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REVIEW

A Review of Methods Currently Used for Assessment of *In vivo* Endothelial Function

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An intact vascular endothelium is critical to the maintenance of normal arterial tone and coagulation status. Endothelial injury leading to dysfunction is thought to be a precursor to most if not all vascular disease, and has been implicated as a critical event in atherosclerosis. At present there are several methods available for detection of in vivo *endothelial function, and the aim of this study was to critically review these methods. Five distinct methods were identified and studied in detail. These methods are diverse and each assesses a different vascular bed. Importantly there is no uniformity among investigators over choice of method and protocol, making it difficult to compare* in vivo *enothelial dysfunction between groups. These issues need to be addressed in large scale comparative analyses so that investigators can agree a common approach to endothelial function assessment.*

Keywords: Endothelium; Endothelial; Dysfunction; Nitric oxide; Smooth muscle; Cardiovascular disease; Venous occlusion plethysmography; Flow-mediated dilatation; Iontophoresis; Coronory angiography; Microvascular; Artery.

Introduction

An intact vascular endothelium is essential in maintaining short term control of arterial tone and coagulation status, and longer term control of smooth muscle cell proliferation and extra-cellular matrix production. Injury to the vascular endothelium is likely to be a preliminary event in most if not all vascular disease. Endothelial dysfunction has been implicated in a wide range of diseases from diabetes mellitus and essential hypertension, to vasospastic conditions such as systemic sclerosis and primary Raynaud's phenomenon. Furthermore, it is postulated that endothelial dysfunction is a precursor to frank atherosclerosis;[1–4] indeed it has been identified *in vivo* in healthy individuals exposed to cardiovascular risk factors such as cigarette smoking, obesity, increasing age and male sex. Cardiovascular disease is currently a leading cause of morbidity and mortality in the Western world,[5] a fact which has provided a strong impetus for the development of methods that facilitate *in vivo* assessment of endothelial function. For many, the long term goal is none other than the establishment of a diagnostic tool that can detect disease early and monitor therapeutic responses.

There are at least five distinct methodologies that are currently being used for *in vivo* endothelial function assessment (Table 1). Some methods, such as venous occlusion plethysmography (VOP), are well established and have been in use for many years, while others are still at the developmental stage. Importantly, there appears to be very little uniformity among investigators regarding method selection and protocol. The aim of this study, therefore, is to critically review the methods currently being used for peripheral endothelial function assessment.

Venous Occlusion Plethysmography

Venous occlusion plethysmography (VOP) has been used to study forearm blood flow for many years. In

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Table 1. Methods used for *in vivo* endothelial function testing

Technique	Vascular bed	Invasiveness	Accuracy/reproducibility
Venous occlusion plethysmography (VOP)	Forearm resistance vessels	Moderately invasive	Highly accurate and reproducible. Regarded by some as the 'Gold Standard'
Flow-mediated dilatation (FMD)	Brachial (conduit) artery	Non-invasive	Accuracy depends on quality of equipment/software. Observer variations an issue
Laser Doppler iontophoresis	Forearm skin microvessels	Non-invasive	Conflicting reports. No clear data as yet
Pulse wave analysis	'Global' endothelial function	Non-invasive	Unclear how accurately global arterial stiffness reflects endothelial function
Retinal arterial architecture	Retinal arterioles	Non-invasive	Not yet assessed

fact, it was first described in 1909 by Hewlett and van Zwaluwenburg,[6] and aside from the incorporation of computer technology, the method has remained essentially unchanged since then. The underlying principle involves the arrest of venous outflow from the forearm such that it begins to swell. The rate and degree of swelling reflects forearm vascular resistance, which is a function of normal vascular endothelial function.

The method is widely used, with most practitioners using the protocol established by Wilkinson and Webb.[7] When performing the procedure it is critical that baseline vasomotor tone remains constant, so factors which may affect vasoreactivity are carefully avoided. Thus, VOP is performed in a quiet, temperature-controlled room, with the subject relaxing in a reclining position. Subjects are asked to abstain from fatty meals, alcohol, caffeine and tobacco in the preceding 6 h. Blood pressure cuffs are placed around the arm and around the wrist, and the arm is held above the level of the heart. The arm cuff is inflated just enough to occlude venous outflow while preserving arterial inflow (around 40 mmHg), and the hand is excluded from the circulation by inflating the wrist cuff to supra-systolic pressures (200 mmHg). As the isolated forearm begins to swell, forearm volume, measured by a voltage dependent strain-gauge, increases in direct proportion to forearm blood flow. The hand is excluded because it's blood flow is highly temperature sensitive, and it contains a high proportion of arterio-venous shunts. A degree of hand ischaemia is inevitable, and limits the testing period to usually no more than 10 min. Both arms are usually studied at the same time, the contra lateral arm providing a contemporaneous control.[7] The increase in forearm volume is taken to represent blood flow in the resistance vessels of the forearm (muscle, soft tissues and skin).

VOP provides a convenient platform for testing forearm vascular resistance. In turn, vascular resistance can be readily manipulated by administering vasoactive drugs directly into the brachial artery using a small (27 G) cannula. Changes in vascular resistance reflect endothelial function. Investigating pharmacological effects in a closed circuit in this fashion has the advantage of avoiding systemic infusions of potentially dangerous drugs, and although there is a theoretical risk of critical forearm ischaemia when investigating vasoconstrictors, this is seldom seen in practice. Complications arising from repeated brachial arterial cannulation are rare,[8] nevertheless the procedure should be regarded as moderately invasive.

Over the years, VOP has proved itself to be a robust and reliable tool for the investigation of vascular function. Despite an image of being cumbersome and somewhat dated, the procedure is well tolerated and continues to be popular. Using intra-arterial infusions of acetylcholine and sodium nitroprusside, VOP has been used to associate endothelial dysfunction with a range of cardiovascular risk factors such as smoking, hypertension, diabetes and aging.[9–14] Most authors have found good reproducibility,[15,16] and some even regard the technique as the 'gold-standard' for the assessment of vascular function.[7,17]

Brachial Artery Flow-mediated Dilatation

Blood vessels have the capacity to adjust blood flow in response to luminal physical and chemical stimuli. This ability to self-regulate vasomotor tone allows the vessel to respond to changes in the local environment. An increase in blood flow will result in an increase in the 'shear stress' to which the local vascular endothelium is subjected and the vessel responds by dilating, a phenomenon called flow-mediated vasodilatation (FMD)[1,18–20] Using a high resolution ultrasound scanner, it is possible to monitor and record