

Assessment of Endothelial Reactivity by Measurement of Vascular Material Response to Shear Stress: A Feasibility Study*

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Abstract— Flow-mediated dilation (FMD) evaluates the relative change in arterial diameter during hyperemia to assess the endothelial response due to a shear stimulus. However, conventional FMD measures diameter response alone and the alterations in the arterial wall's material properties during reactive hyperemia, which also influence dilation, go unaddressed. In this work, we examine the material response (MR) of the artery during reactive hyperemia using clinically relevant stiffness markers for the assessment of endothelial reactivity (ER). For this, we have developed an in-house brachial cuff control (BCC) system to continuously acquire brachial pressure which can be integrated with simultaneous measurement of brachial diameter and used to quantify the relative changes in wall property during hyperemia non-invasively. The assessment of endothelial reactivity using material response (ERA_{MR}) was conducted on 20 healthy participants (12M/8F) and the results were compared with conventional FMD (FMD%). The mean pressure response gave an inverse trend to that of diameter response with varying magnitudes during reactive hyperemia (18.71% from baseline for diameter and 2.45% for pressure), there was a significant difference in the measurement of FMD and ERA_{MR} ($P < 0.05$). The larger distribution of ERA_{MR} compared to FMD% in box-plots further implies the inclusion of within-subject variations. Hence, ERA_{MR} can be a potential estimate of ER, given the need for intensive validations in this line on larger cohorts.

Clinical Relevance— This study demonstrates the independent role of arterial wall material properties to quantify endothelial reactivity in response to a shear stimulus.

I. INTRODUCTION

The blood vessel wall is a multilayered structure whose innermost layer is lined by vascular endothelial cells. The endothelial layer is essential for maintaining vascular homeostasis, specifically controls extracellular matrix synthesis, and regulates smooth muscle cell proliferation. Further, it helps in the modulation of arterial tone and coagulation state by releasing vasodilators like nitric oxide

(NO) [1][2] which would immediately diffuse and dominate arterial dilatation in the arterial wall. Endothelial reactivity (ER) refers to the response of endothelial cells to an input shear stimulus which manifests as functional changes in artery stiffness. Age-related impairments in endothelial structure and function impair ER. This is due to arterial wall remodelling during ageing and is expressed as structural changes in arterial stiffness, which is hastened by the presence of cardiovascular (CV) risk factors [3]. Endothelial dysfunction being a precursor to clinically observable vascular deformations suggests that early detection halts disease development through proper interventions [4].

Flow-mediated dilation (FMD) is a non-invasive method of assessing ER where the brachial artery is subjected to temporary ischemia via cuff inflation (placed distal to the elbow) for ~300 s. The baseline and recovery diameter measurements are taken at pre-inflation (~120 s) and post-deflation (~180 s) of the distal cuff. Following transient ischemia, downstream vascular resistance decreases significantly, resulting in a rise in shear-induced NO and vasodilation of the brachial artery when the cuff deflates [5]. The percentage flow-mediated dilation index (FMD%), which is defined as the relative change in artery diameter during hyperemia, has been commonly used to quantify FMD and is said to be a strong predictor of future CV events [6]. However, conventional FMD measures only the diameter response (DR) to shear stress and the changes in material properties (MP) of the artery which influence its dilation [7], [8] are unaccounted. This can be addressed by estimating the arterial material response during reactive hyperemia (RH) using simultaneous and continuous brachial BP and diameter measurements, which are inherently responsible for the actual dilation of the artery [9].

In this work, we have proposed a method for ER assessment using arterial material response (ERA_{MR}) during RH. We have measured the beat-to-beat blood pressure (BP) and diameter simultaneously to calculate the functional stiffness changes as a marker of material response to shear. An in-house brachial cuff control system (BCC) was developed to continuously acquire brachial pressure pulse in conjunction with B-mode ultrasound imaging for brachial artery diameter measurement. Simultaneous measurements of pressure and diameter of the brachial artery during RH were taken on 20 healthy participants, the data was processed and beat-to-beat measurements of arterial stiffness matrices were obtained. Further, the relative change in arterial stiffness

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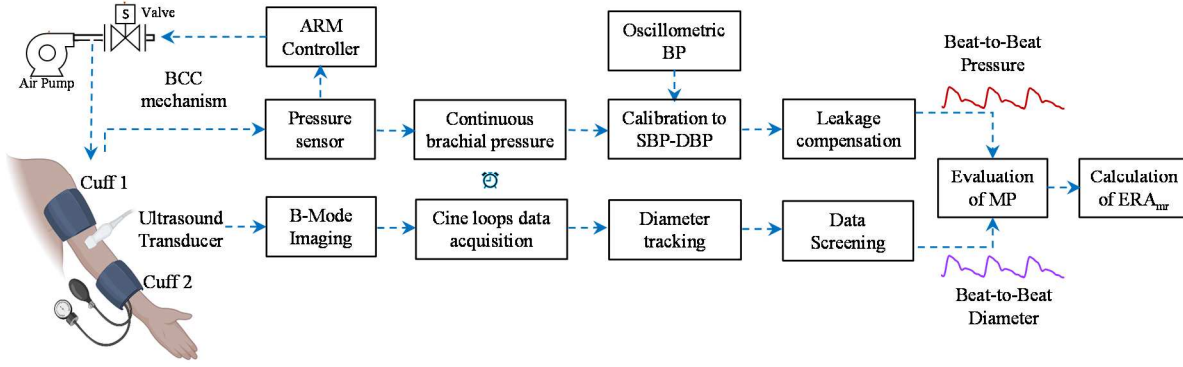


Fig 1. The architecture of the measurement system illustrating the algorithm for ERA_{mr} and the BCC mechanism.

indices during hyperemia was estimated and used to calculate the proposed ERA_{MR} index those accounts for the material response of the artery during RH. Further, ERA_{mr} was compared against FMD (or ERA_{DR}) to analyze the differences in measurements possibly due to the influence of pressure.

The details of the measurement principle, measuring system and the conducted study is presented in Section II. A detailed discussion of the results and observations is discussed in Section III, followed by limitations and future scope. The study conclusions are outlined in Section IV.

II. MATERIALS AND METHODS

A. Principle of Measurement

To assess the arterial material response to an input shear stress, beat-to-beat measurements of arterial stiffness matrices namely, specific stiffness index (β), pressure-strain elastic modulus (E_p), arterial compliance (AC), and local pulse wave velocity (PWV) using the Bramwell-Hill equation (refer [10] for equations) were required since they are inclusive of both pressure and material property in addition to diameter. This demands simultaneous measurement of pressure and diameter from the target artery. Continuous cuff-based pressure measurements were taken using the BCC system and diameter measurements using B-mode scanning of the brachial artery (Fig. 1). In the developed system, the set pressure in the brachial cuff (cuff 1) was maintained at sub-diastolic pressure using a cuff control mechanism to capture pulsations without intervening with the measurements. Timestamps were enabled at each setpoint (start of baseline, intervention and recovery) to guarantee synchronization with the diameter data. A distal cuff (Cuff 2) is used to induce ischemia.

The material properties (ϵ) were calculated from the pressure-diameter measurements. The extrema stiffness post ischemia ($\epsilon_{extrema}$) and baseline stiffness pre-ischemia ($\epsilon_{baseline}$) is identified to derive the ERA_{mr} index given by,

$$ERA_{MR} \% = \frac{\epsilon_{extrema} - \epsilon_{baseline}}{\epsilon_{baseline}} \times 100 \quad (1)$$

B. Continuous Diameter measurement

The diameter signals were obtained by longitudinal B-mode scanning of the brachial artery (Sonix Touch+, BK Medicals, US) with a high-resolution ultrasound probe (7-12 MHz). Endothelium-dependent vasodilation corresponding to

the diameter information was analyzed using continuous edge-detection and wall-tracking software (FMD Studio, Quipu, Netherlands). The data was screened and instantaneous diameter information was saved for analysis.

C. Continuous pressure measurement system

The overall system architecture and algorithm of the cuff control system is given in Fig. 1. The system consists of a 32-bit ARM microcontroller (LPC4370FET256, NXP Semiconductors), pressure sensor (Honeywell 24PCCFA6D), air pump (12V DC 380mA 500mmHg), solenoid valves, and a bladder cuff (cuff 1). The microcontroller uses a feedback control to maintain the cuff at sub-diastolic pressure. The LPC43XX's GPIO lines controls the driver circuitry to regulate the pump and solenoid valves for inflating and deflating the brachial cuff. The pressure sensor monitors cuff pressure, and the amplified pressure signals are digitized by the LPC4370's analog-to-digital converter (12-bit ADC). A dedicated power supply circuitry ensures all the required voltage levels for the device (± 5 V, ± 3.3 V) from a 9V DC source.

The digitized raw data by the microcontroller was transferred to a computer via USB using an application software created in LABVIEW 2019 (National Instruments, USA) and the uncalibrated pressure signals were saved for further offline analysis. The uncalibrated signals were calibrated to the reference systolic and diastolic BP (SBP, DBP) measured using Oscillometric technique (SunTech@247™, SunTech Medical, Halma, UK). Any leakage due to tear or wear of the cuff was compensated using an averaged leakage trendline observed at different set points with a minimum error (< 3 mmHg).

The instantaneous diameter information was used to calculate FMD% and the beat-to-beat pressure-diameter cycles during, baseline and RH, were used to estimate the material properties and $ERA_{MR}\%$.

D. Study Population

An *in-vivo* study was conducted on 20 healthy participants (aged 25 ± 4 years) for ER assessment by estimation of their brachial artery MR to shear stimulus. The study was conducted in a controlled laboratory setting at the Healthcare Technology Innovation Centre, Indian Institute of Technology Madras, India. Participant information such as

TABLE I. SUBJECT DEMOGRAPHY AND PARAMETER DESCRIPTION

Parameter	Value (Mean \pm SD)
Number of subjects	20
Male/Female	12/8
Age (years)	25 \pm 4
Systolic blood pressure (mmHg)	122.79 \pm 6.19
Diastolic blood pressure (mmHg)	72.60 \pm 6.77
Systolic-peak diameter (mm)	3.68 \pm 0.73
End-diastolic diameter (mm)	3.23 \pm 0.43
FMD%	15.82 \pm 9.044
β	6.65 \pm 3.41
Ep (kPa)	80.59 \pm 35.52
AC (mm ² .kPa ⁻¹)	0.0049 \pm 0.0020
Local PWV (m.s ⁻¹)	6.89 \pm 0.93

lifestyle, medical history etc. were collected prior to the study. People undergoing medications, with cardiometabolic disorders, and with a history of tobacco, smoking etc. were excluded from the study. The recommended guidelines [11] were taken into consideration while the study protocol was examined. The experiment data collection adhered with the principles of the Helsinki Declaration (revised in 2013) and was approved by the Institutional Ethics Committee, IIT Madras (IEC/2021-01/JJ/07). The study's goals and methods were explained and formal informed consent was collected from all participants.

E. Study Protocol and Measurement scheme

The protocols used to prepare the subjects were created based on expert guidelines [8]. Before the measurements, the individuals were assigned a slot and advised to fast for 6 hours. Additionally, participants had to refrain from coffee, alcohol, and vitamin supplements for 24 hours before their trials, as well as engage in strenuous physical activities. The study took place in a serene, temperature-controlled lab setting (23 \pm 2 $^{\circ}$ C). Before the examination, the participants were advised to rest for 10-15 minutes in a supine posture meanwhile the operator connected the cuff 1 for continuous blood pressure measurements. Cuff 2 was wound around the forearm, for inducing shear stimulus. Oscillometric BP measurements were measured before and after the exam.

The operator identifies the location of the brachial artery and captures B-mode images using an ultrasound machine with a linear probe (7.5 MHz) accounting for the tissue depth considerations. Baseline diameter is examined before cuff inflation for a period of 2 minutes. Meanwhile, continuous BP is recorded using the BCC system at the start of baseline measurements. At the end of baseline, cuff 2 is inflated to supra systolic (50 mmHg > SBP) pressure to induce ischemia for 5 minutes and released thereafter to measure the reactive hyperemia changes in blood pressure and diameter for 3 minutes. The data was recorded and saved for offline analysis.

E. Statistical Analysis

Continuous variables are presented as mean \pm standard deviations. Multiple means were compared using the F-test (ANOVA) and statistical significance of difference between two measurements using the students' t-test in terms of the p-value. A p-value less than 0.05 (α) is considered statistically

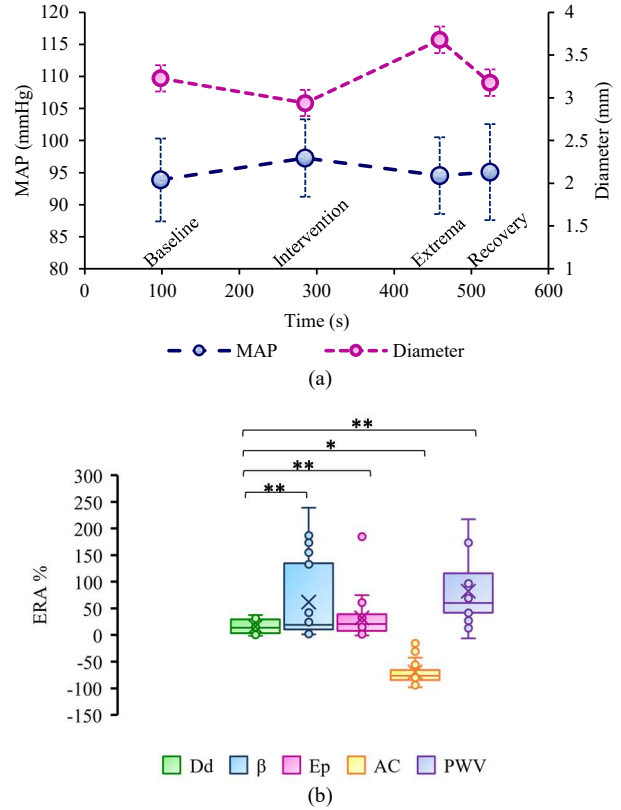


Fig 2. Plots for (a) pressure and diameter during baseline, intervention peak and recovery averaged for all participants and (b) Mean values of ERA_{MR} versus ERA_{DR}

significant. The grouping and variability of the measured parameters are displayed using box-and-whisker diagrams.

II. RESULTS AND DISCUSSION

The subjects' baseline demographics, FMD% and arterial stiffness estimates are detailed in TABLE I.

A. Response of blood pressure and diameter to shear stimulus

Fig 2. (a) depicts the response of mean arterial pressure (MAP) and diameter during baseline, intervention, extrema post intervention and recovery phases of measurements. The mean diameter during baseline was 3.23 \pm 0.43 mm which reduces 6.82% during ischemia and peaks to 18.71% during RH. Whereas, the mean pressure observed during baseline was 95.52 \pm 6.47 mmHg, it increased by 3.60% during ischemia and reduced 2.45% during RH. The mean pulse pressure during baseline was 39.78 \pm 3.17 mmHg and varied by < 1.42% during ischemia and RH. Both diameter and pressure recover to the baseline measures.

The pressure response to shear stimulus has an inverse trend during RH against that of diameter in the given sample population. This is analogous to previous observations reporting the dependance of vasodilation during RH with decreasing transmural pressure [5][12]. Our interest was to examine the influence of the pressure response to shear in ERA by measuring the corresponding changes in MR.

B. Arterial material response to shear stimulus

β , Ep, and PWV escalated by 126.11%, 117.60% and 44.05% respectively during RH corresponding to the observed 18.71% increase in diameter and 2.45% decrease in pressure. This is in line with the studies reporting direct association of pressure and diameter on artery stiffness [13][14]. Whereas, AC reduced by 15.26% which is evident from the inverse relation of compliance with pressure. The sensitivity of material property response during RH is higher compared to that of diameter. This is reflected in the group average values of ERA_{DR} versus ERA_{MR}. ERA_{DR} had a group average of $20.21 \pm 9.32\%$. Whereas, ERA_{MR} had a group average value of $155.98 \pm 103.64\%$ for β , $174.12 \pm 121.56\%$ for Ep, $-48.82 \pm 20.99\%$ for AC and $57.09 \pm 31.63\%$ for PWV. There exists a significant difference ($P < \alpha$) between the means of MR and DR.

The box-whisker plot in Fig. 2 (b) shows the difference in measurement of ERA_{DR} with ERA_{MR}. As evident from the plot, both the measurements have a significant difference ($P < \alpha$) which means the behavior or response of material properties to shear stress is not identical to that of diameter alone. Hence, detailed analytical methods needs to be developed and extensive validations implemented to analyze the individual values of ERA_{MR} versus ERA_{DR}. Such an analysis may prove the reliability of ERA_{MR} over FMD, and then ERA_{MR} can be a potential marker to quantify ER in CV risk assessment. Similar studies have also recommended future researches that relate functional stiffness with endothelial dysfunctioning [15], [16]. Studies measuring simultaneous pressure and diameter are in line [17] but none have measured the continuous brachial BP throughout the intervention. Multimodal image-free systems for simultaneous measurements of pressure [18], diameter [19] and stiffness estimates [20] can further improve the measurements to meet clinical standards.

C. Limitations and Future Scope

Reported observations should be interpreted by underlining the study limitations, majorly the use of a smaller sample size of 20 participants. However, data was obtained under controlled trials and can be expanded for future research to confirm the proposed assumptions. Thus, the reported observations are reliable and corroborate with allied in-vivo studies to a large extent. Further efforts are in progress to extend this study into a clinical setting by recruiting large human cohorts and to develop models that relate arterial stiffness to ER. Above all, there is a lack of an instrumentation that can perform a combined estimation of the transmural pressure, luminal diameter, and blood flow velocity from an arterial site for accurate and synchronized measurement of said indices. Such devices and comprehensive models that can provide reliable estimates of ER are underway.

IV. CONCLUSION

This work investigates the response of arterial stiffness to an input shear stimulus. 20 healthy participants were recruited for the study and expert FMD guidelines were followed. Similar to the difference in the distribution of material

properties to diameter during RH, a significant difference was observed between the arterial stiffness response to shear stress and FMD % which affirms the impact of change in material properties during endothelial dependent vasoconstriction. A larger study may further confirm the potential of arterial stiffness-dependent vasodilation due to shear using simultaneous pressure-diameter cycles. Efforts in this line are in progress to realize methods and systems employing models that relate stiffness to ER in affordable, non-invasive devices.

REFERENCES

- [1] T. A. Alam *et al.*, "A Review of Methods Currently Used for Assessment of In vivo Endothelial Function," vol. 276, pp. 269–276, 2005.
- [2] Z. Ungvari *et al.*, "Endothelial dysfunction and angiogenesis impairment in the ageing vasculature," *Nat. Rev. Cardiol.*, vol. 15, no. 9, pp. 555–565, 2018.
- [3] K. Abboud *et al.*, "Temporal changes in vascular reactivity in early diabetes mellitus in rats: Role of changes in endothelial factors and in phosphodiesterase activity," *Am. J. Physiol. - Hear. Circ. Physiol.*, vol. 297, no. 2, 2009.
- [4] J. Swampillai *et al.*, "Clinical assessment of endothelial function – an update," pp. 1–5, 2005.
- [5] J. Tremblay and K. Pyke, "Flow-mediated dilation stimulated by sustained increases in shear stress: a useful tool for assessing endothelial function in humans?," *Am. J. Physiol. Heart Circ. Physiol.*, vol. 314 3, pp. H508–H520, 2017.
- [6] D. J. Green *et al.*, "Flow-Mediated Dilation and Cardiovascular Event Prediction Does Nitric Oxide Matter?," 2011.
- [7] D. Thijssen *et al.*, "Assessment of flow mediated dilation (fmd) in humans : a methodological and physiological guideline," *Am J Physiol Heart Circ Physiol*, 300(1), H2-H12, 2011
- [8] D. Thijssen *et al.*, "Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans.," *Eur. Heart J.*, 2019.
- [9] Jin W *et al.*, "An in silico simulation of flow-mediated dilation reveals that blood pressure and other factors may influence the response independent of endothelial function. *Am J Physiol Heart Circ Physiol*, 318(5), H1337-H1345, 2020.
- [10] J. Joseph *et al.*, "Assessment of Carotid Arterial Stiffness in Community Settings with ARTSENS®," *IEEE J. Transl. Eng. Heal. Med.*, vol. 9, no. September 2021.
- [11] A. Greyling *et al.*, "Adherence to guidelines strongly improves reproducibility of brachial artery flow-mediated dilation," *Atherosclerosis*, vol. 248, pp. 196–202, 2016.
- [12] B. Jiang *et al.*, "Flow-mediated dilation of the radial artery is offset by flow-induced reduction in transmural pressure," *Hypertension*, vol. 57, no. 6, pp. 1145–1150, 2011.
- [13] L. V. Van Bortel *et al.*, "Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity.," *J. Hypertens.*, vol. 303, pp. 445–448, 2012.
- [14] P. M. Nabeel *et al.*, "Arterial compliance probe for local blood pulse wave velocity measurement," no. 1, pp. 5712–5715, 2015.
- [15] D. A. Duprez, "Arterial Stiffness and Endothelial Function Key Players in Vascular Health," pp. 612–613.
- [16] B. Sidnawi *et al.*, "Characterization of arterial flow mediated dilation via a physics-based model," *J. Mech. Behav. Biomed. Mater.*, vol. 107, no. February, p. 103756, 2020.
- [17] T. Ma *et al.*, "Flow-mediated dilation analysis coupled with nitric oxide transport to enhance the assessment of endothelial function," *J. Appl. Physiol.*, vol. 131, no. 1, pp. 1–14, 2021.
- [18] J. Joseph *et al.*, "Arterial compliance probe for cuffless evaluation of carotid pulse pressure," pp. 1–19, 2018.
- [19] A. K. Sahani *et al.*, "Automatic measurement of end-diastolic arterial lumen diameter in ARTSENS," *J. Med. Devices, Trans. ASME*, vol. 9, no. 4, pp. 1–11, 2015.
- [20] V. Raj Kiran *et al.*, "Evaluation of Local Pulse Wave Velocity using an Image Free Ultrasound Technique," *IEEE Int. Symp. Med. Meas. Appl. Proc.*, pp. 1–6, 2018.