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Introductory Chapter: Microcirculation in Health and Disease

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Microcirculation is the terminal vascular network where the exchange of substances between the blood and the tissues occurs. Usually, the term refers to functional unit comprising vessels with a diameter of less than 100 μm , including arterioles, capillaries, and venules. Appropriate vascularization of tissues and an intact microcirculatory bed are prerequisites for adequate tissue perfusion and thus normal organ functioning. The unique feature of microcirculation is its large area which in conjunction with low velocity of blood flow enables appropriate conditions for the exchange of substances between the blood and the tissues. Another important issue is its adaptability either to acute changes of organ demands or to chronic changes in its environment. These tasks are primarily accomplished by arterioles as the main targets and effectors of vascular tone regulation including the vascular endothelium and smooth muscle cells, as well as adjacent cells and humoral and nervous factors all playing in a fine orchestrated concert. The principal characteristics of fluid and substances trafficking across the capillary wall according to classical Fick and Starling principles and depending on the concentration gradients of substances transported, on capillary permeability, and on hydrostatic and oncotic pressures are described elsewhere.

1. Structural and functional organization: some general and special features

The structural organization of the microcirculation is tightly coupled to its physiologic function. As organs serve different functions and have different metabolic demands, the microcirculatory networks differ among organs. The complexity and heterogeneity in blood flow and metabolism in respect to one tissue as well as in different tissues have been confirmed in animal experiments and in patients due to advanced imaging techniques.

The book starts with a comprehensive overview of the coronary microcirculation written by Fonseca et al., thoroughly describing the characteristics of coronary microcirculation, from anatomical and histological aspects to physiology with emphasis on the regulatory mecha-

nisms, and finally elucidating some pathophysiologic mechanisms in relation to systemic cardiovascular diseases. Of interest is the impact of pericytes to the regulation of microcirculation as pointed out in the chapter, as well as a rather neglected field of venous part of the microcirculation [1]. The chapter specially focuses on the regulation of coronary microvasculature with the presentation of all known mechanisms that might be applied also to other vascular beds, i.e., the myogenic control, the endothelial component, and the metabolic regulation [2]. The latter presumably plays the most important role in the coronary microcirculation due to constantly changing demands of the working heart muscle [3]. Additionally, systemic factors such as the autonomic nervous system and humoral mediators are elucidated. As diseases of the cardiovascular system nowadays represent the leading cause of morbidity and mortality all over the world, the most frequent pathologies of the coronary vessels are presented [4]. The heart vessels are very prone to structural remodeling, vascular rarefaction, and perivascular fibrosis finally culminating in luminal obstruction what might be detrimental for the patient; apart from macrocirculation, it is the microcirculation that is suggested to play a key role in the coronary pathophysiology [5]. The impact of risk factors, oxidative stress and inflammation, and the interplay between various regulatory mechanisms, all predisposing to heart disease development are comprehensively exposed with a brief link to some metabolic diseases.

The dependence of the cellular metabolism on tissue blood flow and vice versa is more extensively described in the following chapter written by Kolka, which focuses on the microcirculation of skeletal muscle and also proposes some therapeutic interventions in targeting the skeletal muscle microcirculation to treat both vascular and metabolic diseases. The skeletal muscle microcirculation is subjected to the greatest variations of blood flow and nutrients breakdown during strenuous exercise as compared to resting conditions. During resting conditions, only about 20% of capillaries are perfused: the blood flow is estimated to amount 5–10 mL/min/100 g, compared up to 80–100 mL/min/100 g during exercise [6], pointing to complex mechanisms of blood flow regulation. Worth to mention is the difficulty to estimate these changes and microvascular dynamics at the capillary level with the available techniques. In the chapter, an interesting scientific approach for the evaluation of substrate exchange in the microenvironment of skeletal muscle is presented, namely, the lymph sampling [7, 8]. Taking into account great variations in the metabolic rate as well as the type and rate of substrates metabolized regarding intensity and mode of exercise, it is compelling to speculate that changes in blood flow will affect metabolism. In this respect, vasoactive compounds affecting vascular tone and perfusion could also indirectly affect the metabolism. Insufficient perfusion might in turn lead to deranged metabolic pathways making one more susceptible to metabolic diseases, such as diabetes and obesity [9]. Interestingly, nitric oxide (NO) as a key endothelial vasodilator also directly affects metabolism by competing with mitochondria for oxygen and consequently inhibiting the oxidative phosphorylation and potentially switching the metabolism to some other (anaerobic) pathways [10]. Apart from classical vasoactive substances mainly released from the endothelium, many hormones are themselves vasoactive; glucagon-like peptide has been shown to increase capillary perfusion on acute basis and angiogenesis on longer term, and considering this, might represent a potential therapeutic target [11, 12]. In addition, some important interactions among vasoactive compounds and

hormones have been elucidated in the chapter, such as the interference of angiotensin II (Ang) and NO systems [8], and the prevention of endothelin-induced vasoconstriction by insulin and adiponectin [13, 14]. Insulin also directly affects capillary recruitment and at supraphysiologic concentrations, it has been shown to increase blood flow in human skeletal muscles and skin [15]. Capillary density has been directly correlated with insulin sensitivity thus strengthening the hypothesis that capillary recruitment importantly contributes to insulin-mediated glucose uptake [16]. As exposed in the chapter of Kolka, an important question to be resolved is the process of insulin trafficking across the endothelial barrier. At this place, a word should be devoted to endothelial glycocalyx, which presents an additional structural and functional endothelial barrier that in turn affects the composition of the muscle interstitium and consequently the supply of nutrients to the cells. Glycocalyx has thoroughly been investigated as a potential therapeutic target [17].

2. The central role of endothelium in the regulation of vascular tone

Although endothelium also plays an important role in the processes of inflammation, hemostasis, and tissue repair, its prominent role in the physiological regulation of vascular tone along with a vast number of endothelial vasoactive substances and their interactions are most extensively exposed in the book. Accordingly, all endothelial mediators might represent a potential therapeutic target as briefly pointed out in some chapters.

The importance of functionally intact endothelium is already obvious in the newborn as exposed in the chapter of Wright and Dyson. Apart from the involvement of the autonomic nervous system and the sympathoadrenal activation at birth, substances released from endothelium importantly contribute to the delicate balance between vasodilators and vasoconstrictors which is a prerequisite for normal circulatory function. To list just a few, isoprostanes and prostaglandins released in response to increased partial oxygen pressure at birth play a major role in the closure of ductus arteriosus, thus enabling the transition to the adult-type circulation taking place at birth [18]. In preterm infants, this delicate balance is disturbed in terms of enhanced vasodilation and diminished vasoconstriction rendering them more susceptible to hypotension resulting in organ hypoperfusion and potentially irreversible end-organ damage [19]. An interesting feature pointed out in the chapter is greater susceptibility of male preterm newborns to developing potentially fatal hypotension, which has been speculated to be due to increased levels of gaseous neurotransmitter hydrogen sulfide (H_2S) in males [20]. Accordingly, the assessment of urinary concentrations of thiosulfate, a metabolite of H_2S [20, 21] and normetanephrine, a measure of total body sympathoadrenal activity [22], respectively, might predict the outcome. The impact of other gaseous compounds is additionally described in the chapter, with the leading role of NO, presumably involved in the regulation of basal vascular tone, and carbon monoxide (CO), apparently playing a crucial regulatory role in the cerebral circulation during the transition period [23, 24]. While most observations were deduced from animal studies or isolated models, far more complex interplay takes part in humans *in vivo*. A lot of potential cross-talks and the influence of one mediator on the other one could merely be speculated, as ex-

posed in the chapter [23]. Their potential adaptive role and their impact in the neonatal period are emphasized. Of interest, the interplay of NO, CO, and H₂S differ in neonates and in the adults, which in addition to the chapter of Wright and Dyson is also exposed in the chapter of Fonseca et al. [25].

The role of NO as a key endothelial vasodilator has briefly been corroborated in the chapters of Fonseca et al., Zupan, and Schier et al. Yet, other vasodilators presumably are even more important at the level of microcirculation, the central role being played by the metabolites of arachidonic acid (AA), extensively exposed in the chapter of Drenjančević et al. Three important pathways of AA metabolism are presented, including: the cyclooxygenase (COX) pathway, the lipopxygenase (LOX) pathway, and the cytochrome (CYP) pathway. In the chapter, the mostly investigated products of these metabolic pathways and their potential interactions at the level of the vascular smooth muscle are thoroughly presented. Worth to emphasize is the dual role of the above-mentioned enzymes, namely, they catalyze the production of vasoconstrictors as well as vasodilators and it is their delicate balance that finally determines the proper vascular tone. In many diseases, this fine balance is disturbed, such as in obesity, diabetes, hypertension, and other metabolic and cardiovascular diseases. Of the CYP metabolites of AA, epoxyeicosatrienoic acids (EETs) have been implicated as endothelium-derived hyperpolarizing factors (EDHF), contributing to proper vasodilation in the settings with reduced bioavailability of NO [26], such as in increased production of reactive oxygen species (ROS), which uncouple the endothelial nitric oxide synthase (eNOS), and consequently augment additional release of ROS from eNOS itself.

An interesting aspect is the nonenzymatic metabolism of AA, mainly mediated by ROS which nowadays are widely recognized as mediators of cellular immunity, inflammation, and tissue repair, and also indirectly affect vascular tone [27]. The impact of ROS as potential (noxious) vascular messengers is also dealt with in some other chapters.

Additional interesting suggestion presented in the chapter of Drenjančević et al is potentially positive contribution of Ang, which has usually been presented as a foe in the vascular homeostasis. Contrary to the common accepted knowledge, they propose that sufficient levels of Ang actually are essential for normal vascular function, as confirmed in the studies which have demonstrated that a decrease in the circulating levels of Ang lead to impaired microvascular endothelial function [28]. As for Ang, Kolka additionally exposes its effect on blood vessel permeability which indirectly also affects the tissue metabolism [29].

Yet, the effects of endothelial vasoactive compounds are not that straightforward, as there are many interactions depending also on the vascular bed studied and being also tissue and species specific.

The deranged interplay of endothelial mediators in the pathogenesis of various diseases of modern era has long been implicated. As stated in the subsequent subheading, some chapters address the question of endothelial dysfunction as a hallmark of many diseases. Moreover, the dysfunction of endothelium often precedes the clinical manifestation of the disease.

3. Dysfunctional microcirculation is a hallmark of many diseases

The importance of intact and functional microcirculation with preserved adaptability to meet organ metabolic demands has long been appreciated. It has been confirmed in many independent studies that deranged microcirculation compromises normal organ function and finally the organism as a whole. Either deranged vascular control in terms of deranged autonomic nervous system as pointed out in the research chapter of Malan et al. as well as in terms of endothelial dysfunction have been implicated. Increased sympathetic tone or disturbed responsiveness to adrenergic challenges might induce increased vasoconstrictor tone finally leading to hypertension and inappropriate structural remodeling of the vessel wall. Endothelial vasoconstrictors and increased oxidative stress also augment the vasoconstrictor component causing ischemia and tissue failure on a larger time scale. Microvascular dysfunction has been shown to be the primary event in the pathogenesis of many metabolic and cardiovascular pathologies which is shortly mentioned in other chapters. On the other hand, injury and inflammation subsequently trigger angiogenesis and structural adaptation that have the potential of restitution, which takes part after say surgical procedures as pointed out in the chapter of Schier et al. The potential to reconstitute *ad integrum* strongly depends on the preoperative state of the microcirculation and on other known vascular risk factors such as hypertension, smoking, diabetes, obesity, etc. [30]. Potential risk factors can partly be overcome by changes in lifestyle and some interventions such as exercise. Moreover, the latter may strongly affect the outcome of a therapeutic procedure as stressed in the chapter of Schier et al. [31].

A good model of microvascular dysfunction potentially leading to impairment of the central nervous system and causing high mortality and morbidity is leukoaraiosis. The term, potentially unfamiliar to broader medical public, denotes diffuse confluent changes in the cerebral white matter often accidentally detected on neuroradiological imaging. Its prevalence in the population aged between 50 and 75 years has been estimated to comprise up to 25% and, as such, undoubtedly must be regarded as highly clinically significant in terms of predisposing to various degrees of cognitive impairment, ischemic events, and stroke [32]. In his chapter, Zupan thoroughly describes the pathogenesis of leukoaraiosis, which includes a spectrum of factors, often apparently discordant, ranging from endothelial dysfunction to leaky blood-brain barrier on one side [33], to ischemia on the other [34], yet all causing chronic perfusion impairment. Similar factors and causes could actually be applied also to other microcirculatory networks. In his chapter, Zupan reports that the prevalence of leukoaraiosis is higher in the Blacks than in the Whites. This might be connected to increased prevalence of hypertension in the Blacks which has extensively been discussed also in the chapter of Malan et al. [35]. Interestingly, Malan et al. also showed a close link between depressive disorders and vascular dysfunction of the retinal artery in terms of sympathoadrenal disbalance. The correlation was significantly more pronounced in the Blacks compared to the Whites pointing out an important role of ethnic predisposition and genetic susceptibility on one side, but also risk factors on the other side [36]. Interestingly, chronic depression has been related to attenuated cortisol levels which would impact the synthesis of epinephrine [37], as proposed in the chapter of Malan et

al. All these facts must be taken into account when designing the therapeutic strategies and a proper follow-up of patients.

Another interesting aspect of endothelial dysfunction addressed in the chapter of Drenjančević et al. is the impact of high salt diet on vascular function [38]; vascular pathologies linked to increased salt intake undoubtedly represent a great burden of the civilized world. As already stated, other aspects of endothelial dysfunction are extensively presented also in the chapters of Fonseca et al. with emphasis on the involvement of coronary microcirculation in the pathogenesis of cardiovascular events, and in the chapter of Kolka on skeletal muscle microcirculation and its involvement in metabolic (obesity, diabetes) and cardiovascular diseases (hypertension).

While those chapters mainly deal with mechanisms of endothelial dysfunction, more clinical aspects in terms of the determination of microvascular and endothelial dysfunction are illustrated in the chapters of Tamas-Szora et al. and Todea et al. They present some potential diagnostic tools for an early detection of microvascular dysfunction as well as for tracing the outcome and the evaluation of the effectiveness of treatment.

4. *In vivo* applicability of some methods for clinical evaluation of microcirculation

Modern techniques with relatively high spatial resolution have enabled a timely detection of the disease, which is a prerequisite for an adequate treatment. Within the noninvasive imaging, sound- and light-based imaging techniques are able to provide high resolution and clinically relevant information in assessing microcirculation.

Mostly applied optical imaging techniques for clinical evaluation of the microcirculation today include (dynamic) capillaroscopy, confocal microscopy, two photon imaging, and stimulated emission depletion microscopy for tracing superficial structures; optical coherence tomography, hyperspectral imaging, side stream dark field imaging, and incident dark field imaging for assessing subsuperficial microvascular beds; and diffusion correlation spectroscopy, functional near infrared spectroscopy, and photoacoustic tomography to assess deeper structures [39]. Obviously, each technique is designed for determination of special microcirculation network and its position regarding the depth of a tissue.

In spite of many methods available, in the book, only two noninvasive techniques are extensively presented, namely, the contrast enhanced ultra-sonography (CEUS) and the laser Doppler (LD) fluxmetry (LDF) in the chapters of Tamas-Szora et al. and Todea et al., respectively. Their applicability in the assessment of vascular dearrangement in tumor evolvment, angiogenesis, inflammation, and some other pathologies and in the assessment of dental pathologies, respectively, is presented along with some advantages and disadvantage of both. Both methods are based on optical and acoustic penetration of a tissue and exploit the Doppler effect causing the frequency shift of illuminated light and sound, respectively, due to reflections from moving particles, i.e., predominantly erythrocytes. Both chapters give insight into

potential clinical applicability of CEUS and LDF for tracing microcirculation and emphasize the need and importance of performing *in vivo* studies on humans.

In their chapter, Tamas-Szora et al. comprehensively describe the principles governing CEUS as well as some modifications and their various applicability, substantiated with representing illustrative figures that accompany the text and enable a better perception of the method for unfamiliar readers. CEUS has rendered itself a valuable clinical tool for assessing vascularization in various tissues, specially parenchymal organs, such as liver [40], testicles, kidney [41], and mammary glands [42]. Yet, the limitation inherent to all sonographic methods described so far is that CEUS enables the discrimination of the vessels of size of around 100 μm and, in this respect, does not accurately evaluate the proper “microcirculation” [43]. Nevertheless, it is highly applicable in the clinical settings as it enables the detection of blood flow down to velocities less than 2 cm/s, and the discrimination between inflammatory and degenerative pathology as in musculoskeletal diseases [44]. In this regard, capillary perfusion could be indirectly estimated, i.e., either increased perfusion in say inflammation or cessation of blood flow in ischemic tissues.

To improve the CEUS technique, different contrasting agents that augment the signals under observation might be applied; yet, they increase the diagnostic costs. The advantages of CEUS over some other methods include repeatability, lack of harmful effects as in computer tomography (CT) caused by ionizing radiation, and high spatial and temporal resolution to list just a few. Target-specific structures might additionally be detected by combining the contrast agent with specific antibodies.

LDF and its update, LD imaging [45] with its varieties remain the gold standard for clinical evaluation of microcirculation as described in the chapter of Todea et al. In the chapter, some results of authors' own experiments evaluating the outcome of therapy in terms of microvascular function are exposed. LDF has proven to be an effective tool of choice to evaluate microcirculation in the oral cavity, preferentially of the gingiva and dental pulp, respectively [46, 47]. LD techniques enable assessment of tooth vitality after various procedures including bleaching, tooth implants and prepared teeth, surgical intervention after trauma, as well as tracing microcirculation following treatment of gingival disease, such as inflammation, and gingival blood flow resolution after surgical procedures. Supposedly, LDF also enables to evaluate the redistribution of blood flow through arteriovenous anastomoses that are a unique feature of the cutaneous and mucous microcirculation.

5. Therapeutic interventions at the level of microcirculation: potential role of endothelial progenitor cells (EPCs)

Interventional studies have focused on various aspects of microvascular function, as already outlined in the chapters of Fonseca et al., Kolka, Drenjančević et al., and Zupan. Many vasoactive compounds might represent therapeutic targets, either by targeting their endothelial receptors or interfering with their synthesis by acting on the corresponding intracellular

enzymes. In addition, interfering with the renin-angiotensin-aldosterone [48] system seems a promising therapeutical intervention to treat vascular and associated metabolic diseases as pointed out in the chapter of Kolka. The effects of phosphodiesterase inhibitors (Sildenafil and Tadalafil), and thiazolidinediones on vascular function, capillary recruitment, and consequently metabolism have also been investigated, as exposed by Kolka. The potential intervention on the level of glycocalyx has already been mentioned [17]. An interesting target affecting the metabolism of fat and increasing energy expenditure and angiogenesis might be brown adipose tissue, as briefly exposed in the chapter of Kolka [49]. Moreover, the supplementation of L-arginine might ameliorate vascular complications in patients suffering from neurodegenerative and vascular diseases as mentioned in the chapter of Zupan [50].

Apart from the above-mentioned targets, endothelial progenitor cells have evolved over the last few years as a promising new strategy for targeting microvascular and subsequently organ dysfunction. Nowadays, many studies are being conducted on how the injection of EPCs on the site of injury or damaged organ affects potential improvement of organ function. Apart from acute adjustments, chronic adjustments in terms of increased angiogenesis are crucial for tissue regeneration. Angiogenesis and vasculogenesis are key events in directing proper organ function, not only during fetal life, but also later in adulthood. EPCs also play an important role in vascularization in pregnancy [51]. They are important component in tissue regeneration and, in this respect, might represent a potential therapeutic niche. Thus, improvements of techniques to obtain sufficient number of EPCs from the peripheral circulation, or from the bone marrow, proper harvesting and breeding are prerequisites for efficient therapy. Some important aspects of EPCs are presented in the chapter of Nova-Lamperti et al., where the crucial technical steps in obtaining and manipulating the cells are presented as well as the results of some studies investigating the effects of therapy with EPCs. Furthermore, the stimuli for migration, recruitment, and differentiation of stem cells affecting angiogenesis *in vivo* are corroborated. Unfortunately, the potential of EPCs for proper angiogenesis strongly depends on the clinical condition and risk factors of the individuum. Namely, it has been shown that the number of EPCs conversely correlated with cardiovascular risk factors, such as hypercholesterolemia, hypertension, smoking, diabetes mellitus, and dyslipidemia. Moreover, lower numbers of EPCs as compared to healthy ones have been shown in patients suffering from unstable angina, myocardial infarction, as well as atherosclerosis, and erectile dysfunction, as described in the chapters of Schier et al. and Nova-Lamperti et al. On the other hand, some cytokines, hormones, drugs, and physical activity increase the number and function of EPCs. In this respect, the importance of physical activity could not be overemphasized. Some good and positive examples are presented in the chapter of Schier et al. who have shown that even short lasting submaximal exercise performed preoperatively to assess the cardiopulmonary status of a patient might significantly improve the outcome after major surgery in patients [31, 52]. In this respect, regular exercise should be strongly encouraged in all groups of patients, let alone in healthy populations in general practice. Clinical applicability of EPCs has already been confirmed in many clinical trials, when *ex vivo* expanded EPCs were injected into the damaged area of tissue, such as in the treatment of acute myocardial infarction [53], in the recovery from deep venous thrombosis, in the recanalization of organized venous thrombi

[54], in pulmonary arterial hypertension [55], in attenuation of peripheral artery disease [56], and in liver regeneration [57]. Yet, the disadvantage of such therapy is a very low number of EPCs in peripheral blood and relatively high costs of mobilization. Nevertheless, EPCs seem to represent a promising future therapeutic approach for the treatment of “modern era” diseases.

6. Conclusion

In the introductory chapter, I intended to briefly sum up the content of the book, exposing some interesting features of separate chapters. Basic principles of the microvascular blood flow and vascular tone regulation are briefly presented with endothelium playing a central role. Today, researchers are focused on complex interactions of vasoactive compounds trying to elucidate their potential interplays in health and disease, which would accomplish therapeutic strategies. Prototypes of alternative therapeutic approach presented in the book might be exercise as a type of self-governed therapy on one side, and EPCs as a kind of complex hospital-based therapy. As diseases of the cardiovascular system are the leading cause of morbidity and mortality in modern world, additional efforts in establishing new diagnostic tools and efficient therapies are urgently needed. The authors have provided comprehensive overviews and opened up new challenging questions that I hope would be useful to scientists involved in the microcirculation which remains an unlimited field of inspiration. A lot has already been unrevealed, and the rest is yet to be discovered.

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