MICROCIRCULATION IN THE FOCUS OF RESEARCH

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Introduction

Profound concepts of biomechanical regularities of circulatory blood flow are needed for physiological observations and their possible subsequent clinical inferences.

In consideration of blood circulation *in toto* a fundamental biophysical difference exists between the blood flow in vessels > ~100 µm diameter and those < 100 µm, which is defined by the Reynolds number (Re). The Reynolds number is dimensionless and describes the ratio of inertial forces to viscous forces in a flowing fluid. It quantifies the relative importance of these two types of forces for given flow conditions and is a guide to when turbulent flow will occur, as in blood for example:

$$\mathrm{Re} = \frac{v \cdot l}{\eta}$$

where v is the current speed, l the characteristic length and η the kinetic viscosity of the current's medium (blood).

A value of Re > 1 characterizes the macrocirculation (arteries and veins), while Re < 1 characterizes the microcirculation (arterioles, capillaries and venules). In the microcirculation blood changes its flow characteristics as a consequence of certain segregation phenomena of the plasma-blood cell mixture whereby the enormous peripheral flow resistance is overcome and a blood circulation is even possible.

In the macrocirculation a certain cardiac output (CO) is moved in the vasculature through the contraction force and myocardial contractibility of cardiac activity (Stroke Volume - SV, and Heart Rate - HR). The CO effects the necessary pressure differential between arterial (high pressure) and venous (low pressure) systems.

$$CO = SV \,.\, HR$$

The peripheral flow resistance and the elastomechanical behavior of the artery walls (in particular the aorta) dictate the blood flow characteristics and periodicity in the macrocirculation.

The microcirculation is functionally the most important part of the human circulation as the locus of material exchange with the tissue cells and the first site of immunoreactivity. The known fluid mechanics laws, derived from the Euler continuity equation, lose their validity in this area. Blood in the microcirculation is a mixture of plasma and cells of varying sizes that no longer approximates the behavior of a physical fluid but rather resembles bulk material on a conveyor belt.

Dysfunction or disturbances of the microcirculation contribute to a large number of illnesses. These microcirculatory limitations are either causal factors or contributory to the course of the disease. Disturbances in the microcirculation are often triggered by disturbances in the macrocirculation but show a repetitive tendency to develop with their own dynamic, often therapy resistant, substantial independence

from macrocirculatory events. For example, *Ulcus cruris* with stage III chronic venous insufficiency, circulatory disorders and necrotic formations with *Diabetes mellitus* Type II, and various chronic wound healing disorders.

A demand-oriented circulatory adaptation in response to diverse organ material exchange requirements is the most important prerequisite for a high human physical and mental performance capacity. Maintenance or regeneration of an efficient regulation of blood circulation to organs is therefore central to prophylactic and therapeutic measures. An optimal regeneration, restitution or healing progression is not possible without adequate participation of the microcirculation.

To understand the microcirculation's physiological laws impacting the local regulation of blood circulation in organs, their dysfunction and possible therapeutic options, several physicochemical, physiological, flow-mechanical and elastomechanical parameters need to be taken into consideration.

Aspects of current biophysical and physiological knowledge of the microcirculation

The flow area of the microcirculation encompasses the arterioles, capillaries and venules (Fig. 1). The wall architecture of the large caliber arterioles consists on the lumen side of a singular endothelial layer, the basal membrane and two to three layers of smooth muscle cells. The small caliber arterioles only have a single layer of smooth muscle cells at their disposal that become intermittently spaced towards the capillaries. A corresponding situation occurs in the small- and large-caliber venules. The capillary walls exist only of the lumen-surrounding endothelial sheath and basal membrane.

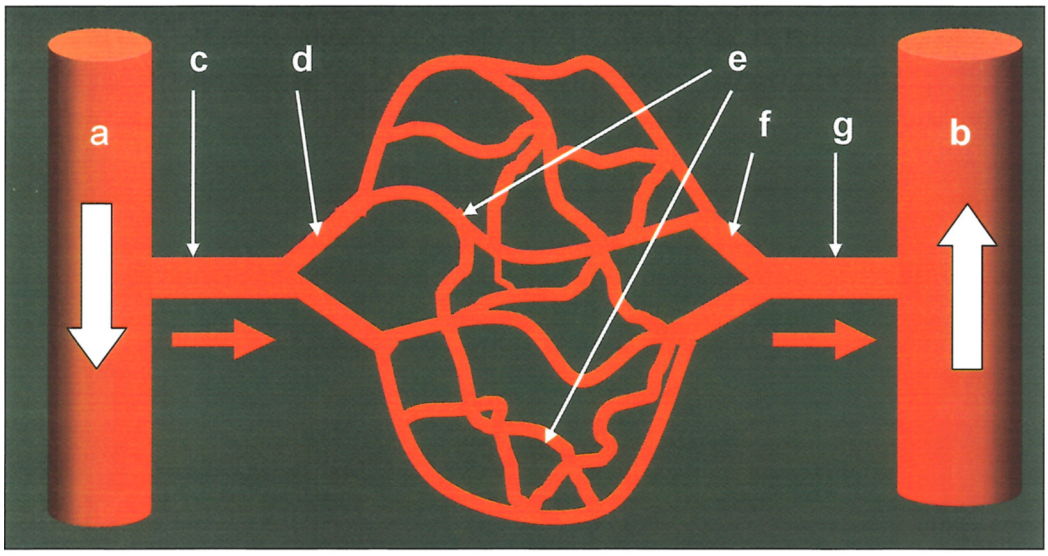


Figure 1. Schematic area of microcirculation between a small artery (a) and a small vein (b); large caliber arteriole branch (c), small caliber arteriole branch (d), capillary network (e), small caliber venule branch (f), large caliber venule branch (g).

The material exchange occurs in the capillaries. The blood plasma substrate transports nutrients in and metabolic end products are transported out. The transit pathway is the transcapillary fluid current along a capillary due to the interactions of hydrostatic and colloid osmotic pressures in the arterial and venous flanks of a capillary. Further, plasma factors of the immune system are also transported in the plasma. The hemoglobin in the erythrocytes are responsible for the exchange of blood gases (oxygen and carbon dioxide) due to their respective concentration gradients between blood and tissue cells. The paradigm of a

dysfunctional microcirculation is the disturbance of diffusion. To focus the consideration on this part of the material exchange, the diffusion law generally states:

$$\Delta m = D\frac{A \cdot \Delta c \cdot \Delta t}{\Delta x}$$ where m is mass, D the diffusion constant, A the area through which a diffusion takes place, c the concentration, t the time and x the diffusion path.

Of particular biological interest is the relationship between diffusion path and diffusion time, which cannot be simply inferred from the diffusion law. Solving the diffusion equation for D:

$$\Delta x \sim \sqrt[2]{\Delta t}$$

This means that a ten-fold increase in the diffusion path x results in a one hundred-fold increase of the diffusion time. This dependence of the diffusion time on the diffusion path is the biophysical cause for the varying capillary thicknesses of the different tissues corresponding to their material exchange requirements. Consequently, the disturbed microcirculation is in the first order a distribution disruption of the plasma-blood cell mixture in the capillary network.

For a principal consideration of current flow the simple assumption is made that blood behaves like a homogenous fluid (continuum mechanics). The flow stream Q is the Quotient of a flow volume ΔV over the unit of time Δt:

$$Q = \frac{\Delta V}{\Delta t} \qquad \left[\frac{\mu m^3}{\text{sec}}\right]$$

A prerequisite of any flow is the pressure differential Δp, and furthermore the flow resistance R must be considered (OHM law):

$$Q = \frac{\Delta p}{R}$$

The flow resistance R in a pipe is dependent on the radius r of the vessel, the overall length of the single vessel l_1, and of the quotients of the shear stress τ and the shear rate γ in the pipe diameter, resulting in:

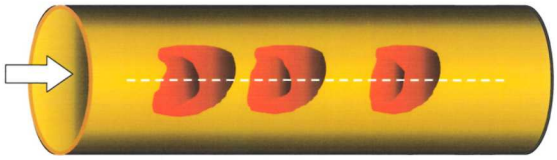
$$Q = \xi \frac{r^4 \cdot \Delta p}{\frac{\tau}{\gamma} \cdot \sum_{i=1}^{i=n} l_i}$$ where ξ = proportionality factor

This equation can strictly only be applied qualitatively because of the idealized acceptance of homogeneity of the blood as a circulation medium. Of eminent significance is the dependence of the flow stream Q on the Radius r of the vessel:

$$Q \sim r^4$$

Under the assumption that all dimensions remain constant, a doubling of the radius r means a 16-fold increase of the flow stream Q. A resultant increase in blood flow to the capillary network is commensurate with the increase in radius of the large caliber arterioles. Figure 2 illustrates an intravital microscopic observational example. As can be inferred from the above-mentioned flow equation, a lowering of the flow resistance is coupled to this circulation flow. The state of contraction in the large caliber arteriole segments is realized by neural or hormonal instructions to the smooth muscle cells in the microvasculature wall (formerly known as resistance vessels).

The full explanation for regulation of organ perfusion is not yet complete. A further biophysical law is to be considered to explain the segregation of the plasma and blood cells in microvessels < 100 μm.



Note that flow velocity of the circulation causes blood cells and plasma to move at differential speeds in the circulation, which causes their segregation.

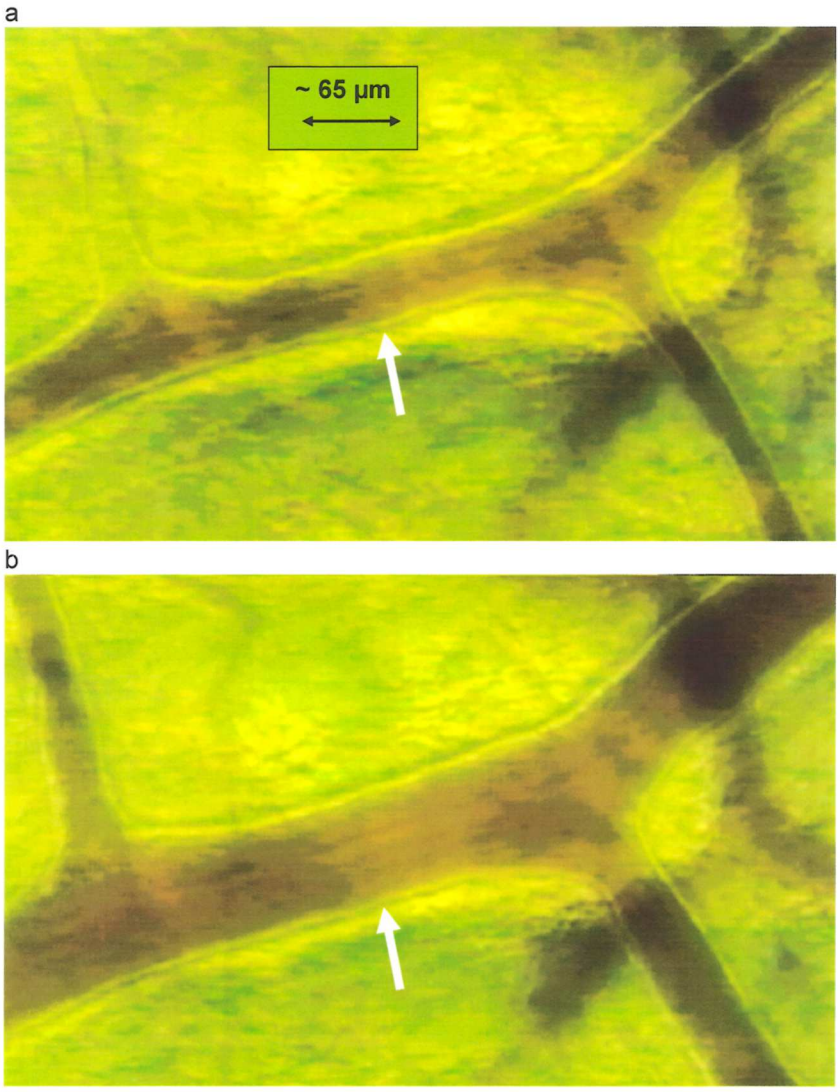


Figure 2. Intravital microscopic observational example of an intestinal arteriole (arrow) showing differential vasodilatation of the same microvasculature region at two separate observational points in time.

The particles with a higher kinetic energy (mass) in a fluid medium have, for physical reasons, a higher flow velocity. In the context of blood as a flow medium, those particles would be the blood cells, which are directed towards the axial current. The slower blood plasma moves preferentially in the peripheral currents. This separation of the plasma-blood cell mixture is more pronounced as the flow velocity of the blood increases.

The consequences of shear stress τ and shear rate γ for flow stream Q in the above-mentioned equation can be clarified by a simple experiment under idealized assumptions (Figure 3). Assume a fluid between two plates broken down in lamellae on surface A. The lower plate is motionless and only the upper plate moves through force F parallel to the fluid lamellae. Observations show that because of the differential effect of inter-lamellar frictional force F_R the individual lamellae are moved along at different speeds. A velocity gradient now exists $\Delta v/\Delta x$, mentioned above. The Newtonian friction law applied to solve F_R is as follows:

$$F_R = \eta^* A \frac{\Delta v}{\Delta x}$$

where η is the proportionality constant (material constant of the flowing medium, kinematic toughness-viscosity). Because the idealized assumptions of the flow medium are not strongly realized, a sham-viscosity η^* is used in the following viscosity equation, yielding:

$$\eta^* = \frac{\left(\frac{F_R}{A}\right)}{\left(\frac{\Delta v}{\Delta x}\right)} = \frac{\tau}{\gamma}$$

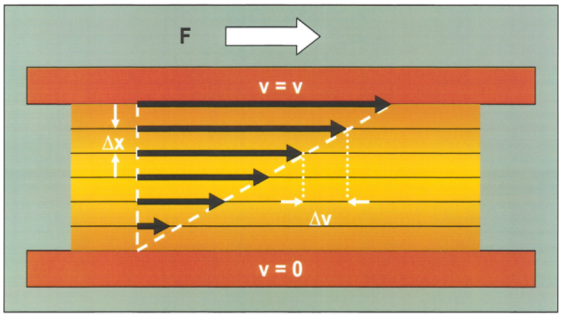


Figure 3. A flow profile in a flow-through pipe.

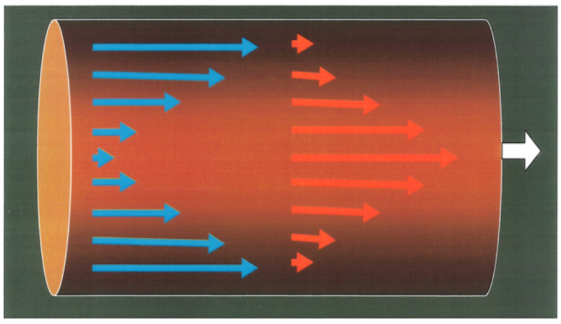


Figure 4. Shear stress τ and shear rate γ in pipe

The relationship of shear stress τ and shear rate γ is hereby clarified in the conditional equation of the flow stream Q of the blood. In a flow-through vessel the velocity of the flow medium v is highest when the frictional forces are lowest (in the axial current) and the opposite when the velocity is lowest and the frictional forces highest (at the vessel wall). The flow velocity also has an effect on the morphology of the red blood cells. The cell membrane displays certain plasticity characteristics (stiffness, rigidity) that are influenced by the momentary flow velocity. Figure 5 illustrates schematically that with increasing contributions of flow velocity blood becomes increasingly thinner.

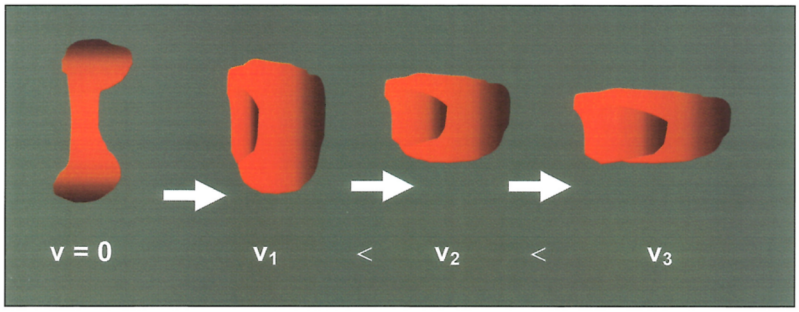


Figure 5. Schematic cross-sectional representation of red blood cells at varying flow velocities v.

The next consideration is the distribution of the plasma-blood cell mixture in the microvasculature, in particular the capillary network. Examination of the same organ microvasculature region at various activity levels of the supporting parenchymal cells reveals that all available capillary flow paths are not perfused with blood cells at all times (Fig. 6). During a resting phase of the organ few capillaries are perfused with blood cells. Longer diffusion pathways are tolerated. Metabolic requirements of the body cells and material exchange increase with activity, requiring shorter transportation pathways. At this stage a larger number of capillaries, which heretofore were predominantly perfused with plasma, transport red blood cells.

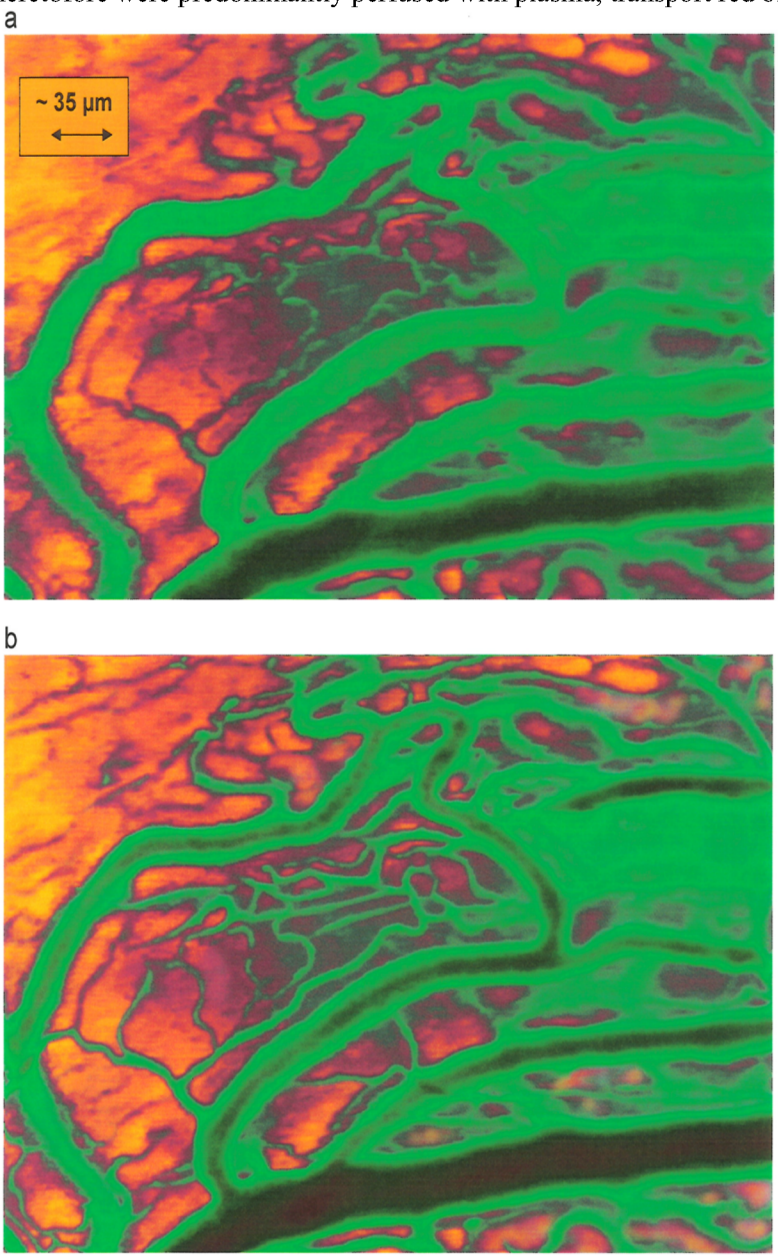


Figure 6. Variable distribution status of the plasma-blood cell mixture in the microvasculature network (a: resting phase, b: active phase) of the subcutis observed with intravital microscopy. Color correction of the original frames showing blood cell perfusion in green.

Figure 7 schematically illustrates the changes of the plasma-blood cell mixture distribution status in the capillary network.

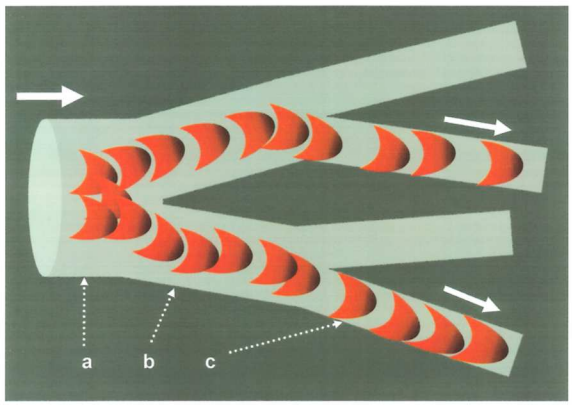


Figure 7. Schematic representation of the distribution of the plasma-blood cell mixture in the microcirculation (arterioles and capillaries); a. large caliber arteriole, b. small caliber arteriole branch; and, c. capillary branch.

The hydrostatic pressure differential of arterioles at a particular point in time is not the same in all capillary branches of a network entity. Red blood cells transported in the axial current of the arterioles are directed to the capillaries exhibiting the greatest pressure differential. The other capillaries are predominantly perfused by plasma. The capillary states switch continuously between predominantly blood cell and plasma perfusion. Independent thereof, a lesser number of network capillaries are perfused with blood cells in a resting organ with lower material exchange requirements, while an increase in organ activity level requires a greater need for material exchange and blood cells. The most important characteristic of a dysfunctional microcirculation is a distribution disturbance of the plasma-blood cell mixture in the capillary networks. The consequences are elongated distribution pathways.

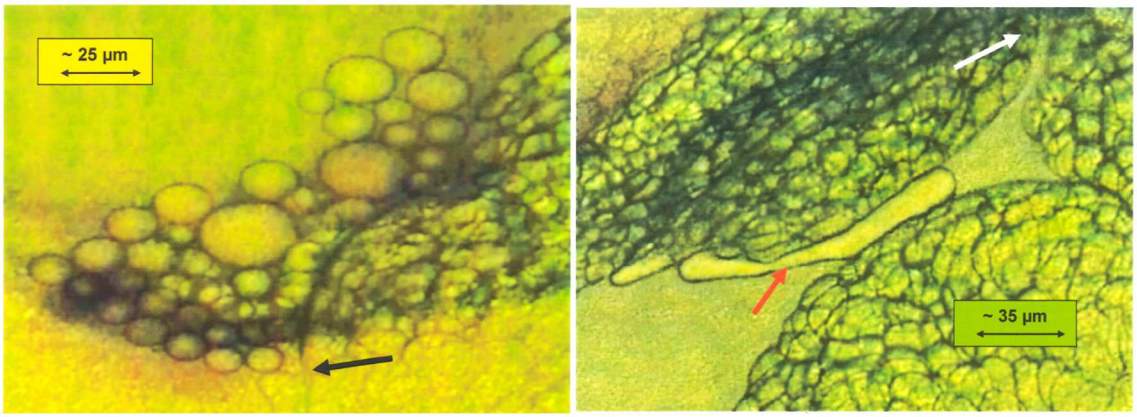


Figure 8. a (left). Observational example from intravital microscopy of the initial lymph flow exit of a fat droplet from the blood plasma of a venule into the extravasal space; b (right) example of the initial lymph movement of a fat-phase (red arrow) that developed from numerous fat droplets due to minimization of fluid surfaces to a lymph capillary (white arrow).

An undisturbed microcirculation is the prerequisite for an unobstructed course of the first steps of a cellular immune reaction. The first steps are as follows: the transport and arrival of white blood cells, the adhesion of white blood cells to the endothelium of the microvessel, and transmigration of the white blood cells into the tissue. Therefore, a connection exists between the functional state of the microcirculation and the immune reactions.

A large portion of the cellular immune reactions occurs in the venules. Figure 9 illustrates the mechanical flow problem of the transport of white blood cells through capillaries to the venular outflow. In

contrast to the red blood cells, the cumbersome and unshapely white blood cells must be pushed through the capillaries from behind by the red blood cells. At first the white blood cells transported from the small caliber arterioles block the capillary whereafter the following red blood cells build up a higher hydrostatic pressure. Once the pressure buildup is sufficient a movement of the white blood cells in the direction of the blood flow is enacted.

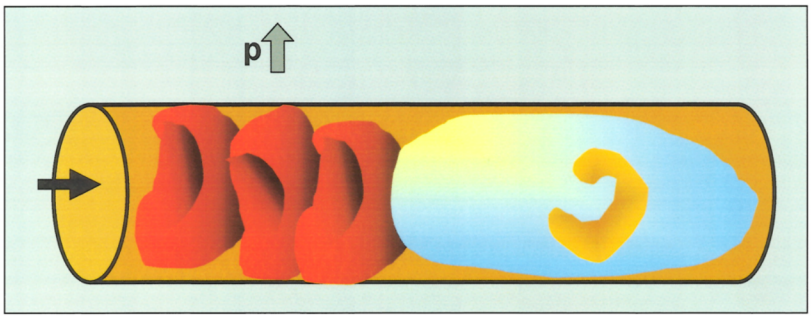


Figure 9. Schematic of white blood cell transport through a capillary.

Transmigration of white blood cells into the tissue in the context of an immune reaction occurs preferentially in the venules. Figure 10 shows the accumulation and partial adhesion of white blood cells from an intravital microscopy example.

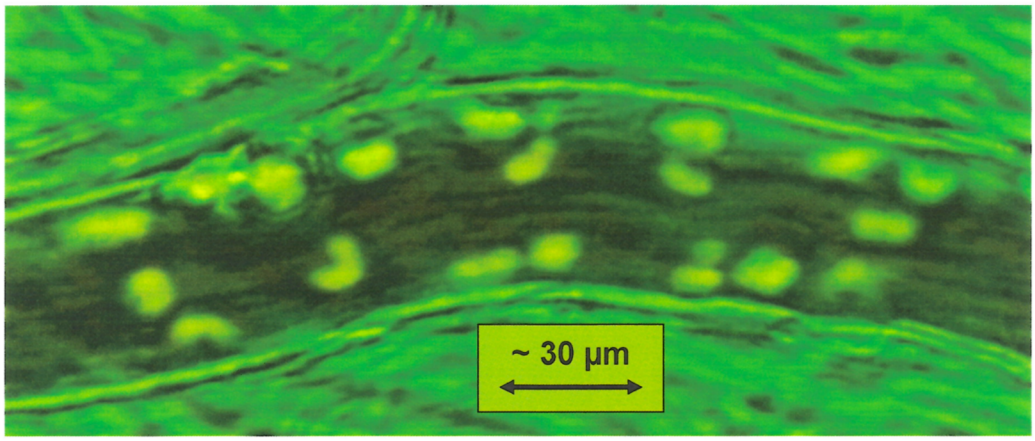


Figure 10. Accumulation of white blood cells in a venule and partial adhesion to the endothelium.

The physiological regulation mechanisms of organ perfusion are now in focus. The principal biomechanical flow relationship of the microvasculature's plasma-blood cell mixture (under idealized assumptions) was easily understandable whereas processes now become more complex.

The discussion now involves the perfusion regulation in large-caliber arteriole sections (cf. Fig.) Receptors are located in this region for neural and hormonal instructions for diameter adjustment that is realized through the contraction state of the smooth muscle cells in the vessel wall. This is called arterial vasomotion. This becomes an effective regulation agent because of $Q \sim r^4$. In the course of minutes to hours periodic diameter changes occur from which pressure gradients develop that effect a demand-oriented adaptation of the total perfusion volume in this vascular zone. The distribution and flow pathways of blood volume that reaches the narrowing small caliber arteriole branches and the subsequent capillary network is effected by a different, local mechanism. This regulation mechanism is not determined by central orders because this section of small caliber arterioles does not contain receptors for such neural or hormonal commands. The diameter changes of small caliber arterioles occur by autorhythmic contractile movements

of smooth muscle cells that surround these vessel sections in a single-layered and intermittent manner. This is referred to as spontaneous autorhythmic vasomotion, the most important localized regulation mechanism of organ perfusion because it is responsible for determining the segregation phenomena of the plasma-blood cell mixture. This local mechanism has therefore the deciding influence on the distribution of the plasma-blood cell mixture in the capillary networks (cf. Fig. 7). This mechanism is so effective that even central neural and hormonal influences on the large caliber arterioles can often under certain circumstances be overridden or compensated for. Explanation of the spontaneous autorhythmic vasomotion lies with the smooth muscle cells that phylogenetically belong to the oldest cells of the human organism and have conserved the characteristic of independent contraction in response to a physical stimulus. This capacity has been lost by other cells over the course of evolution.

The periodicity of vasomotion in the large caliber arterioles and the spontaneous autorhythmic vasomotion of the small caliber arterioles have differing biorhythms yet are in close physical proximity (cf. Fig. 1). From intravital microscopic observations under physiological flow conditions approximately 1 to 5 (median 3) vessel wall oscillations per minute can be discerned. Under illness conditions (e.g., an older patient with diabetic insufficiencies) oscillatory movements of the small caliber vessel wall are only discernible at greater intervals of multiple minutes coupled with reduced amplitudes. From these observations distribution disturbances of the plasma-blood cell mixture in the downstream capillary network are detected with the known effects of material exchange and immune reactions.

The mechanism by which the synchronization of the various biorhythms in the large- and small-caliber arterioles takes place is under current investigation. What is known is that this occurs under physiological flow conditions, and mainly under acute stress. The effect of an acute stress-mediated narrowing of the large caliber arteriole on the material exchange can be compensated for by an elevated frequency of the spontaneous vasomotion of the small caliber arterioles. This is not the case under chronic stress conditions. Under long-term stressor influences it is understood that the spontaneous autorhythmic vasomotion of continually narrowed large caliber arterioles can no longer sufficiently equalize the distribution of the blood in the capillary networks and sometimes reacts in an opposing manner. The consequences span from limitations of material exchange, functional reduction of support to the tissues, and cellular damage (necrosis, ulcerations) to cell death.

The spontaneous periodicities are initiated by the shear stress-dependent, endothelium-mediated arteriolar tone regulation. The state of contraction of the vessel wall smooth muscles is influenced by the released, highly diffusive Nitric Oxide (NO) that is formed in the endothelium (cf. Fig. 4). The endothelium is the central modulator of vascular functions. The endothelial lumen surface influencing shear stress τ releases a molecular enzymatic mechanism in the endothelial cells. This enables NO to have a relaxing effect on the smooth muscle cells from the activation of the soluble guanylyl cyclase.

Several therapeutic options are currently available for the treatment of vasomotor dysfunction. In the area of large caliber arterioles (with corresponding receptor stock) a successful medication-based treatment is possible (e.g., beta-receptor blockers). This achieves not only a diameter change of the arteriolar section with a corresponding effect on the pressure gradient and the distribution state of the blood, but also influences the related reduction of the peripheral flow resistance of the systemic blood pressure. A medication-based influence on the spontaneous autorhythmic vasomotion is not possible, because of the absence of corresponding receptors. A directed influence on the autorhythmic vasomotion periodicity is clearly only possible from an inherent natural stimulation by a corresponding physical stimulus. This is supported by more precise knowledge of the oscillation behavior of the small caliber arteriole vessel walls.

Figure 11 shows measurement results of the flow stream of small caliber arterioles with an extreme, high-resolution research method (measurement data collected at 20 ms intervals). The periodicity of stream flow Q_{RBC} is an expression of the oscillation behavior of the vessel wall, for which the contraction behavior

of the smooth muscle cells is relevant. A very similar oscillation curve is obtained from comparable measurements of the diameter of the microvasculature as a function of time. Every smooth muscle cell delivers a harmonic component that synchronizes with its amplitudes and frequencies to produce compound oscillations. Every compound oscillation exists of a base oscillation and harmonics. In this 'orchestra' of harmonics specialized cells (smooth muscle cells) exist that show the slightest oscillation frequency (base oscillation f_0). They function in essence as 'pacemaker' cells and transfer the oscillation progression to the next highest oscillation frequency f_1 (1st harmonic), which in turn transfers to another cell with the next highest oscillation frequency f_2 (2nd harmonic) etc., as illustrated in Figure 12.

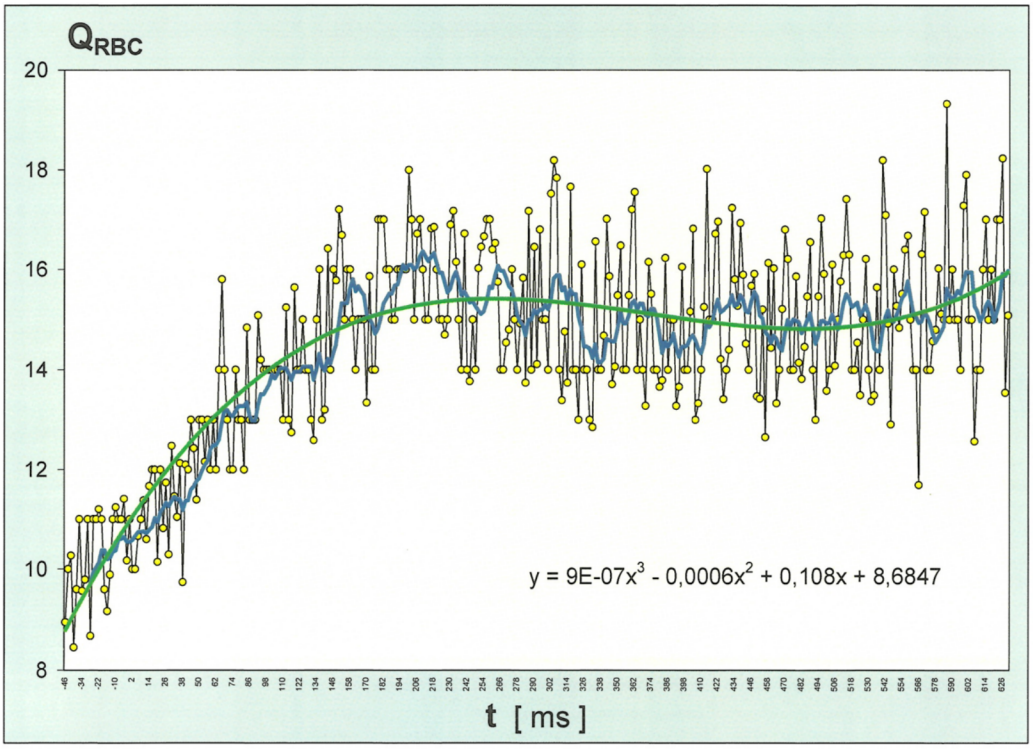


Figure 11. Biorhythmic behavior of the flow stream Q_{RBC} in a small caliber arteriole (extract of a measurement protocol). Ordinate: flow stream Q_{RBC} in relative values, abscissa: time (ms). Blue is sliding average, green is polynomial.

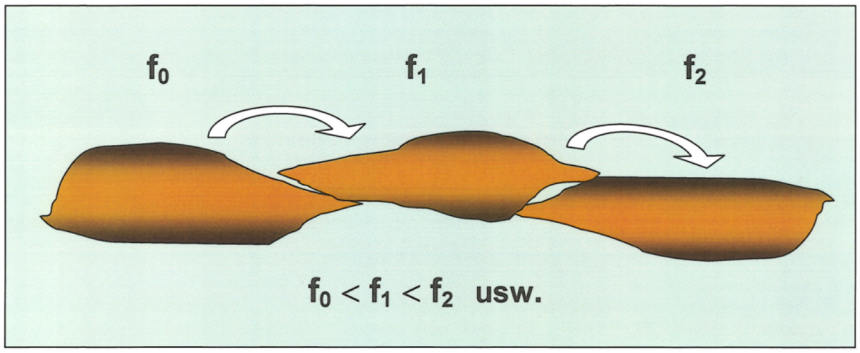


Figure 12. The development of harmonics (f_1 and f_2) excited through a base oscillation f_0 of the smooth muscle cells in the microvessel wall.

The elastomechanical behavior of the arteriolar vessel wall is the next consideration. A force F working on a vessel wall surface A, i.e., a stress $\sigma = F/A$, results in a length change Δl in reference to the original length l_0. Per Hooke's law of elasticity, the equation becomes:

$$\sigma \sim \frac{\Delta l}{l_0}$$

In the tension-extension diagram a comparatively small area of direct proportionality (Hooke's elasticity) is shown and a larger area of plastic distortion. The elastic restoring forces are less than the active processes (energy-dependent activities of the smooth muscle cells). Despite this an analysis can be made of the composite oscillation activity of a small caliber arteriolar vessel wall. The composite oscillation is broken down into its harmonic (sinusoidal) components (base oscillation and higher mode harmonics). For a periodic function $f(t)$ with Period T the Fourier analysis produces:

$$f(t) = a_0 + \sum_{n=1}^{\infty} \left[a_n \cos\left(2\pi nt / T\right) + b_n \sin\left(2\pi nt / T\right)\right]$$

with

$$c_n = \sqrt{a_n^2 + b_n^2} \qquad \text{and} \qquad \varphi_n = arctg\left(a_n / b_n\right)$$

where c_n are the amplitudes and φ_n the phase constants.

The knowledge of these periodicities is thereby of significance for the understanding of the spontaneous autorhythmic vasomotion. Figure 13 provides the amplitude-frequency spectrum. The content of the defined A_{VM} area (broken line) is considered the functional level of vasomotion. This is how, for example, lesser oscillation amplitudes of the small caliber arterioles through restriction of the segregation phenomena between blood cells and plasma achieve a decline of the blood perfused capillaries and therefore an elongated diffusion pathway. The consequences are limitations of the material exchange and therefore limitations of organ functions.

In summary, the spontaneous autorhythmic arteriolar vasomotion is the most important regulation mechanism of organ perfusion. The shear stress-dependent, endothelium-mediated arteriolar tone regulation is only possible with fast flowing blood (microhemodynamic 'window' of an optimal material exchange-adequate perfusion regulation).

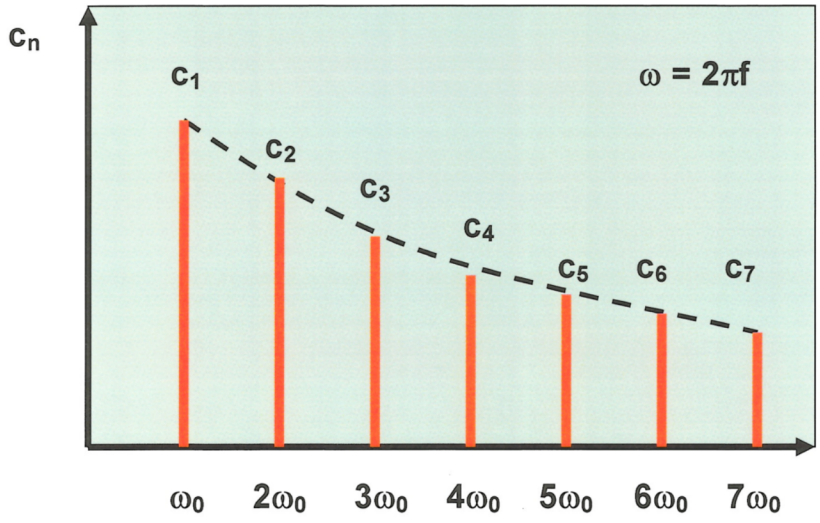


Figure 13. Amplitude frequency spectrum of a composite oscillation. Frequency of base oscillation ($\omega_0 = 2\ \pi f$) and harmonics $\omega_n = n\ \omega_0$ (with n=2,3,4...); c_n amplitudes; φ_n phase constants.

Further details of the performance regularity of the venous outflow from cellular functions, among other aspects, are not discussed here in the context of this topic. Klopp's book *Microcirculation* (2nd ed.) is currently in preparation to incorporate the enormous increase in knowledge in this field of the past 15 years.

The most important consequences of a disturbed microcirculation are finally summarized in Figure 14.

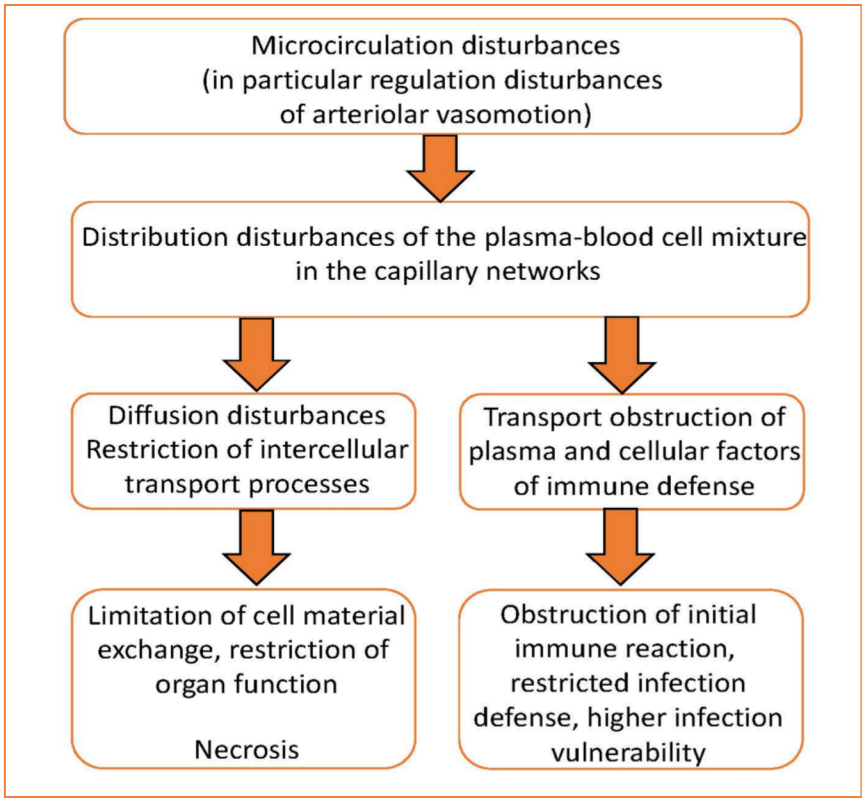


Figure 14. Consequences of a disturbed microcirculation.

A physical stimulation of the deficient spontaneous autorhythmic arteriolar vasomotion

The following concept is central to all clinical pathophysiological thinking: In the deficient state it is to stimulate bodily regulatory mechanisms via a suitable physiological stimulus to be able to by themselves clear the disturbance that has occurred. Such physiological stimulus must be recognized by the system as a stimulant.

As previously discussed a series of highly effective pharmacological therapy options are available for the large caliber arteriolar sections, that are not applicable for the small caliber arteriolar branches. This is the cause for numerous shortfalls of therapeutic success and therapy resistance.

The effective influence of various vascular medications, e.g., beta-receptor blockers, on macrocirculatory factors and some microcirculatory effects on large caliber arteriolar sections has in retrospect been known for a long time (Klopp et al. 2006; 2007). The importance of the local perfusion regulation through the small caliber arteriolar sections has only come to light in the last 10 to 15 years of intensive research. Because the spontaneous autorhythmic vasomotion is determined by a physical stimulus, the shear stress of flowing blood, it was advisable to search for a physical therapy option by which the inherent bodily regulatory factors in the deficient case would be stimulated externally. The end result of

these considerations was the analysis of the oscillation behavior of the small caliber vessel wall under physiological and pathological conditions (cf. Figs. 11, 12, 13).

The complexity of the physiological regulation processes in the small caliber arteriolar sections and the molecular-biological processes in the context of this treatise could only be discussed on a basic level. It could not be anticipated *a priori* that causal therapeutic effects through such a physical stimulation were achievable, but rather in the best case scenario an adjunctive treatment therapy would be discovered.

As carrier wave of a suitable stimulation signal both elastomechanical and electromagnetic waves were considered of which the electromagnetic wave ultimately prevailed.

Natural conditions were of significant importance for the establishment of the energy contributions that are transferred through the physical stimulation signal (transfer of much lower energy contributions from the shear stress-dependent, endothelial-mediated tone regulation of the small caliber arterioles). Very small energy contributions are sufficient for the endogenous regulation from the shear stress of flowing blood on the lumen side of endothelial surfaces and to provide the endothelial bio-catalytic converters (enzymes) the necessary activation energy for the molecular-biological processes to be lowered for the regulation to take place under physiological flow conditions. An effective physical stimulus of electro-magnetic flux density of ~100 μTesla was deemed sufficient.

The establishment of the appropriate signal configuration was determined by the results of the oscillation analyses of the natural physiological oscillation behavior of the arteriolar wall. With the help of an electromagnetic field as an energy carrier to the tissues the physical stimulus of the amplitude-, frequency-, and phase-modulated complex signal (composed of the sinusoidal harmonics) is transported in a specified frequency window of approximately 5 to 40 Hz.

As part of a placebo-controlled study of a biometrically defined sample of middle-aged male subjects exposed to mild chronic stress and mixed infections the representative characteristics of microcirculation in the abdominal subcutis were measured by way of high-resolution investigative methods after application of different electromagnetic alternating fields of the same flux densities and research conditions (Klopp et al. 2013d). As the imaging measurement method a laser Doppler vital-microscopic unit was used in a combined incident-transmitted light method with secondary computer-based image processing.

Measured values collected were: a) number of blood cell-perfused nodes in a defined tissue volume unit nNP (as measure of the state of distribution of the plasma-blood cell mixture of the microvascular network); b) venular oxygen saturation ΔpO_2; and, c) area under the envelope of the amplitude-frequency spectrum of the spontaneous arteriolar vasomotion A_{VM}. For the statistical analysis of the measured data the Wilcoxon rank sum test at the significance level $\alpha = 0.05$ was used.

The test equipment used was TD1: placebo-device, TD2-TD6: devices to elicit electromagnetic alternating fields of simple square-, triangular-, sawtooth-, sinusoidal- and amplitude-modulated sinusoidal oscillations, and TD7: device with amplitude-, frequency- and phase-modulated signal in accordance with physiological oscillatory behavior of small caliber vessel walls, a biorhythmically defined stimulation signal).

The treatments were administered under defined conditions in a 3-day interval twice daily for 10 minutes at 2-hour intervals. The measurement times of the daily data collection were: D0 - determination of initial values one day prior to start of study; D1, D2, D3 - immediately after the 2nd treatment of the corresponding day; D4 - determining the decline of characteristic changes after the end of treatments. Figures 15, 16 and 17 depict the obtained data.

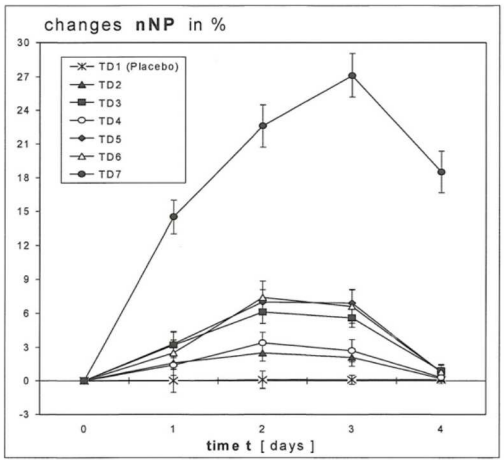


Figure 15. Measured values of the characteristic "number of blood cell perfused nodes in the defined network unit, nNP" (means and standard deviations) following application of test devices TD1-7.

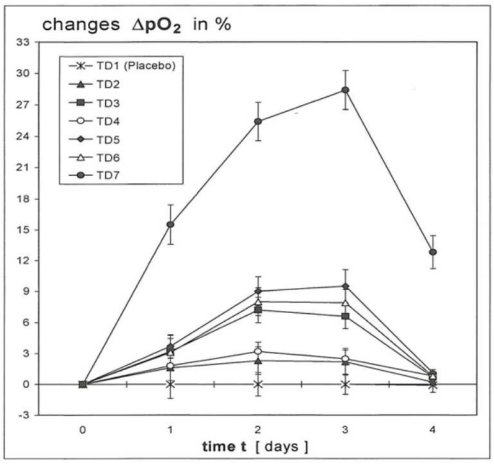


Figure 16. Measured values of the characteristic "venular oxygen saturation pO_2" (means and standard deviations) following application of the test devices TD1-7.

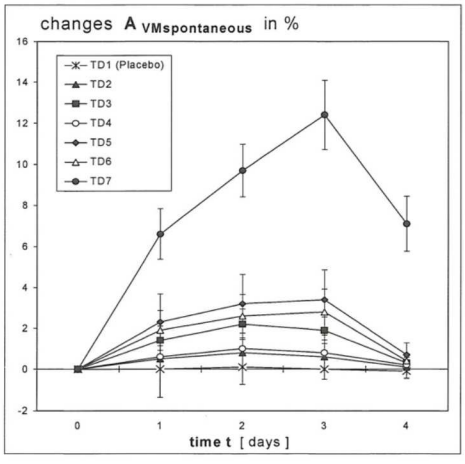


Figure 17. Measured values of the characteristic "area under the envelope of the amplitude-frequency spectrum of spontaneous arteriolar vasomotion A_{VM}" (means and standard deviations) following application of test device TD1-7.

The examinations showed that only a targeted, biorhythmically defined stimulus could affect arteriolar vasomotion and thus microcirculatory blood flow regulation in a therapeutically relevant manner and therefore was suitable for prophylactic and complementary-therapeutic use. TD 7 was the only test unit meeting this requirement, while the placebo device had no effect and TD2-TD6 effected only minimal changes.

In another placebo-controlled study Klopp et al. (2014) examined a biometrically defined sample of elderly patients with diabetes mellitus type II and wound healing disturbances to determine the extent to which a complementary application of a biorhythmically defined physical stimulus can contribute to the therapeutic treatment success of dysfunctional spontaneous arteriolar vasomotion. During the 27-day treatment period two subsamples were examined: a) control group of standard clinical treatment; and, b) test group standard clinical treatment and complementary adjuvant application of a biorhythmically defined physical stimulation signal.

As the imaging measurement method a laser Doppler vital-microscopic unit was used in a combined incident-transmitted light method with secondary computer-based image processing. The examined characteristics were: a) number of blood cell-perfused nodes in a defined tissue volume unit nNP (as measure of the state of distribution of the plasma-blood cell mixture of the microvascular network); b) venular oxygen saturation ΔpO_2; c) area under the envelope of the amplitude-frequency spectrum of the spontaneous arteriolar vasomotion A_{VM}; and, d) number of white blood cell adhesions in a defined venular inner wall surface A = 18,000 μm^2, nWBC/A (first stage of cellular immune reaction). For the statistical analysis of the measured data the Wilcoxon rank sum test at the significance level $\alpha = 0.05$ was used.

The adjuvant treatment during the 27-day period occurred at intervals of every 3 days (2 applications of 12 minutes with 2-hour intervals). The following measurements were taken under constant constraints of the immediate treatment day after the last daily treatment: D0 - determination of initial values prior to start of study; D3, D6, D9,...D27 - immediately after the 2nd treatment of the corresponding day. Measurement locus was a defined subcutaneous tissue region (corresponding to the wound region, ankle or foot area). Measurements were taken in the wound periphery (8mm from the wound edge). Figures 18-21 depict the obtained data.

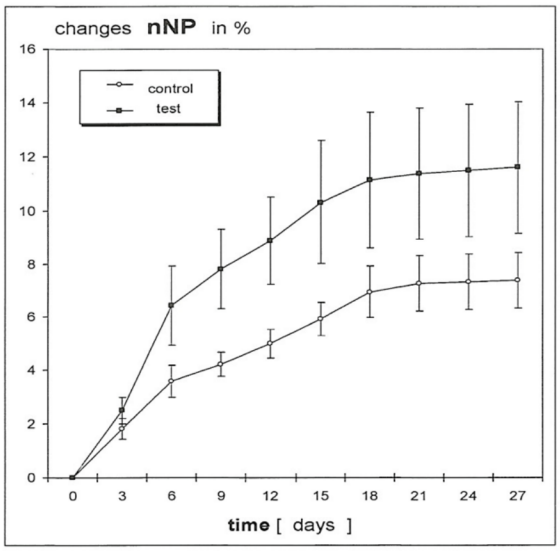


Figure 18. Measured values of the characteristic "number of blood cell perfused nodes in the defined network unit, nNP" (means and standard deviations).

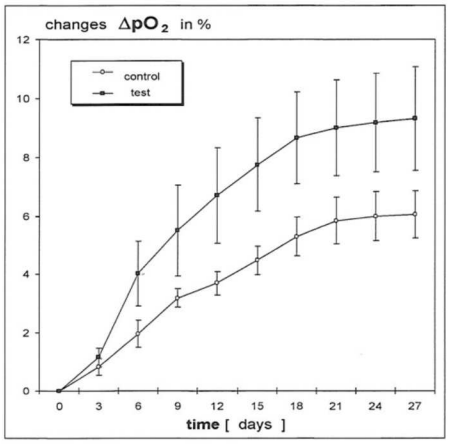


Figure 19. Measured values of the characteristic "venular oxygen saturation pO_2" (means and standard deviations).

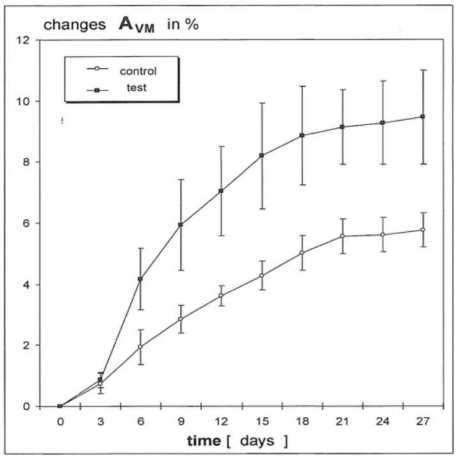


Figure 20. Measured values of the characteristic "area under the envelope of the amplitude-frequency spectrum of spontaneous arteriolar vasomotion A_{VM}" (means and standard deviations)

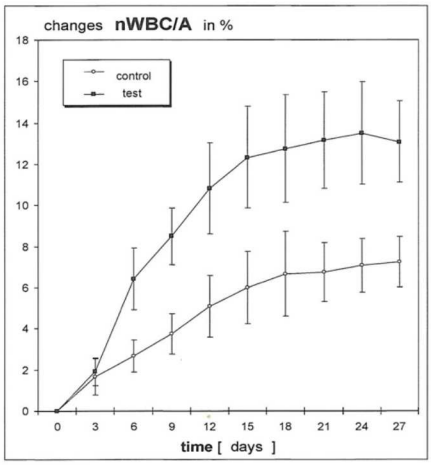


Figure 21. Measured values of the number of adhering white blood cells on a defined venular inner wall surface nWBC/A (means and standard deviations).

The planimetric investigation of the wound surface resulted in significant value differences between samples. On D27 the wound surface of the control group patients had shrunk by ~ 41% while the patients in the test group shrunk by ~ 64%. The obtained measurement data support an adjuvant application of a biorhythmically defined physical stimulus for optimal therapeutic treatment of material exchange under inadequate perfusion regulation and also for promising supplementation of prophylactic measures. These achieved research results and others (Klopp et al. 2013a; 2013b; 2013c; 2014; 2016) can be considered encouraging for the conduct of further investigations in this field.

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