to researchers. However, multimodal intravital microscopy has improved significantly during the last decades and could be adapted to probe the coronary microcirculation. The establishment of 'coronary microcirculatory observatories' providing cutting-edge experimental technology and relevant animal models to researchers to test promising pathophysiological hypotheses might be a route to solve the described dilemma to benefit the respective patients.

Authors' contributions

A.R.P. and B.R. performed statistical analysis, handled funding and supervision, acquired the data, conceived and designed the research, drafted the manuscript, and made critical revision of the manuscript for key intellectual content.

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