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Original article

# A novel system for the investigation of microvascular dysfunction including vascular permeability and flow-mediated dilatation in pressurised human arteries

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ABSTRACT

**Introduction:** Adverse drug reactions may be manifested through changes in microvascular function (e.g. angioedema) or by subtle modification of the mechanisms controlling vascular tone, such as flow-mediated dilatation. Until now the early detection of such adverse drug reactions has been hampered by the lack of a predictive *in vitro* model. This *in vitro* model can be utilised to test potential effect of drugs on the normal responses of the vascular system. **Methods:** The PM-1, a new automated perfusion myograph, allows detection of the external and internal dimensions of tubular biological structures and regulates both the intraluminal pressure and flow independently. Drugs can be infused intraluminally or extraluminally (by adding to the bathing solution) to determine effects on constriction, relaxation or modulation of vascular tone. The novel imaging system also facilitates the measurement of vascular permeability using dyes introduced intraluminally into the vessel. **Results:** To assess effects on flow-mediated dilatation we increased flow rate in pressurised human subcutaneous arteries (<500 μm diameter) in the absence and presence of various drugs. Increasing flow from 0.04 ml/min to 0.3 ml/min resulted in a 39 ± 3% relaxation of a U46619 pre-constriction ($10^{-6}$ M). This was enhanced in the presence of Ivermectin and inhibited in the presence of 100 μM L-NAME (316 ± 169% and 16 ± 1% respectively).To assess effects on vascular permeability we infused albumin-bound Evans blue dye through the lumen of human subcutaneous arteries as a marker, in the absence and presence of a modulatory drug. Infusion of thrombin (0.5 units/ml) through the vessel lumen caused an 11.8% increase in vessel permeability compared to vehicle infusion. **Conclusion:** The development of the PM-1 allows new drugs to be tested in relevant human or animal tissues at an early stage allowing crucial go/no-go decisions to be made early in development and giving a more complete picture of the overall effects of test compounds on vascular function.



## 1. Introduction

Disrupted microvascular function is a factor in many conditions including hypertension, diabetes, heart failure and sepsis. Such microvascular changes might affect the regulation of vascular tone, in particular by inhibition of endothelial relaxant factors or may occur via changes in the integrity of endothelial tight junctions that regulate vascular permeability. As such, a great deal of research has been conducted into the regulation of vascular smooth muscle tone and the influence of endothelial factors; however, less attention has been paid to the combined effect of drugs and disease on the regulation of the vasculature. Undesirable adverse drug reactions may be manifested through changes in microvascular function, for example, angioedema, vasoconstriction or vasodilatation, or perhaps a more subtle modification of the mechanisms controlling vascular tone such as flow-mediated vasodilatation.

Angioedema is caused by an increase in vascular permeability leading to a swelling of the deep layers of subcutaneous or submucosal tissues. Drug-induced angioedema is a well documented adverse effect of ACE inhibitors, SSRIs (selective serotonin reuptake inhibitors), COX-II inhibitors, angiotensin II antagonists, statins and proton pump inhibitors (Salih & Thomas, 2006).

In addition to vascular permeability changes, vascular function is determined by smooth muscle tone that controls vessel diameter and hence vascular resistance. The direct effect of many drugs on vascular tone is well characterised; for example the effect of 5-$HT_{1B/1D}$ agonists on cerebral and coronary arteries and the decrease in flow-mediated relaxation associated with protease inhibitors and angiogenesis inhibitors. Many of these effects are of course a balance between beneficial effects (disrupting blood flow to a tumour) and adverse effects (reduced ability to perfuse tissues when blood flow increases); however, the ability to predict whether a drug will produce microvascular side effects such as angioedema or altered flow-mediated vasodilatation could lead to safer drug development and reduce the number of late withdrawals of drugs from the market. Until now such predictions have been hampered by the lack of a

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relevant *in vitro* model system that can easily measure changes in permeability and diameter of blood vessels.

Measurements of vascular permeability are typically conducted using *in vivo* animal models such as the Evans blue dye model, where extravasation of the dye into the wall of blood vessels is used as a marker of vascular permeability. Such models suffer from disadvantages including potential species differences, an inability to track changes in real-time and the requirement to sacrifice animals. Current cell-based permeability assays, even if using human cells, might not reflect the true 3-D architecture of human blood vessels and are therefore not entirely satisfactory. The use of fresh isolated human blood vessels *in vitro* overcomes many of these problems.

The investigation of the structure and function of small vessels and other tubular tissues often utilises the technique of isometric wire myography, however this technique has drawbacks. The mechanical deformation of the tissue ring under force loading is non-physiological and the addition of drug compounds to the bathing solution is undirected. The isobaric pressure myograph was developed to address some of these limitations as the vessel geometry and loading are typically more physiological and the delivery of the test drug can be limited to intra- or extraluminal surfaces. Perfusion studies are particularly important for the basic understanding of vascular physiology; however, isobaric pressure myography has until now been an inherently difficult technique. Most perfusion experiments have been performed without intraluminal flow, where drug compounds are simply added to the extraluminal bathing solution. However due to the emerging importance of flow-mediated dilatations in the overall role of regulating vascular function as outlined above, it is often important to perfuse drugs directly into the lumen of blood vessel, a technique which itself is challenging and makes a difficult technique even harder. A Pubmed search for papers utilising pressure myography in conjunction with intraluminal flow returns only 12 citations from the year 2000 to the present day.

Biopta has developed a new technology (the PM-1) allowing sensitive, accurate measurements of the function of pressurised small vessels in the presence of flow, which allows dyes to be introduced into the lumen of the vessel. This instrument, with its novel image analysis method and automated fluid handling, has allowed us to measure changes in flow-induced vascular function and vascular permeability in isolated fresh human arteries. The present paper outlines the method and applications of the system in the study of vascular function.

## 2. Methods

### 2.1. Tissue samples

Ethical approval was received in advance of the research and informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Skin samples were obtained from residual tissue of patients undergoing elective cosmetic surgery. Excised tissue was placed in AQIX solution for transport to Biopta. With the aid of a dissection microscope, vessel segments roughly 4 mm in length and free from any side branches were dissected free from the surrounding tissue taking care not to damage the vessel wall.

### 2.2. Perfusion myography

Biopta's instrument, the PM-1, is the first automated perfusion myograph which in conjunction with detection of the external and internal dimensions of tubular biological structures, regulates both the pressure and intraluminal flow independently, allowing automated changes in flow rate and addition of drugs intraluminally. Previous perfusion systems required a high level of operator skill and time-consuming nursing of the apparatus through the experiment. The present system has deskilled the tissue handling process and sensitive control of flow through accurate syringe pumps and automated fluid handling with 12 programmable drug vials, which means that once the protocol has been initiated no further user involvement is required. A programmable test scheduler allows the operator to plan and run an experiment without manual intervention.

Segments of arteries with 100–2000 μm internal diameter were mounted in the removable tissue bath on stainless steel cannulae which were sized matched to the vessel (14 gauge to 35 gauge cannula: 2.11 mm to 0.13 mm external diameter). Glass cannulae are easily broken; stainless steel cannulae are more robust and can be re-used; however, to study vessels smaller than 100 μm glass cannulae are required. Vessels were tied onto the cannula using two single strands of thread at each end. Once mounted, the vessels were heated to the required temperature (28–40 °C) and pressurised to a set pressure (5–200 mm Hg).

Fluid lines were primed automatically to remove air from the system before the vessels were mounted and pressurised, because air in the system prevents the vessel from reaching the desired pressure.

The PM-1 simultaneously measures the transmission of two different wavelengths of light to give a precise measure of vessel diameter (red wavelengths provide measure of inner diameter, blue wavelengths outer diameter; international patent WO/30276). A digital image of the vessel is displayed on a CCD screen and snapshots of the digital image can be saved for later analyses (Fig. 1).

### 2.3. Flow-mediated dilatation

Small human subcutaneous arteries (diameter<500 μm) were mounted on the PM-1 system and pressurised to 60 mm Hg following which they were perfused with physiological saline solution (PSS) at low flow (0.04 ml/min) for 10 min to equilibrate and maintain a steady tone and diameter.

The endothelial responses of the artery were assessed by exposing the arteries to 300 nM U46619 (thromboxane $A_2$ mimetic), either intraluminally or extraluminally (by addition to the bathing solution) to induce a constriction. On plateau of the U46619 response, acetylcholine was added (either intraluminally or extraluminally) to determine whether endothelium-dependent vasorelaxation responses were present. To wash out the drugs, the bathing solution was then flushed with fresh PSS, or the lumen contents were flushed with PSS.

The flow-mediated relaxation was assessed by first constricting the arteries using 300 nM U46619 at a low flow rate. The flow–diameter relationship was then studied by flowing PSS through the lumen of the artery at a high flow rate of 0.3 ml/min in the presence and absence of compounds that were known to affect the flow-mediated response (in the continued presence of 300 nM U46619).

### 2.4. Vascular permeability

Evans blue dye (EBD) is widely used to investigate *in vitro* cellular permeability and vascular leakage; however, EBD itself is a small molecule and easily passes through gaps in the vascular endothelial layer, and therefore it is necessary to conjugate the dye to a larger molecule such as bovine serum albumin (BSA) to prevent passage of the dye through the endothelium and into the vascular wall. Previous studies from the literature have used EBD conjugated to BSA at a ratio of 10:1 (Stopa et al., 2006). At this ratio EBD does not readily pass through the endothelial layer of unstimulated vessels.

Subcutaneous resistance arteries were loaded with the EBD by flowing BSA-conjugated dye through the lumen of the vessel. Using the PM-1 we were able to capture real-time images of the vessel throughout the experimental protocol. Analysis of these images gave data on the permeability of the vessel wall, or the accumulation/