Differential Effects of Shear Forces and Pressure on Blood Vessel Metabolism and Function in a Perfusion Model

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Abstract— **Tissue engineering protocols commonly incorporate mechanical stimuli like pulsatile flow and luminal pressure to adapt the constructs to the biomechanical requirements of the target circulation. This study investigated the effects of shear forces and of luminal pressure on blood vessel metabolism and function using bovine veins as a model. Veins were perfused for four days in M199 at 40 ml/min. Shear forces were increased by adjusting the viscosity of the medium to that of blood. Some veins were additionally treated with luminal pressure. Control groups were either denuded or perfused with 60 ml/min to assess the influences of endothelium and of elevated flow rates, respectively. Physiological shear forces were sufficient to maintain endothelial integrity, unless superphysiological flow rates were applied. Reductive capacities were not affected by any of the perfusion conditions. Responses to the receptor-dependent vasoconstrictor noradrenalin were increased after perfusing with physiological shear forces and luminal pressure. This also induced a shift of the metabolism from glycolysis and lactate fermentation to the use of alternative substrates other than glucose. Mechanically denuded control vessels were prone to apoptosis and showed an altered metabolism as well. However, denuded vessels treated with luminal pressure also showed improved responses to vasoconstrictors compared to denuded controls. These results indicate that mechanical challenges are required to maintain endothelial integrity and to improve contractile function of vessels in perfusion systems. Metabolic patterns change with mechanical challenges, and care should be taken to provide sufficient amounts of substrates other than glucose for energy metabolism.**

Keywords— **tissue engineering, vascular function, perfusion bioreactor, metabolism.**

I. Introduction

Atherosclerosis and endothelial dysfunction affect an increasing number of patients especially in western countries. The resulting vascular dysfunction causes common diseases such as lower limb ischemia and coronary artery disease. Ultimately, many patients require bypass surgery to revascularize the affected limbs or organs. In contrast to their successful use in larger vessels, synthetic materials have not achieved acceptance as small caliber vessel grafts due to their thrombogenic properties and compliance mismatches[1]. Therefore, autologous vessels are still a mainstay of revascularization. However, harvesting these vessels causes an additional health risk for patients, and approx. 6% of eligible patients cannot provide suitable autologous vessels due to diseases or prior removal.

Tissue engineering attempts to create tissue or organ replacements by combining biological or biocompatible matrices with autologous cells, thus providing mechanically stable and immunologically inert replacements for failing structures. Several approaches were published in terms of vascular tissue engineering [2]. Most of these procedures have in common a prolonged perfusion of the constructs in a bioreactor in order to adapt them to the target circulation. This includes appropriate settings of flow rate, luminal pressure, and shear forces. The influences of these parameters on vascular function and metabolism have not been fully elucidated yet. In the current study we have used bovine veins as model vessels in a perfusion bioreactor to address these questions.

II. Materials and Methods

A. Vessel Harvesting

Bovine lateral saphenous veins were harvested as pedicles from freshly sacrificed animals and transported to the lab in Krebs-Henseleit buffer at 4°C. Veins were dissected free from connective tissue under a sterile hood. Branches were ligated as required. Segments of 8 cm in length were used for perfusion experiments, and shorter segments were used as fresh controls for functional analyses.

B. Perfusion System

Three separate bioreactors (Fig. 1) consisted of medium reservoirs (1), neonatal membrane oxygenators (2), independent peristaltic pumps for perfusion and superfusion circulations (3,4), variable pulse dampeners (5), and vessel chambers (6). The perfusion circulations also included

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pressure probes (7) and pressure-operated starling resistors (8). Oxygenators were flushed with a mixture of 20% oxygen and 5% carbon dioxide (balance nitrogen). Sterile ports allowed retrieval of medium samples for blood gas analysis. Media were replaced every other day.

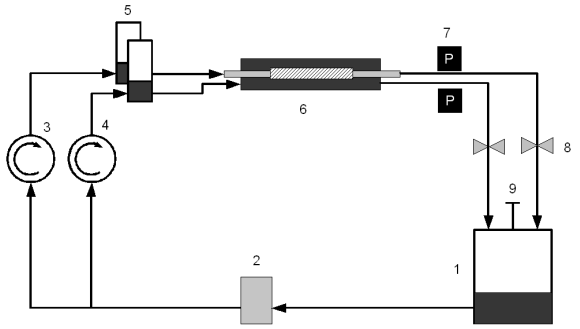

Fig. 1 Perfusion bioreactor

C. Perfusion Conditions

Vessels were perfused with M199 supplemented with 20% fetal calf serum and standard antibiotics and antimycotics for 4 days at 37°C. The conditions are outlined in Table 1. Shear forces were increased by adjusting the viscosity to that of blood using dextran. Additional luminal pressure above the control values was applied by means of the starling resistors. Some vessels were denuded mechanically prior to perfusion to check the contribution of endothelium to any of the observed effects.

Table 1 Perfusion conditions

group	dextran	luminal pressure (mm Hg)	endothelium-denuded	luminal flow (ml/min)
1	-	-	-	40
2	12%	-	-	40
3	12%	20	-	40
1	-	-	+	40
2	12%	-	+	40
3	12%	20	+	40
1a	-	-	-	60
2a	12%	-	-	60
3a	12%	20	-	60

D. Organ Bath Studies

Responses to vasoactive substances were assessed isometrically in an organ bath as described previously [3]. Samples were immersed in Krebs-Henseleit buffer and bubbled with carbogen. Responses to 150 mM KCl and to a concentration series of noradrenalin (NA) were determined. Endothelium-dependent relaxation was assessed by measuring the response to a concentration series of acetylcholine (ACh) after constricting the vessels to 80% using NA.

E. Tetrazolium Dye Reduction

Vessel segments were opened longitudinally and mounted luminal side facing up in a vessel holder. Tetrazolium dye reduction, indicating the reductive capacities of the tissues, was determined according to standard protocols in the wells provided by the holder top.

F. Histology

Short vessel segments were fixed in phosphate-buffered formaldehyde and embedded in paraffin. Thin sections were analyzed by H&E staining, TUNEL, and anti-CD31 antibodies to visualize the general structure, apoptosis, and endothelial cells, respectively.

G. Statistics

Data are presented as mean±standard deviation and were compared using t-test or ANOVA as appropriate. Differences were accepted as significant if $p<0.05$.

III. Results

A. Vessel Denudation

Mechanical denudation did not affect responses of the vessels to KCl or to NA. Reductive capacity was also not affected. However, the vessels no longer responded to ACh as expected. Therefore mechanically denuded vessels were considered valid controls to investigate the role of endothelium.

B. Influence of Perfusion Conditions on Vessel Structure

The gross structure of the outer layers of the vessel wall (Tunica media and T. externa) were not altered by any of the perfusion conditions. However, endothelial cells were lost in the absence of dextran (group 1) and, to varying

degrees, at higher perfusion flow rates (groups 1a through 3a). Apoptosis was not detectable in groups 1 through 3, whereas denuded vessels showed clear signs of apoptosis. Groups 1a through 3a showed varying degrees of apoptosis concomitant with their loss of endothelial cells.

C. Oxygen and Glucose Consumption

The oxygen gradients across the vessels were close to the detection limit and indicated a low oxygen consumption which was easily met by the applied membrane oxygenation. pH values remained within the physiological range under all perfusion conditions.

Glucose consumption and lactate production indicated a glycolytic conversion of glucose without oxidative phosphorylation in groups 1, 2, 2–, and 1a through 3a. In contrast, vessels of groups 3, 1–, and 3– produced more lactate than glucose could provide, indicating a shift to other energy sources in the presence of luminal pressure (Table 2).

Table 2 Glycolytic indexes

group	value	group	value	group	value
1	1.16	1–	1.94	1a	1.17
2	1.01	2–	1.54	2a	1.08
3	1.58	3–	1.82	3a	1.02

D. Responses to Vasoactive Compounds

Contractions induced by KCl were lower in groups 1– and 2–, indicating beneficial effects of luminal pressure even in the absence of endothelium (Fig. 2).

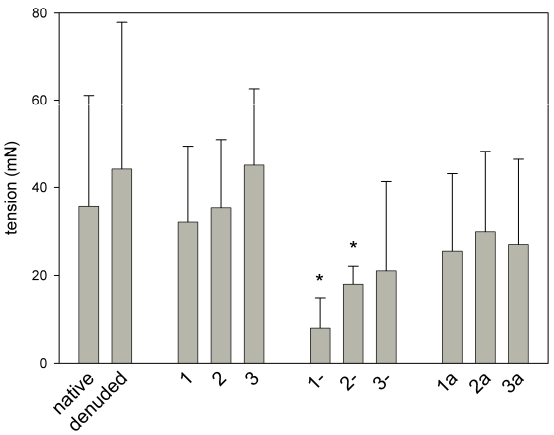

Fig. 2 Maximum constriction to 150 mM KCl

Maximum contractions induced by NA were significantly stronger in group 3 and significantly lower in denuded vessels (groups 1– through 3–) after 4 days of perfusion (Fig. 3). There were no significant changes in all other groups. The relaxing effect of ACh was not detectable in any group after perfusion.

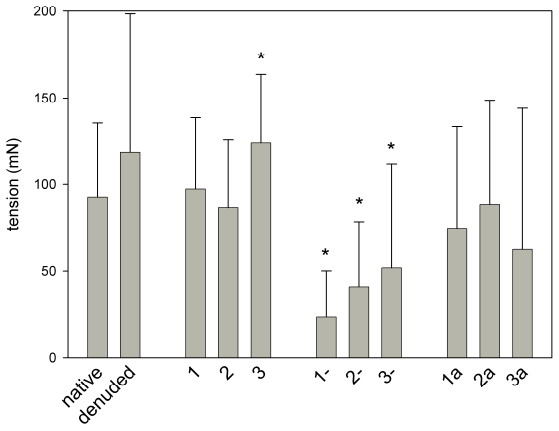

Fig. 3 Maximum constriction to 1E-5M noradrenalin

E. Reductive Capacities

Reductive capacities were unaltered after perfusion in all groups except 1– and 2–, which correlates with their attenuated responses to KCl.

IV. Discussion

Flow rates, pressure, and shear forces showed a noticeable effect on blood vessel structure and function in our perfusion model. In terms of structure, the most important result was the protective effect of physiological shear forces on the preservation of endothelial cells after 4 days of perfusion. However, if the flow rates were increased above levels the veins were adapted to, a loss of endothelial cells within 4 days occurred regardless of the perfusion conditions. These results agree well with the "ramped pressure" approach used by many tissue engineering protocols [4], i.e. increasing flow rates only gradually to allow time for the constructs to adapt to the altered flow conditions. Vessels perfused at venous flow rates did not show any signs of apoptosis, even if endothelial loss occurred (in the absence of dextran). In contrast, denuded vessels showed varying degrees of apoptosis. Apoptotic nuclei were detectable throughout the vessel wall in the absence of dextran, whereas in its presence only few stained nuclei appeared close to the luminal side. A similar distribution, but at a much lower density, was also observed in vessels perfused at elevated flow rates. As judged by the

histological results, the veins performed best under venous flow conditions. Increased shear forces helped maintain endothelial integrity. However, beneficial effects of increased shear forces were also visible in the absence of endothelium.

The oxygen consumption of the vessels was negigible and could be easily met by the membrane oxygenators. This confirms earlier reports about the energy metabolism of smooth muscle cells or of tissue slices in organ baths [5]. This also agrees well with glucose metabolism and lactate production in the bioreactors. Under a variety of perfusion conditions, one mol glucose was converted to two mol of lactate, indicating lactate fermentation as the main source of energy. This also explains the low oxygen consumption as oxidative phosphorylation is not required by fermentative metabolism. Only three of the applied perfusion conditions caused a deviation from this metabolic pattern. Most notably, increased shear forces and luminal pressure (group 3) caused an increase of the glycolytic index without any changes to the vessel structure. As more lactate was produced than glucose could deliver, the vessel walls obviously used other energy sources. Intracellular glycogen stores are unlikely to last for several days, so other medium components like amino acids and fatty acids [5] are more likely energy sources under these conditions. Increases of glycolytic indexes were also observed in groups 1– and 3–. However, these may have other causes as vessels of these groups show alterations of the structure as a consequence of denudation. These results indicate that a sufficient supply of energy sources other than glucose should be present in perfusion media if the vessels or constructs are mechanically exercized.

Contractile responses to KCl were not affected by perfusion except in endothelium-denuded vessels. Again, increased shear forces and luminal pressure showed a beneficial effect here, as there was no significant decrease of KCl repsonses in group 3–. These results match the reductive capacity data, as groups 1– and 2– were the only ones that showed a decrease between days 0 and 4. Hence, the reduced contractile responses may be attributed to a more general failure of cellular metabolism as a consequence of apoptosis. It is interesting to note that luminal pressure showed salutary effects in the absence of endothelium. In all other groups contractile responses and reductive capacities were not different before and after perfusion. Responses to the receptor-dependent vasoconstrictor noradrenalin were similar in general. However, vessels of group 3 showed stronger responses to this compound after perfusion compared to the day 0 controls. This again highlights the salutary effects of shear forces and pressure on blood vessel function in a perfusion system.

V. Conclusions

The results of the current study emphasize the need to monitor the substrate consumption of vessels and tissue-engineered constructs in perfusion bioreactors, as mechanical challenges like shear forces and luminal pressure significantly affect vessel wall metabolism. Perfusion media compositions should include sufficient amounts of alternate energy sources besides glucose. Oxygen partial pressures comparable to ambient air are not limiting. Mechanical stimulation is beneficial for contractile function. Sufficient shear forces appear to be essential to maintain endothelial integrity and to avoid aptoptosis, whereas distension by increasing luminal pressure apparently exerts its positive effects even in the absence of endothelium.

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