African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)
www.abjournals.org

ETHNIC MINORITY MICROPARTICLES HAVE DISTINCT PRO-THROMBOTIC AND PRO-OXIDATIVE PHENOTYPES AND INTERACT DIFFERENTIALLY WITH ENDOTHELIAL CELLS *IN VITRO*: IMPLICATIONS FOR RISK TO CARDIOVASCULAR DISEASE

Christopher J. Pritchard[1], George A. Lacey[2], Amon B. Hackney[1], Michelle Henshaw[1], Alicia J. Kulbicki[1], Manveer S. Saund[2], Alexius Akubueze[1], Anwar R. Baydoun[1] and Naomi Martin[1]

[1]Faculty of Health and Life Sciences, De Montfort University, Leicester, LE1 9BH, UK

[2]Department of Respiratory Sciences, University of Leicester, Leicester, LE1 7RH, UK

Corresponding E-mail: naomi.martin@nottingham.ac.uk

Cite this article:

Pritchard C.J., Lacey G.A., Hackney A.B., Henshaw M., Kulbicki A.J., Saund M.S., Akubueze A., Baydoun A.R., Martin N. (2023), Ethnic Minority Microparticles have Distinct Pro-Thrombotic and Pro-Oxidative Phenotypes and Interact Differentially with Endothelial Cells in vitro: Implications for Risk to Cardiovascular Disease. African Journal of Biology and Medical Research 6(2), 43-70. DOI: 10.52589/AJBMR-YTFDLV0G

Manuscript History

Received: 19 April 2023

Accepted: 8 June 2023

Published: 4 July 2023

ABSTRACT: *Ethnic minority individuals are disproportionately susceptible to endothelial dysfunction and cardiovascular disease (CVD). Microparticles (MP) are biologically active membrane-bound nanovesicles released from cells that act as biomolecular shuttles. Plasma MP was isolated from healthy White, Black African, and South Asian individuals and analysed using flow cytometry. Their effects and interactions were assessed using fluorescence, confocal, and scanning electron microscopy. Total MP and a sub-population of smaller MP associated with dysfunction and disease progression were significantly increased in Black African individuals. Pro-thrombotic and pro-oxidant MP were substantially more numerous in Black African individuals. The tissue factor activity of ethnic minority MP was significantly greater than White MP. Ethnic minority MP induced significantly greater functional changes and morphology to an endothelial cell line in vitro and integrated into endothelial cells noticeably more than White MP. These data imply distinct differences in ethnic minority MP, suggesting a role in CVD susceptibility.*

KEYWORDS: Ethnic Minority; Cardiovascular Disease; Microparticle; Thrombosis; Endothelial Dysfunction

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)

www.abjournals.org

INTRODUCTION

Black African and South Asian ethnic minority individuals are disproportionately susceptible to cardiovascular disease (CVD) associated with endothelial dysfunction (Khunti et al., 2013). Still, the cellular and molecular reasons for this are unclear. Ethnic minority individuals present with premature and more severe and aggressive CVD with higher mortality rates, which is not explained by traditional risk factors (Thorpe et al., 2016; Palaniappan et al., 2018). These individuals may have a genetic predisposition to CVD, but studies are compounded by the high degree of genetic diversity and the spectrum of vascular diseases (Mensah et al., 2019). It is also possible that different mechanisms lead to their susceptibility to CVD. In this regard, Black African and South Asian individuals have an increased chronic inflammatory profile with increased endothelial oxidative stress and dysfunction (Feairheller et al., 2011). Ethnic minorities also have an enhanced pro-thrombotic state and increased incidences of venous thromboembolism (Zakai et al., 2011). Differences in biomarkers affecting coagulation mechanisms and haemostasis might be important.

Microparticles (MP) are biologically active nanovesicles shed from cells in response to activation, inflammation, apoptosis, and stress (van Niel et al., 2018). They act as biomolecular vehicles, transporting effector molecules to distant sites, affecting recipient cell function. MP originates mostly from platelets and other cell types, including leukocytes and endothelial cells. They are composed of the surface proteins and the cytoplasmic contents of their parent cell. MP is involved in inflammatory processes, disease pathophysiology, and coagulation. The prevailing view is that MP is harmful and contributes to an individual's disease risk due to the effector molecules they transport and deliver to cells systemically (Viera et al., 2012). Their circulating levels are often elevated in CVD associated with endothelial dysfunction (França et al., 2014). However, their role in predisposing ethnic minorities to CVD is unclear, and there are limited publications on differences in circulating levels or on the pathological nature of MP between healthy White, Black African, and South Asian individuals. Studies in patients with systolic heart failure have, however, reported higher circulating levels of MP in South Asian compared to White patients (Agouni et al., 2019), and human umbilical vein endothelial cells (HUVECs) derived from Black individuals released significantly more endothelial-derived MP following treatment with tumour necrosis factor-α when compared to HUVECs from white donors (Brown et al., 2011). These studies did not investigate ethnic differences in healthy individuals or the function of MP produced, which is critical in determining the pathological predisposition of ethnic minorities to CVD as a consequence of elevated MP.

There are several mechanisms via which MP might contribute to the pathogenesis of CVD, including the transportation of effector molecules such as phosphatidylserine (PS) and tissue factor (TF, CD142), which are involved in the regulation of haemostasis and thrombosis (Antonova et al., 2019). Exposure to PS may advance vascular dysfunction and decrypt non-functional cryptic TF into its pro-coagulant-active form via calcium influx (Darabi et al., 2016). This exposure is promoted by reactive oxygen species (ROS) released from endothelial cells, which affects vascular homeostasis and the regulation of effector cells (Panth et al., 2016). MP also possesses NADPH oxidases and generates ROS, which may be an additional mechanism to affect endothelial dysfunction in CVD (Brodsky et al., 2004). Thus, MP and their actions may contribute to the disproportionate susceptibility of healthy ethnic minority groups to CVD, and studies that would unequivocally establish the profile of expression, pro-thrombotic and pro-oxidative phenotype, and actions of MP between ethnic groups are therefore required as these do not currently exist in the literature.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

In this study, we hypothesize that the pro-thrombotic phenotype of circulating MP and their interactions with endothelial cells is enhanced in healthy Black African and South Asian individuals and might represent a mechanism of CVD susceptibility and pathogenesis. The aim of this study, therefore, was to assess the thrombotic and oxidative phenotype of ethnic minority MP and to evaluate their interactions with endothelial cells in order to investigate their potential role in CVD and endothelial dysfunction in ethnic minority individuals.

LITERATURE/THEORETICAL UNDERPINNING

Black African and South Asian ethnic minority individuals have a higher risk of diseases associated with endothelial dysfunction but the reasons for this are not clear (Khunti et al., 2013; Thorpe et al., 2016; Palaniappan et al., 2018; Mensah et al., 2019). These individuals have an increased pro-thrombotic and pro-inflammatory state and higher rates of venous thromboembolism and CVD (Zakai et al., 2011). Variations in biomarkers that are involved in coagulation mechanisms and haemostasis could contribute to these differences.

MP have crucial roles in mediating intercellular communication and delivering effector molecules systemically and may contribute to the development of CVD via the transportation of pro-thrombotic molecules, playing important roles in processes such as haemostasis and thrombosis, contributing to CVD pathogenesis and risk (Antonova et al., 2019). Studies of the MP of healthy individuals could help in the understanding of the disproportionate endothelial dysfunction disparities in ethnic minorities.

We hypothesise that healthy Black African and South Asian individuals have elevated circulating MP counts, a pro-thrombotic phenotype and that their MP have enhanced interactions with endothelial cells *in vitro* and have a potential role in CVD in these individuals.

METHODOLOGY

Participants

White study participants had a median (range) age of 27 (20-48) years (55% male, 45% female). Black African study participants had an age of 24 (20-59) years (73% male, 27% female), and South Asian study participants had an age of 30 (22-47) years (50% male, 50% female). There were no significant differences between the ages of the ethnic groups (ns, P=0.875). White study participants had a median (range) BMI of 23.2 (21.0-29.6) kg/m^2. Black African study participants had a BMI of 22.7 (21.8-40.0) kg/m^{2,} and South Asian study participants had a BMI of 24.4 (18.5-35.5) kg/m^2. There were no significant differences between the BMIs of the ethnic groups (ns, P=0.655) and no correlations between BMIs and MP counts (R^2 values: White 0.031, Black African 0.0004, South Asian 0.030). Participants analysed for each study are detailed in Appendix 1.

Inclusion criteria were age 20-60, body mass index >18.5 kg/m^2, and participants identifying as White, Black African, or South Asian ethnicity. As these were healthy individuals with no reported or diagnosed illnesses, no clinical characteristics such as renal, liver, or cardiac function data, inflammation, or coagulation biomarkers were collected. Exclusion criteria

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

included pregnancy or breastfeeding, immunological conditions, diabetes mellitus, smokers, acute disease or infection, cardiovascular disease, or any conditions known to increase circulating MP counts.

Phlebotomy and microparticle isolation

Blood was drawn using a dry syringe with a 21-gauge needle and then discarded; further blood drawn was immediately aliquoted into a sodium citrate pre-treated monovette (Sarstedt AG, Nümbrecht, Germany) as recommended (Kailashiya et al., 2018). Blood was mixed with anticoagulant by gentle inversion, transported carefully, kept at room temperature (RT), and processed within 2 hrs of collection. Plasma samples were anonymised, so researchers were unaware of the ethnicity of the samples. All plasma samples were collected, processed, stained, acquired, and analysed similarly. Platelet-poor plasma was prepared from blood samples following two centrifugation steps of 2500 x g for 20 min at RT and then stored in aliquots at -80˚C until use. MP in cell-free plasma was prepared by serial centrifugation according to Biró *et al* (2004). Briefly, platelet-poor plasma was centrifuged at 18000 x g for 30 min at RT then 90% of the supernatant was replaced with the same volume of MP buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4; 0.2 µm-filtered), this was repeated to produce platelet-free plasma, i.e., isolated MP. In order to confirm that the MP prepared were closed and not cellular fragments, MP was centrifuged at 18000 x g for 30 min at RT then incubated with CellTracker™ Red at a final concentration of 1 µM in serum-free media for 45 min at 37˚C and then washed. An aliquot was resuspended in MP buffer and analysed using flow cytometry.

Flow Cytometry

Aliquots of MP were incubated either alone with MP buffer or with Annexin V labelled with fluorescein isothiocyanate (FITC) as a marker of PS expression or with mouse anti-human phycoerythrin (PE)-labelled antibody to tissue factor CD142 (all BD Biosciences, Oxford, UK). All antibody incubations were carried out in the dark for 25 min at RT and then samples were diluted with MP buffer before acquisition. All flow cytometric acquisition was done using a BD Accuri™ C6 Plus and all analysis was carried out using BD Accuri™ C6 software (both BD Biosciences). MP was acquired using a gating defined by size-calibrated fluorescent Megamix™ beads according to the manufacturer's instructions (BioCytex, Marseille, France) and according to a standardized calibrated-bead strategy (Robert et al., 2009). All samples were acquired using the slowest flow rate for a fixed period of 120 seconds with an SSC threshold of 10,000. To measure ROS, MP was incubated with or without 2',7'-Dichlorofluorescin diacetate (DCFH-DA) at a final concentration of 5 µM for 15 min at 37°C, diluted with 300 µl MP buffer and then analysed using flow cytometry.

Tissue Factor Activity assay

MP were isolated as above. An aliquot was placed in the MP buffer for counting using flow cytometry so that TF activity could be reported relative to the number of MP conferring activity. The tissue factor activity of MP was assayed as the ability of TF to activate factor X to factor Xa was measured over a period of 25 min according to the manufacturer's instructions (Abcam, Cambridge, UK). The amount of factor Xa produced was quantitated using a specific factor Xa substrate to release yellow para-nitroanaline chromophore. The change in absorbance at 405 nm, which was directly proportional to TF activity, was measured. TF activity was reported as total TF activity and also as activity per 10^6 MP.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)

www.abjournals.org

Effects of the incubation of microparticles with endothelial cells *in vitro*

EA.hy926 endothelial cell lines from ATCC (ATCC Cat# CRL2922, RRID: CVCL_3901) (Manassas, Virginia, USA) were maintained according to the manufacturer's instructions; they were cultured in cDMEM (complete Dulbecco's Modified Eagle Medium) supplemented with 10% (v/v) heat-inactivated foetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin (all Fisher Scientific, Loughborough, UK) and maintained at 37˚C in a humidified 5% CO_2 atmosphere. Endothelial cells were seeded at $6x10^4$/well in a 96-well plate and left overnight. MP was prepared as above; an aliquot was taken into the MP buffer for flow cytometric counting. Media was removed from the wells and replaced with 180 µl fresh media plus 20 µl of MP; these were then incubated for 24 hours. The following day, supernatants were removed, and cells were rinsed with phosphate-buffered saline (PBS; 0.2 µm-filtered) and dissociated using 0.25% Trypsin-EDTA (Fisher Scientific). Media was added to inactivate Trypsin, then cells were washed in PBS at 200 g for 5 min and resuspended in PBS. Aliquots of cells were stained with mouse anti-human vWF antibody directly conjugated to FITC or mouse anti-human CD142 (TF) antibody directly conjugated to PE (both BD Biosciences), for 25 min at RT, and then samples were diluted with PBS prior to acquisition.

Imaging interactions of microparticles with endothelial cells *in vitro* using scanning electron microscopy

EA.hy926 endothelial cells were seeded onto sterile 13 mm round glass coverslips (Fisher Scientific) at $1.5x10^4$/well in a 24-well plate and left overnight. The media was removed and replaced with 460 µl fresh media plus 40 µl of MP; these were then incubated for 24 hours. Treated cells were fixed in 4% paraformaldehyde (Sigma, Gillingham, UK) and dehydrated through an alcohol concentration series before being dried using hexamethyldisilazane (Hazrin-Chong et al., 2012) (Sigma) in ethanol. The cells were then sputtered with 2 nm gold and imaged on a 60° tilted stage. Images were acquired using a Carl Zeiss EVO LS 15 scanning electron microscope (Carl Zeiss Ltd, Cambridge, UK) under high vacuum, 10 kV accelerating beam voltage, 20 µm beam aperture, and 100 pA probe current.

Imaging interactions of microparticles with endothelial cells *in vitro* using fluorescence microscopy

EA.hy926 endothelial cells were seeded at $6x10^4$/well in cDMEM in a 96-well plate and incubated overnight. The fluorescent membrane integrator Calcein (Fisher Scientific) was then used to label MP to identify their interactions with endothelial cells fluorescently. MP was incubated with Calcein at a final concentration of 50 µM and incubated at 37˚C in a humidified 5% CO_2 atmosphere for 30 min, as Gray *et al.* (2015) described. After staining, wash with MP buffer to remove any non-integrated Calcein. An aliquot of stained MP was taken into MP buffer to confirm fluorescence using flow cytometry. Media was removed from the endothelial cells and replaced with 180 µl fresh media plus 20 µl of Calcein-stained MP; these were then incubated for 24 hours. The media supernatant was carefully removed, and PBS was added. Fluorescent microscopic images were taken using EVOS cell imaging (Fisher Scientific) with transmission and GFP lenses. After imaging, cells were harvested as described above in section 4.5, then spun at 200 g for 5 min and resuspended in PBS. Aliquots of cells were acquired using flow cytometry and analysed for fluorescence to indicate interactions with MP.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)

www.abjournals.org

Imaging interactions of microparticles with endothelial cells *in vitro* using confocal microscopy

EA.hy926 endothelial cells were incubated with CellTracker™ Green at a final concentration of 5 µM in serum-free media for 45 min at 37˚C with agitation, then centrifuged at 200 g for 5 min and resuspended in cDMEM before seeding at 4.2x 10^6/well in a 12-well plate as in described in section 4.6 and incubated overnight. MP was subsequently incubated with CellTracker™ Red, as described in the section. The media was removed from the endothelial cells and replaced with 660 µl fresh media plus 340 µl of stained MP, which were then incubated for 24 hours. The media was removed, and the cells were rinsed gently with PBS and incubated with Hoechst 33342 for 20 min at 37˚C in the dark, then rinsed again. For image acquisition, 50 µl PBS was dropped onto borosilicate glass slides (VWR International, PA, USA), and the glass coverslips were removed from the wells using flat-tipped tweezers and inverted on to the PBS to prevent the sample from drying out. Samples were imaged using a Zeiss LSM 900 confocal microscope with Airyscan 2 and Colibri 7 using x63 oil immersion objective (Plan-Apochromat 63x/1.4 Oil DIC M27). Images were acquired using Zeiss Zen Blue software (Carl Zeiss, Jena, Germany). Z-stacks were taken sectioning the cells' height, acquired with 1 µm steps, and imaged with 1024 x 1024 pixel scanning.

Statistical Analysis

Results were compared using a Kruskal-Wallis one-way analysis of variance followed by post hoc Dunn's multiple comparison tests to determine significant differences between ethnic groups. The time course was analysed using repeated measures two-way analysis of variance with Geisser-Greenhouse correction, post-hoc Tukey's multiple comparison test. Data is presented as median (range). Statistical significance was accepted at $P<0.05$. Statistical analysis was performed using Prism™ software (Graphpad 9.5.0 (730), California, USA).

RESULTS/FINDINGS

Circulating microparticle count and size

MP were confirmed to be closed spherical bodies when stained with the fluorescent dye CellTracker™ Red which freely enters into and is retained by cells or membrane-bound bodies. There was no significant difference between the number of unstained MP acquired compared to CellTracker™ Red stained MP with all ethnicities (all ns, P=0.109, data not shown).

There were significantly fewer circulating MP in healthy White individuals (2.43 (1.42-5.29)x10^6/ml) compared to healthy Black Africans (4.52 (2.24-11.06)x10^6/ml) ($P<0.03$), but South Asians showed no significant difference in circulating MP when compared to either White or Black African individuals (3.67 (2.66-5.52) x10^6/ml) (both ns, P=0.749 and P=0.642 respectively) (Figure 1).

Interestingly, two distinct sizes of MP populations were observed in all EV samples: larger (HiFSC (high forward scatter)) and smaller (LoFSC (low forward scatter)) (Figure 2a). Latex beads of different sizes were acquired using flow cytometry to indicate the approximate size of larger HiFSC and smaller LoFSC MP gating (Figure 2b). There were fold-increases of 1.6 and 2 in the number of smaller LoFSC MP of approximately 500 nm diameter in both Black African

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

and South Asian ethnic minorities, respectively, when compared to White individuals (Figure 2c). The number of smaller LoFSC MP was significantly fewer in healthy White individuals (1.56 (0.36-3.05)x10^6/ml) compared to those in Black African individuals (2.43 (1.45-8.68) x10^6/ml) (P<0.04). However, neither was significantly different from those from South Asians (3.13 (1.56-8.18) x10^6/ml) (White vs. South Asian (ns, P=0.091), Black African vs. South Asian (ns, P=0.999)). The number of circulating larger HiFSC MP of approximately 600 nm from White individuals (0.68 (0.04-3.06)x10^6/ml) were a third of the levels in Black Africans (2.02 (0.18-4.50)x10^6/ml) and almost half of those in South Asians (1.58 (0.23-2.67)x10^6/ml). None of these differences were significant (all ns, White vs. Black African (ns, P=0.380), White vs. South Asian (ns, P=0.999), Black African vs. South Asian (ns, P=0.999)) (Figure 2d).

Pro-thrombotic phenotype and activity of microparticles

The pro-thrombotic phenotype of MP were investigated to assess their role as CVD risk factors. MP was stained with antibodies against PS and TF and analysed using flow cytometry. Data is presented as the median (range) number of circulating MP. The pro-thrombotic PS-expressing MP in Black African and South Asian individuals were respectively almost three (0.26 (0.13-0.69)x10^6/ml; P<0.03), and two times (0.19 (0.07-0.33) x10^6/ml) higher compared to those from White individuals (0.09 (0.01-0.33)x10^6/ml). There was no statistical difference between South Asian individuals and White (ns, P=0.449) or Black African individuals (ns, P=0.634)) (Figure 3a).

The number of circulating pro-thrombotic TF-expressing MP was similar in White (0.26 (0.11-0.59) x10^6/ml), Black African (0.17 (0.08-0.32) x10^6/ml) and South Asian individuals (0.19 (0.04-0.31) x10^6/ml) and showed no significant difference between groups(White vs. Black African (ns, P=0.474), White vs. South Asian (ns, P=0.808), Black African vs. South Asian (ns, P=0.999)) (Figure 3b).

Tissue factor activity of White, Black African, and South Asian MP, assayed as the ability of TF to activate factor X to factor Xa, was measured over a period of 25 min. There was no difference in the total TF activity of MP between ethnicities (ns, P=0.632). However, the TF activity of MP from White individuals increased marginally but not significantly over the 25 min incubation period (5 min 28704 (23426-32580) pM vs. 25 min 30222 (23923-33928) pM) (ns, P=0.055). By comparison, there were significant increases in the TF activity of both Black African (5 min 29204 (23164-35582) pM vs. 25 min 31019 (25609-34479) pM) (P<0.007) and South Asian (5 min 29545 (25167-34599) pM vs. 25 min 31384 (27995-34692) pM) (P<0.0005) MP over this period (Figure 4a).

Further, an aliquot of MP counted using flow cytometry to determine TF activity relative to the number of MP conferring activity revealed no significant differences in the TF activity per 10^6 MP from White individuals over 25 min (5 min 1256 (349-1742) pM/10^6 MP vs. 25 min 1308 (375-1773) pM/10^6 MP) (ns, P=0.161). Interestingly, the difference in TF activity of the ethnic minority MP was retained. There were small but significant increases in the TF activity per 10^6 MP of both Black African (5 min 1148 (728-2296) pM/10^6 MP vs 25 min 1194 (798-2215) pM/10^6 MP) (P<0.02) and South Asian (5 min 1176 (663-1769) pM/10^6 MP vs 25 min 1260 (726-1774) pM/10^6 MP) (P<0.0007) individuals over this period (Figure 4b).

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

Pro-oxidative microparticles

The pro-oxidative nature of MP was investigated using 2',7'-Dichlorofluorescin diacetate (DCFH-DA), which is a cell-permeant fluorogenic dye that becomes fluorescent upon interactions with ROS. Data are presented as median (range) RFU, DCFH-DA signal calculated as RFU with DCFH-DA minus RFU without DCFH-DA. Circulating MP from White individuals contained significantly reduced ROS (55.28 (18.08-175.30) RFU) compared to MP from Black African individuals (208.90 (148.80-267.70) RFU) ($P<0.007$). Although the ROS content of MP from South Asian individuals (193.30 (87.62-218.90) RFU) was more than three times greater than that of MP from White individuals, this difference was not statistically significant (ns, $P=0.082$) nor different from that of Black African individuals (ns, $P=0.079$) (Table 1).

Effects of ethnic microparticles on endothelial cell function *in vitro*

The expression of TF by endothelial cells incubated with MP from White individuals (230.1 (225.1-290.5) RFU) was significantly less than those incubated with MP from either Black African (340.5 (318.3-407.1) RFU) ($P<0.04$) or South Asian (361.2 (318.3-509.2) RFU) ($P<0.02$) individuals. There was no significant difference in TF expression by endothelial cells incubated with Black African or South Asian MP (ns, $P=0.999$) (Figure 5).

Endothelial cells incubated in the absence of MP were used to determine the gating of positive staining (Figure 6a). A single population of vWF-expressing cells was present in the majority (3/5 samples) of endothelial cells incubated with MP from White individuals (Figure 6b). There were also two distinct populations of vWF-stained endothelial cells, but seen predominantly (7/10 samples) in those which had been incubated with MP from either Black African (Figure 6c) or South Asian (Figure 6d) individuals. The expression of vWF on endothelial cells incubated with ethnic minority MP was three times greater than that of cells incubated with MP from White individuals. These differences were not significant, and vWF expression was similar in endothelial cells incubated with MP from either White (7005 (3689-46013) RFU), Black African (21762 (4326-65744) RFU) or South Asian individuals (21112 (6780-56005) RFU) (all ns, White vs. Black African (ns, $P=0.999$), White vs. South Asian (ns, $P=0.999$), Black African vs. South Asian (ns, $P=0.999$)) (Figure 6e).

The effects of ethnic minority MP on the gross morphology of endothelial cells were investigated using scanning electron microscopy (SEM). Endothelial cells incubated with media alone (Figure 7a) had a similar morphology to those incubated with MP from White individuals (Figure 7b). However, those incubated with either Black African (Figure 7c) or South Asian (Figure 7d) MP had uneven surface morphology, membrane ruffling, and indications of MP adherence and cell interactions.

Interactions of ethnic microparticles with endothelial cells *in vitro*

To assess interactions between MP and endothelial cells, EA.hy926 cells were incubated for 24 hrs with Calcein-treated MP from White and ethnic minority individuals and subsequently imaged using fluorescence microscopy.

The integration of Calcein into MP was confirmed using flow cytometry, whereby unstained MP (Figure 8a) showed no fluorescent signal compared to Calcein fluorescence in stained MP (Figure 8b). No fluorescent signal was observed by fluorescence microscopy in cells incubated

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

with media alone (Figure 8c). Fluorescent MP from White individuals had limited interactions with endothelial cells (Figure 8d). In contrast, the fluorescent MP from both Black African (Figure 8e) and South Asian (Figure 8f) individuals appeared to interact significantly with endothelial cells.

The treated cells were dissociated, harvested, washed , and analysed using flow cytometry to confirm interactions between MP and endothelial cells. The percentage of fluorescent endothelial cells following incubation with MP from White individuals (8.40 (7.85-9.63)%) was not significantly different from those which had been incubated with MP from Black African (10.43 (9.06-14.30)%) or South Asian (12.42 (10.13-14.24)%) individuals (both ns: White vs. Black African (ns, P=0.143), White vs. South Asian (ns, P=0.999)). The percentage of fluorescent endothelial cells incubated with MP from South Asian individuals was, however, 1.5 times greater than the fluorescent cells incubated with MP from White individuals (P<0.03) (Table 2).

The interaction of MP and their potential integration into endothelial cells was also assessed using cells labelled with CellTracker™ Green fluorescent membrane-integrator and MP from White and ethnic minority individuals stained with CellTracker™ Red fluorescent membrane-integrator. No integration of White MP (Figure 9a) was observed, but Black African (Figure 9b) and South Asian (Figure 9c) MP were observed inside endothelial cells.

DISCUSSION

This is the first study to directly compare the circulating count and pro-thrombotic phenotype of MP from healthy White, Black African, and South Asian individuals and to compare their interactions with endothelial cells *in vitro*. We have demonstrated significant increases in the circulating count, the pro-thrombotic and pro-oxidative phenotype in Black African MP, which were not seen in MP from South Asian individuals. We have also demonstrated increased levels of PS and TFA in Black African MP, which may indicate enhanced pro-thrombotic activity. Although this was not confirmed by direct assessment of the biological prothrombinase activity of PS-MP, it is recognised that PS is a critical mediator of thrombosis, and its inhibition inhibits MP thrombin generation (Zlamal et al., 2022; Tripisciano et al., 2017). It is, therefore, reasonable to speculate from our findings that Black African MP may be highly thrombogenic.

We have also demonstrated enhanced effects on cellular dysfunction and interactions with endothelial cells, including morphological changes caused by both Black African and South Asian MP. While our study has limitations in the small cohort size in some experiments, it represents highly novel and interesting observations that may suggest a mechanism for the unusual susceptibility of ethnic minority individuals to endothelial dysfunction in CVD. It requires further investigation using larger study groups.

We observed a significant increase in the circulating MP from healthy Black African compared to healthy White individuals. As MP is considered an independent risk factor for thromboembolic events (Viera et al., 2012), their increase might be a consequence of the increased oxidative profile and endothelial oxidative stress seen in Black African individuals (Feairheller et al., 2011). In contrast, MP from South Asian individuals, although more numerous than seen in White individuals, did not show statistical significance.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

Other studies (Agouni et al., 2019; Kretzschmar et al., 2014; Shantsila et al., 2011; Khan et al., 2017) have reported on the circulating levels of MP in ethnic minority individuals, but these were either in isolation or in participants with co-morbidities who were not 'healthy.' None looked at the comparative profiles of MP in healthy individuals amongst different ethnic groups to determine their predisposition to CVD. Three other reports (Freeman et al., 2018; Eitan et al., 2017; Noren Hooten et al., 2019) have compared healthy Black African and White individuals and found no differences in MP using nanoparticle tracking analysis (NTA) which has been reported to be lacking in detection sensitivity (Maas et al., 2015) and may have failed to identify distinct populations of MP. Cell-specific markers and differences in size presented by Freeman *et al.* (2018) did not differentiate between ethnicities, highlighting limitations in these earlier studies, which we have set out to address in this manuscript.

We utilized flow cytometry, the most robust and appropriate method for analysing distinct MP populations (Cointe et al., 2016). Our study did not determine the parental origin of the MP because it was outside the scope of our study remit. However, it is reasonable to assume that most MP within each plasma sample was of platelet origin, this being the most abundant MP type (van Niel et al., 2018). Analysis of MP revealed the presence of smaller MP in all healthy individuals, which were significantly more numerous in Black African individuals. Few other published works have investigated the presence of smaller MP in healthy Black African or White individuals, and these were significantly smaller in size (Freeman et al., 2018; Eitan et al., 2017; Noren Hooten et al., 2019) and may possibly be exosomes (Cointe et al., 2016). These studies found no difference between ethnicities.

The clinical significance of the smaller MP detected in our study is unclear and requires further investigation but work in our laboratory associates smaller MP with cellular dysfunction and disease progression (Martin et al., 2018), which is supported by others (Noren Hooten et al., 2019; Borghesan et al., 2019; García Garre et al., 2019; Jy et al., 1995). The ethnicity of subjects was, however, not mentioned in these studies. Further analysis revealed that larger MP was more numerous, but not significantly, in ethnic minority individuals. Evidence shows that vesicles of different sizes are enriched with distinct proteins, have differential functions, and locate at distinct sites (Buzás et al., 2018; Kowal et al., 2016). A more detailed analysis of the composition and contents of these smaller and larger MP might provide insight into their roles in CVD ethnic minority individuals.

The previously unreported significant increase in circulating PS-expressing MP from Black African individuals presented here has implications for increased thrombosis, oxidative stress, and adhesion. Thrombin generation appears to be dependent on exposed PS, which acts as a platform for coagulation and possibly also has a role in TF decryption (Spronk et al., 2014), disseminating a hypercoagulable status. Curiously, this increase in PS-expressing MP was not seen in South Asian individuals, and it is possible that alternative factors might direct thromboembolic events in these individuals.

We found no significant differences in the number of TF-expressing MP between all groups, but antigen assays do not distinguish between encrypted and decrypted active forms of TF. Mayr *et al.* (2009) reported decreased *in vivo* LPS-activated MP TF activity in healthy Black individuals, with no differences in baseline activity. This was, however, assayed using plasma rather than isolated MP and could therefore be attributed to platelets and not MP alone. When we assayed the TF activity of isolated MP, we found significant increases in the activity of ethnic minority MP over the 25 min incubation, which was maintained when the data were

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

adjusted as TF activity per 10^6 MP. This was not seen in MP from healthy White individuals. The increases seen in ethnic minority MP are novel and could perhaps be due to differences in expression and/or dynamics of the decryption of TF to its coagulant form. MP may participate in both TF-dependent and alternative TF-independent mechanisms of thrombosis, such as interactions with ROS (Gutmann et al., 2020) or integrin initiation (Schwarz et al., 2002).

ROS has far-reaching modulatory, effector, and activating effects, particularly in disease states where there is an imbalance in homeostasis and is also thought to play a role in regulating coagulation and haemostasis by activating TF (Vara et al., 2014). We observed a significant increase in ROS from Black African MP, which might affect the oxidative phenotype (Feairheller et al., 2011) and endothelial cell dysfunction (Brown et al., 2011) observed previously in ethnic minorities. This increase in ROS was not observed in MP from South Asian individuals, and this difference between ethnic groups may be associated with the excess risk of CVD in Black African individuals (Ho et al., 2022) and may warrant further investigation.

When naïve endothelial cells were incubated with ethnic minority MP *in vitro*, we observed a significant increase in endothelial TF expression. This increase might be due to the uptake and recycling of MP TF, as analysis of endothelial cells incubated with TF-positive MP did not reveal any responsive *de novo* TF mRNA (Collier et al., 2013). Even so, the presence of increased TF on endothelial cells induced by Black African and South Asian MP will most likely affect endothelial dysfunction, perhaps by inducing apoptosis (Aharon et al., 2008).

Interestingly, we observed two distinct populations of vWF staining on endothelial cells incubated with ethnic minority MP which was generally not seen on those incubated with White MP. This could reflect the formation of ultra-large vWF strings on the endothelial cell surface due to reduced ADAMTS-13 activity (Chauhan et al., 2007), which occurs following endothelial activation (Jimenez et al., 2006). We observed no significant differences in endothelial cell vWF expression following incubation with MP. However, those incubated with ethnic minority MP had three times the vWF expression of cells incubated with MP from White individuals, which could promote platelet adhesion and vascular inflammation *in vivo* (Wu et al., 2017).

Our novel findings on the actions of ethnic minority MP with endothelial cells *in vitro* assessed using SEM, fluorescent membrane-integrators, fluorescent microscopy, and flow cytometry, and fluorescent cell-integrators and confocal microscopy, all indicate that both Black African and South Asian ethnic minority MP interact with and integrate into endothelial cells noticeably more than those from White individuals. These interactions are likely to affect adhesion (Rautou et al., 2011), chemokine (Atehortúa et al., 2019) and cytokine (Nomura et al., 2001) production, microRNA delivery (Laffont et al., 2013), amongst other effects. These would contribute to increased endothelial cell activation and oxidative stress and increased leukocyte adhesion on blood vessel walls during pathophysiological processes.

The study of healthy MP, the molecules they transport, and their interactions with cells will increase our understanding of their potential role in cardiovascular disease in ethnic minority individuals and enable the exploration of targeted management strategies to reduce this disease risk factor.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

IMPLICATION TO RESEARCH AND PRACTICE

If ethnic minority MP has a baseline phenotype indicative of chronic inflammation as suggested by this study, their disease effects will be further amplified. In addition, our observations suggest that different processes and contributions of MP in the development of CVD might differ between Black African and South Asian ethnic minority individuals. Ethnic minority individuals are underrepresented in medical research, and further investigations of MP from healthy White and ethnic minority are required to understand the possible mechanisms of cardiovascular health.

CONCLUSION

In conclusion, the results of our study suggest that Black African MP have a baseline phenotype indicative of chronic inflammation, and both Black African and South Asian MP interact with endothelial cells and affect a dysfunctional phenotype. This may be associated with the susceptibility of certain ethnic minority groups to CVD. Our study has some limitations in the small sample of individuals studied. Nevertheless, studying healthy MP and their interactions with cells increases our understanding of their role in disease and their potential as disease progression and treatment efficacy biomarkers.

Future Research

Future research would be to investigate the pro-thrombotic and pro-oxidative phenotype of MP from a larger cohort of healthy White and ethnic minority individuals. A more detailed analysis of the specific effects, protein profile, and miRNA carriage of the smaller MP should be carried out to elucidate their particular role in CVD.

Acknowledgements

The authors graciously acknowledge the assistance of John Rebuck in proofreading the manuscript. The authors acknowledge Dr Rachel Armitage (De Montfort University) for both her expertise and operation of the scanning electron microscope. The authors thank Dr Raymond Allan (De Montfort University) for his expertise and guidance with confocal imaging. The authors thank De Montfort University staff and students who gave up their time to participate in the study.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

REFERENCES

Agouni, A., Parray, A. S., Akhtar, N., Mir, F. A., Bourke, P. J., Joseph, S., Morgan, D. M., Santos, M. D., Wadiwala, M. F., Kamran, S., Sivaraman, S. K., Shuaib, A. (2019) There is a selective increase in pro-thrombotic circulating extracellular vesicles in acute ischaemic stroke and transient ischemic attack: A study of patients from the Middle East and Southeast Asia—frontiers in Neurology, 10, 251. https://doi.org/10.3389/fneur.2019.00251

Aharon, A., Tamari, T., Brenner, B. (2008) Monocyte-derived microparticles and exosomes induce procoagulant and apoptotic effects on endothelial cells. Journal of Thrombosis and Haemostasis, 100, 878–885. https://doi.org/10.1160/th07-11-0691

Antonova, O. A., Yakushkin, V. V., Mazurov, A. V. (2019) Coagulation activity of membrane microparticles. Biochemistry (Moscow), Supplement Series A: Membrane and Cell Biology, 13, 169–186. doi:10.1134/s1990747819030036

Atehortúa, L., Rojas, M., Vásquez, G., Muñoz-Vahos, C. H., Vanegas-García, A., Posada-Duque, R. A., Castaño, D. (2019) Endothelial activation and injury by microparticles in patients with systemic lupus erythematosus and rheumatoid arthritis. Arthritis Research & Therapy, 21. https://doi.org/10.1186/s13075-018-1796-4.

Biro, E., Nieuwland, R., Sturk, A. (2004) Measuring circulating cell-derived microparticles. Journal of Thrombosis and Haemostasis, 2, 1843–1844. https://doi.org/10.1111/j.1538-7836.2004.00937.x.

Borghesan, M., Fafián-Labora, J., Eleftheriadou, O., Carpintero-Fernández, P., Paez-Ribes, M., Vizcay-Barrena, G., Swisa, A., Kolodkin-Gal, D., Ximénez-Embún, P., Lowe, R., Martín-Martín, B., Peinado, H., Muñoz, J., Fleck, R. A., Dor, Y., Ben-Porath, I., Vossenkamper, A., Muñoz-Espin, D., O'Loghlen, (2019) A. Small extracellular vesicles are key regulators of non-cell autonomous intercellular communication in senescence via the interferon protein IFITM3. Cell Reports, 27, 3956–3971. https://doi.org/10.1016/j.celrep.2019.05.095.

Brodsky, S. V., Zhang, F., Nasjletti, A., Goligorsky, M. S. (2004) Endothelium-derived microparticles impair endothelial function *in vitro*. American Journal of Physiology: Heart and Circulatory Physiology, 286, 1910–1915. https://doi.org/10.1152/ajpheart.01172.2003.

Brown, M. D., Feairheller, D. L., Thakkar, S., Park, J. Y. (2011) Racial differences in tumor necrosis factor-α-induced endothelial microparticles and interleukin-6 production. Vascular Health and Risk Management, 7, 541–550. https://doi.org/10.2147/VHRM.S22930.

Buzás, E. I., Tóth, E. Á., Sódar, B. W., Szabó-Taylor, K. É. (2018) Molecular interactions at the surface of extracellular vesicles. Seminars in Immunopathology, 40, 453–464. https://doi.org/10.1007/s00281-018-0682-0.

Chauhan, A. K., Goerge, T., Schneider, S. W., Wagner, D. D. (2007) Formation of platelet strings and microthrombi in the presence of adamts-13 inhibitor does not require P-selectin or β3integrin. Journal of Thrombosis and Haemostasis. 5, 583–589. https://doi.org/10.1111/j.1538-7836.2007.02361.x.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

Cointe, S., Judicone, C., Robert, S., Mooberry, M. J., Poncelet, P., Wauben, M., Nieuwland, R., Key, N. S., Dignat-George, F., Lacroix, R. (2016) Standardization of microparticle enumeration across different flow cytometry platforms: Results of a Multicenter Collaborative Workshop. Journal of Thrombosis and Haemostasis. 15, 187–193. https://doi.org/10.1111/jth.13514.

Collier, M. E., Mah, P. M., Xiao, Y., Maraveyas, A., Ettelaie, C. (2013) Microparticle-associated tissue factor is recycled by endothelial cells resulting in enhanced surface tissue factor activity. Journal of Thrombosis and Haemostasis, 110, 966–976. https://doi.org/10.1160/TH13-01-0055.

Darabi, M., Kontush, A. (2016) Phosphatidylserine in atherosclerosis. Current Opinion in Lipidology, 27, 414–420. https://doi.org/10.1097/MOL.0000000000000298.

Eitan, E., Green, J., Bodogai, M., Mode, N. A., Bæk, R., Jørgensen, M. M., Freeman, D. W., Witwer, K. W., Zonderman, A. B., Biragyn, A., Mattson, M. P., Noren Hooten, N., Evans, M. K. (2017) Age-related changes in plasma extracellular vesicle characteristics and internalization by leukocytes. Scientific Reports, 7. https://doi.org/10.1038/s41598-017-01386-z.

Feairheller, D. L., Park, J. Y., Sturgeon, K. M., Williamson, S. T., Diaz, K. M., Veerabhadrappa, P., Brown, M. D. (2011) Racial differences in oxidative stress and inflammation: *In vitro* and *in vivo*. Clinical and Translational Science. 4, 32–37. https://doi.org/10.1111/j.1752-8062.2011.00264.x.

França, C. N., Izar, M. C., Amaral, J. B., Tegani, D. M., Fonseca, F. A. (2014) Microparticles as potential biomarkers of cardiovascular disease. Brazilian Archives of **Cardiology,** 104, 169–174. https://doi.org/10.5935/abc.20140210.

Freeman, D. W., Noren Hooten, N., Eitan, E., Green, J., Mode, N. A., Bodogai, M., Zhang, Y., Lehrmann, E., Zonderman, A. B., Biragyn, A., Egan, J., Becker, K. G., Mattson, M. P., Ejiogu, N., Evans, M. K. (2018) Altered extracellular vesicle concentration, cargo, and function in diabetes. Diabetes, 67, 2377–2388. https://doi.org/10.2337/db17-1308.

García Garre, E., Luengo Gil, G., Montoro García, S., Gonzalez Billalabeitia, E., Zafra Poves, M., García Martinez, E., Roldán Schilling, V., Navarro Manzano, E., Ivars Rubio, A., Lip, G. Y., Ayala de la Peña, F. (2018) Circulating small-sized endothelial microparticles as predictors of clinical outcome after chemotherapy for breast cancer: An exploratory analysis. Breast Cancer Research and Treatment, 169, 83–92. https://doi.org/10.1007/s10549-017-4656-z.

Gray, W. D., Mitchell, A. J., Searles, C. D. (2015) An accurate, precise method for general labelling of extracellular vesicles. MethodsX, 2, 360–367. https://doi.org/10.1016/j.mex.2015.08.002.

Gutmann, C., Siow, R., Gwozdz, A. M., Saha, P., Smith, A. (2020) Reactive oxygen species in venous thrombosis. International Journal of Molecular Science, 21, 1918. https://doi.org/10.3390/ijms21061918.

Hazrin-Chong, N. H., Manefield, M. (2012) An alternative SEM drying method using hexamethyldisilazane (HMDS) for microbial cell attachment studies on sub-bituminous coal. Journal of Microbiological Methods, 90, 96–99. https://doi.org/10.1016/j.mimet.2012.04.014.

Ho, F.K., Gray, S. R., Welsh, P., Gill, J. M., Sattar, N, Pell, J. P., Celis-Morales, C. (2022) Ethnic differences in cardiovascular risk: Examining differential exposure and susceptibility to risk factors. BMC Medicine, 20. doi:10.1186/s12916-022-w.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

Jimenez, J. J., Jy, W., Mauro, L. M., Lens, K. M., Daghistani, N., Dodson, H. J., Horstman, L. L., Ahn, Y.-S. (2006) Activation of endothelial cells (EC) leads to the release of inhibitory endothelial microparticles and decreased intracellular ADAMTS13 activity. Blood, 108, 1810–1810. https://doi.org/10.1182/blood.V108.11.1810.1810.

Jy, W., Horstman, L. L., Wang, F., Duncan, R. C., Ahn, Y. S. (1995) Platelet factor 3 in plasma fractions: Its relation to microparticle size and thromboses. Thrombosis Research, 80, 471–482. https://doi.org/10.1016/0049-3848(95)00202-2.

Kailashiya, J. (2018) Platelet-derived microparticles analysis: Techniques, challenges, and recommendations. Analytical Biochemistry, 546, 78–85. https://doi.org/10.1016/j.ab.2018.01.030.

Khan, S., Simpson, J., Lynch, J. C., Turay, D., Mirshaidi, S., Gonda, A., Sanchez, T.W., Casiano, C. A., Wall, R. N. (2017) Racial differences in the expression of inhibitors of apoptosis (IAP) proteins in extracellular vesicles (EV) from prostate cancer patients. PLOS ONE, 12(10), e0183122. Https://doi.org/10.13371/journal.pone.0183122.

Khunti, K., Morris, D. H., Weston, C. L., Gray, L. J., Webb, D. R., Davies, M. J. (2013) Joint prevalence of diabetes, impaired glucose regulation, cardiovascular disease risk, and chronic kidney disease in South Asians and White Europeans. PLoS ONE, 8, e55580. https://doi.org/10.1371/journal.pone.0055580.

Kowal, J., Arras, G., Colombo, M., Jouve, M., Morath, J. P., Primdal-Bengtson, B., Dingli, F., Loew, D., Tkach, M., Théry, C. (2016) Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proceedings of the National Academy of Sciences of the United States of America, 113, E968-E977. https://doi.org/10.1073/pnas.1521230113.

Kretzschmar, J., Babbitt, D. M., Diaz, K. M., Feairheller, D. L., Sturgeon, K. M., Perkins, A. M., Veerabhadrappa, P., Williamson, S. T., Ling, C., Lee, H., Grimm, H., Thakkar, S. R., Crabbe, D. L., Kashem, M. A., Brown, M. D. (2014) A standardized exercise intervention differentially affects premenopausal and postmenopausal African-American women. Menopause, 21, 579–584. https://doi.org/10.1097/GME.0000000000000133.

Laffont, B., Corduan, A., Plé, H., Duchez, A.-C., Cloutier, N., Boilard, E., Provost, P. (2013) Activated platelets can deliver mRNA regulatory ago2•microrna complexes to endothelial cells via microparticles. Blood, 122, 253–261. https://doi.org/10.1182/blood-2013-03-492801.

Maas, S. L. N., de Vrij, J., van der Vlist, E. J., Geragousian, B., van Bloois, L., Mastrobattista, E., Schiffelers, R. M., Wauben, M. H. M., Broekman, M. L. D., Nolte-'t Hoen, E. N. M. (2015) Possibilities and limitations of current technologies for quantification of biological extracellular vesicles and synthetic mimics. Journal of Controlled Release, 200, 87–96. https://doi.org/10.1016/j.jconrel.2014.12.041.

Martin, N., Hackney, A. B., Isherwood, J., Runau, F., Arshad, A., Chung, W. Y., Dennison, A. R. (2018) Reduced pro-coagulant phenotype of microparticles in pancreatic cancer patients on omega-3-supplemented gemcitabine treatment. Clinical Nutrition, 37, S13. https://doi.org/10.1016/j.clnu.2018.06.1114.

Mayr, F. B., Spiel, A. O., Leitner, J. M., Firbas, C., Jilma-Stohlawetz, P., Chang, J.-Y., Key, N. S., Jilma, B. (2009) Racial differences in endotoxin-induced tissue factor-triggered coagulation. Journal of Thrombosis and Haemostasis, 7, 634–640. https://doi.org/10.1111/j.1538-7836.2009.03307.x.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

Mensah, G. A., Jaquish, C., Srinivas, P., Papanicolaou, G. J., Wei, G. S., Redmond, N., Roberts, M. C., Nelson, C., Aviles-Santa, L., Puggal, M., Green Parker, M. C., Minear, M. A., Barfield, W., Fenton, K. N., Boyce, C. A., Engelgau, M. M., Khoury, M. J. (2019) Emerging concepts in precision medicine and cardiovascular diseases in racial and ethnic minority populations. Circulation Research, 125, 7–13. doi:10.1161/circresaha.119.314970.

Nomura, S., Tandon, N. N., Nakamura, T., Cone, J., Fukuhara, S., Kambayashi, J. (2001) High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in THP-1 and Endothelial Cells. Atherosclerosis, 158, 277–287. https://doi.org/10.1016/S0021-9150(01)00433-6.

Noren Hooten, N., McFarland, M. H., Freeman, D. W., Mode, N. A., Ezike, N., Zonderman, A. B., Evans, M. K. (2019) Association of extracellular vesicle protein cargo with race and clinical markers of mortality. Scientific Reports, 9, 17582. https://doi.org/10.1038/s41598-019-53640-1.

Palaniappan, L., Garg, A., Enas, E., Lewis, H., Bari, S., Gulati, M., Flores, C., Mathur, A., Molina, C., Narula, J., Rahman, S., Leng, J., Gany, F. (2018) South Asian cardiovascular disease & cancer risk: Genetics & pathophysiology. Journal of Community Health, 43, 1100–1114. https://doi.org/10.1007/s10900-018-0527-8.

Panth, N., Paudel, K. R., Parajuli, K. (2016) Reactive oxygen species: A key hallmark of cardiovascular disease. Advances in Medicine, 9152732. https://doi.org/10.1155/2016/9152732.

Rautou, P.-E., Leroyer, A. S., Ramkhelawon, B., Devue, C., Duflaut, D., Vion, A.-C., Nalbone, G., Castier, Y., Leseche, G., Lehoux, S., Tedgui, A., Boulanger, C. M. (2011) Microparticles from human atherosclerotic plaques promote endothelial ICAM-1–dependent monocyte adhesion and transendothelial migration. Circulation Research, 108, 335–343. doi:10.1161/circresaha.110.237420.

Robert, S., Poncelet, P., Lacroix, R., Arnaud, L., Giraudo, L., Hauchard, A., Sampol, J., Dignat-George, F. (2009) Standardization of platelet-derived microparticle counting using calibrated beads and a cytomics FC500 routine flow cytometer: A first step towards Multicenter Studies? Journal of Thrombosis and Haemostasis, 7, 190–197. https://doi.org/10.1111/j.1538-7836.2008.03200.x.

Schwarz, M., Nordt, T., Bode, C., Peter, K. (2002) The GP IIB/iiia inhibitor abciximab (C7E3) inhibits the binding of various ligands to the leukocyte integrin MAC-1 (CD11B/CD18, αMβ2). Thrombosis Research, 107, 121–128. https://doi.org/10.1016/s0049-3848(02)00207-4.

Shantsila, E., Wrigley, B., Shantsila, A., Tapp, L. D., Blann, A. D., Gill, P. S., Lip, G. Y. H. (2011) Ethnic differences in macrovascular and microvascular function in systolic heart failure. Circulation: Heart Failure. 4, 754–762. https://doi.org/10.1161/CIRCHEARTFAILURE.111.962365.

Spronk, H. M. H., ten Cate, H., van der Meijden, P. E. J. (2014) Differential roles of tissue factor and phosphatidylserine in activation of coagulation. Thrombosis Research, 133, S54–S56. https://doi.org/10.1016/j.thromres.2014.03.022.

Thorpe, R. J., Fesahazion, R. G., Parker, L., Wilder, T., Rooks, R. N., Bowie, J. V., Bell, C. N., Szanton, S. L., LaVeist, T. A. (2016) Accelerated Health declines among African Americans in the USA. Journal of Urban Health, 93, 808–819. https://doi.org/10.1007/s11524-016-0075-4.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

Tripisciano, C., Weiss, R., Eichhorn, T., Spittler, A., Heuser, T., Fischer, M., Weber, V. (2017) Different potential of extracellular vesicles to support thrombin generation: contributions of phosphatidylserine, Tissue Factor, and cellular origin. Scientific Reports, 7. 10.1038/s41598-017-03262-2.

van Niel, G., D'Angelo, G., Raposo, G. (2018) Shedding light on the cell biology of extracellular vesicles. Nature Reviews Molecular Cell Biology, 19, 213–228. https://doi.org/10.1038/nrm.2017.125.

Vara, D., Pula, G. (2014) Reactive oxygen species: Physiological roles in the regulation of vascular cells. Current Molecular Medicine, 14, 1103–1125. https://doi.org/10.2174/1566524014666140603114010.

Viera, A. J., Mooberry, M., Key, N. S. (2012) Microparticles in cardiovascular disease pathophysiology and outcomes. Journal of the American Society of Hypertension, 6, 243–252. https://doi.org/10.1016/j.jash.2012.06.003.

Wu, M. D., Atkinson, T. M., Lindner, J. R. (2017) Platelets and von Willebrand factor in atherogenesis. Blood, 129, 1415–1419. https://doi.org/10.1182/blood-2016-07-692673.

Zakai, N. A., McClure, L. A. (2011) Racial differences in venous thromboembolism. Journal of Thrombosis and Haemostasis, 9, 1877–1882. https://doi.org/10.1111/j.1538-7836.2011.04443.x.

Zlamal, J., Bakchoul, T., Singh, A., Weich, K., Althaus, K. (2022) Platelet phosphatidylserine is the critical mediator of increased thrombus formation in heparin-induced thrombocytopenia. Blood, 140 (S1): 8374–8375. https://doi.org/https://doi.org/10.1182/blood-2022-171209

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)
www.abjournals.org

APPENDIX

FIGURES

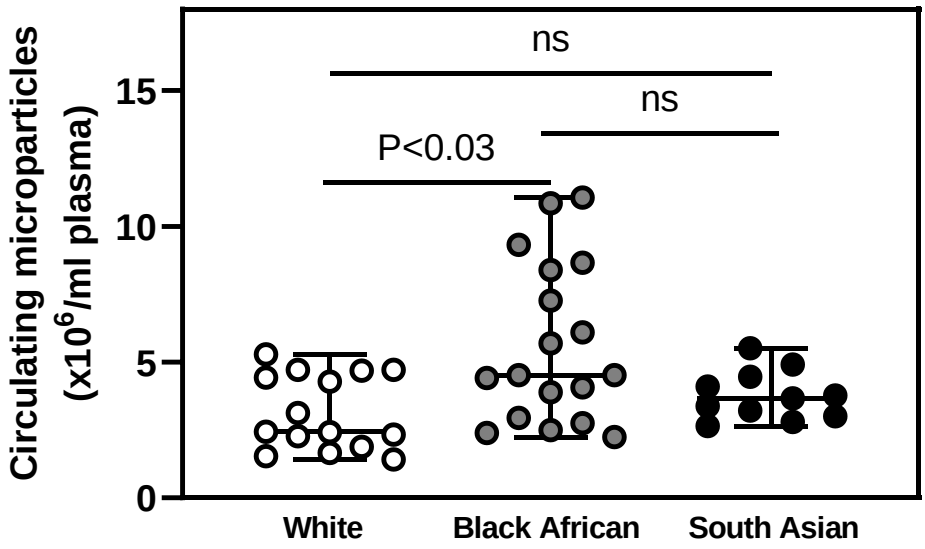


Figure 1. Enumeration of circulating MP in healthy White (open circles, n=15), Black African (grey circles, n=18), and South Asian (closed circles, n=11) individuals. Plasma-derived MP was enumerated using flow cytometry. MP counts are represented as median (range), and significance was set at P<0.05. Abbreviations: MP, microparticles. Statistics: Kruskal-Wallis one-way analysis of variance, posthoc Dunn's multiple comparison test.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)

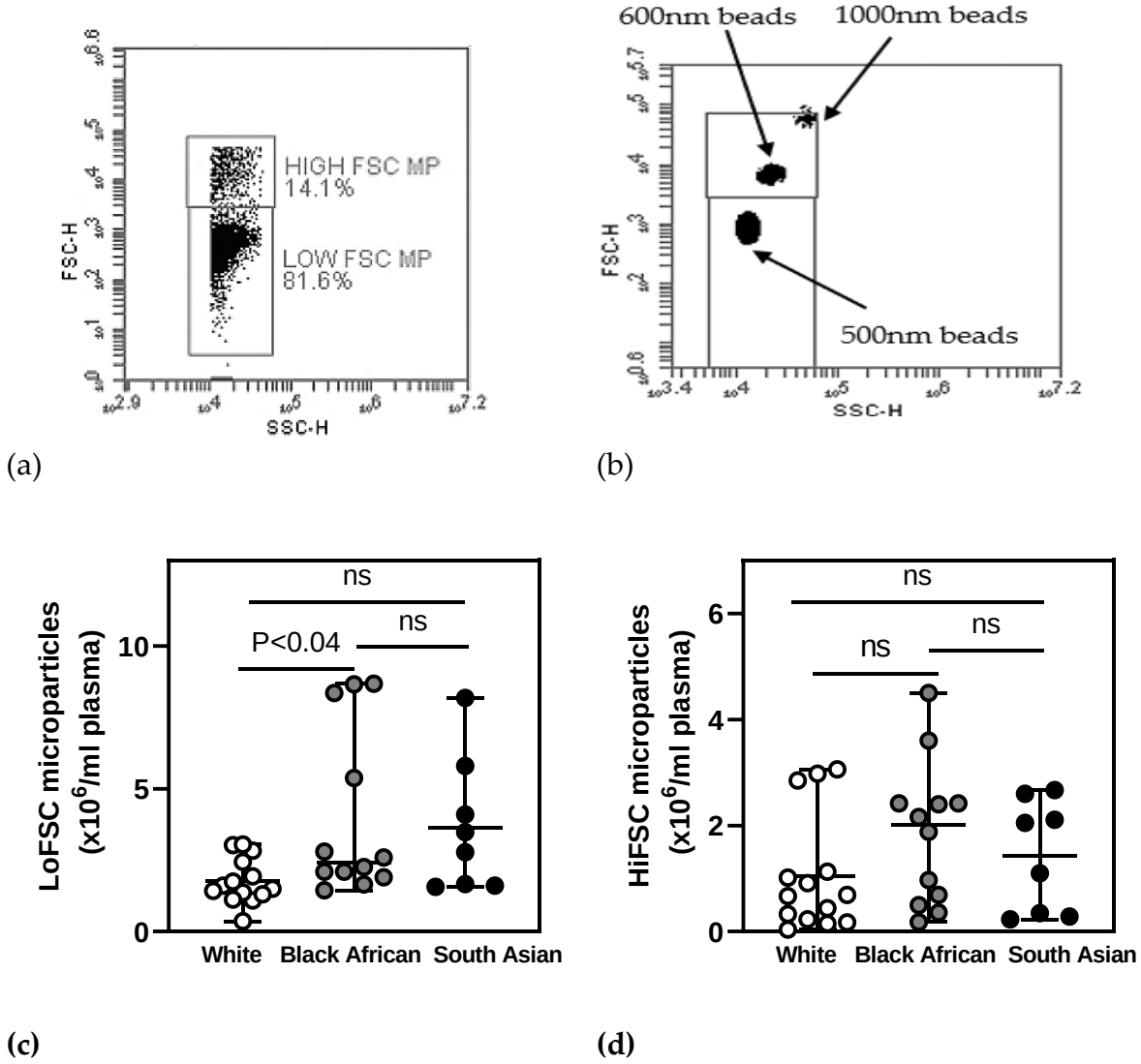
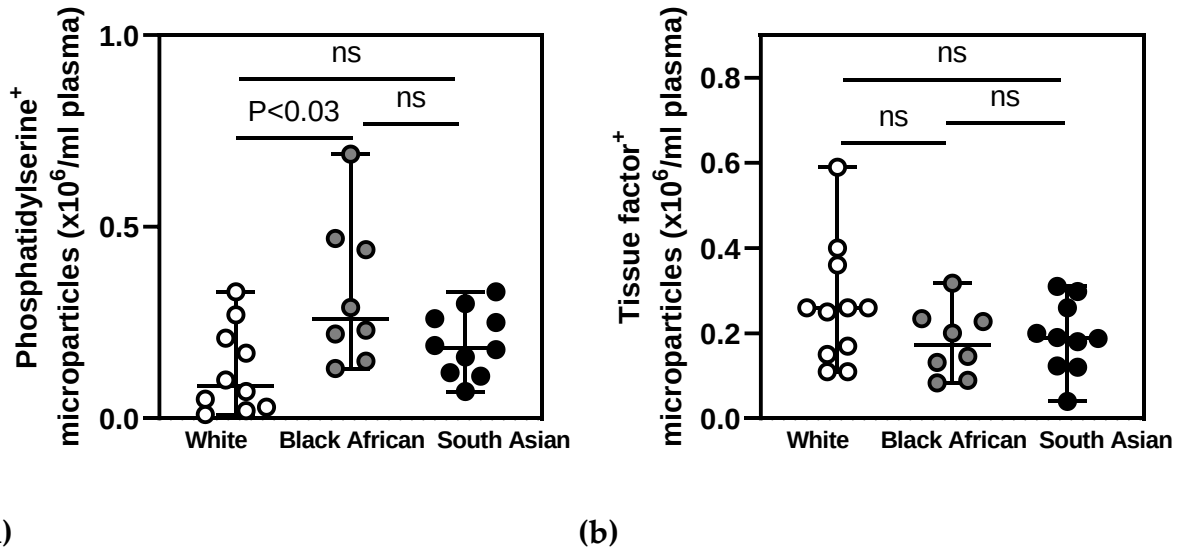


Figure 2. Size of circulating MP. Flow cytometric analysis revealed the presence of two sizes of MP: larger HiFSC and smaller LoFSC. Typical flow cytometric dot plot illustrating HiFSC and LoFSC gating (Figure 2a). Flow cytometric dot plot showing the positions of 500 nm, 600 nm, and 1000 nm latex beads to indicate sizes of larger HiFSC and smaller LoFSC MP gating (Figure 2b). The smaller (Figure 2c) and larger (Figure 2d) MP from White (open circles, n=14), Black African (grey circles, n=12), or South Asian (closed circles, n=8) individuals were enumerated. MP counts are represented as median (range), and significance was set at P<0.05. Abbreviations: MP, microparticles; HiFSC, high forward scatter; LoFSC, low forward scatter. Statistics: Kruskal-Wallis one-way analysis of variance, posthoc Dunn's multiple comparison test.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)

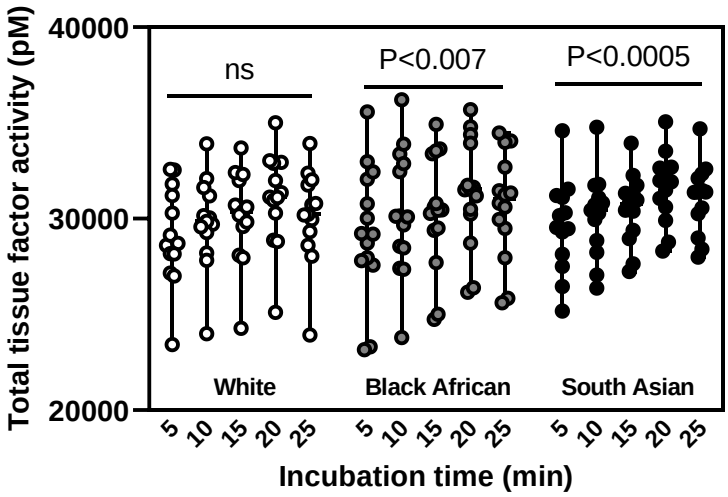


(a) **(b)**

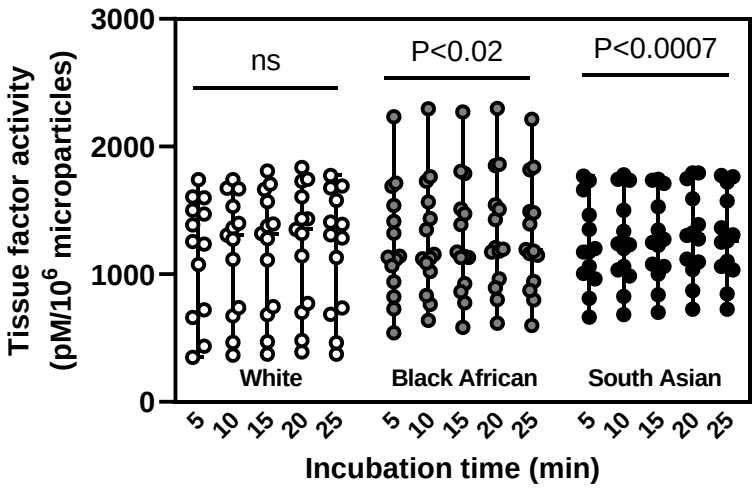
Figure 3. The pro-thrombotic phenotype of MP from White (open circles, n=11), Black African (grey circles, n=8), or South Asian (closed circles, n=10) individuals was determined. MP were stained with Annexin V or CD142 antibody to assess phosphatidylserine (Figure 3a) or tissue factor (Figure 3b) expression respectively and analysed using flow cytometry. MP counts are represented as median (range), and significance was set at P<0.05. Abbreviations: MP, microparticles. Statistics: Kruskal-Wallis one-way analysis of variance, posthoc Dunn's multiple comparison test.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)



(a)



(b)

Figure 4. The tissue factor (TF) activity of MP from White (open circles, n=13), Black African (grey circles, n=14), or South Asian (closed circles, n=13) individuals was determined as the ability of TF to activate factor X to factor Xa and measured over a period of 25 min. Total MP TF activity (Figure 4a) and TF activity per 10^6 MP (Figure 4b) are presented. MP TF activity is represented as individual values, and significance was set at P<0.05. Abbreviations: MP, microparticles; TF, tissue factor. Statistics: repeated measures two-way analysis of variance with Geisser-Greenhouse correction, post hoc Tukey's multiple comparison test.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)

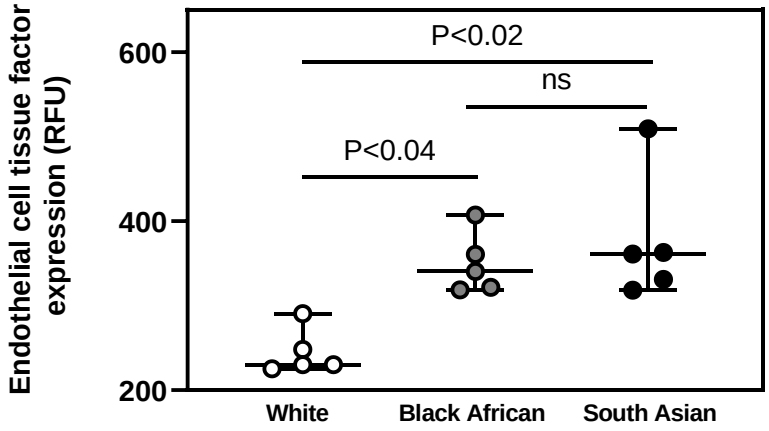
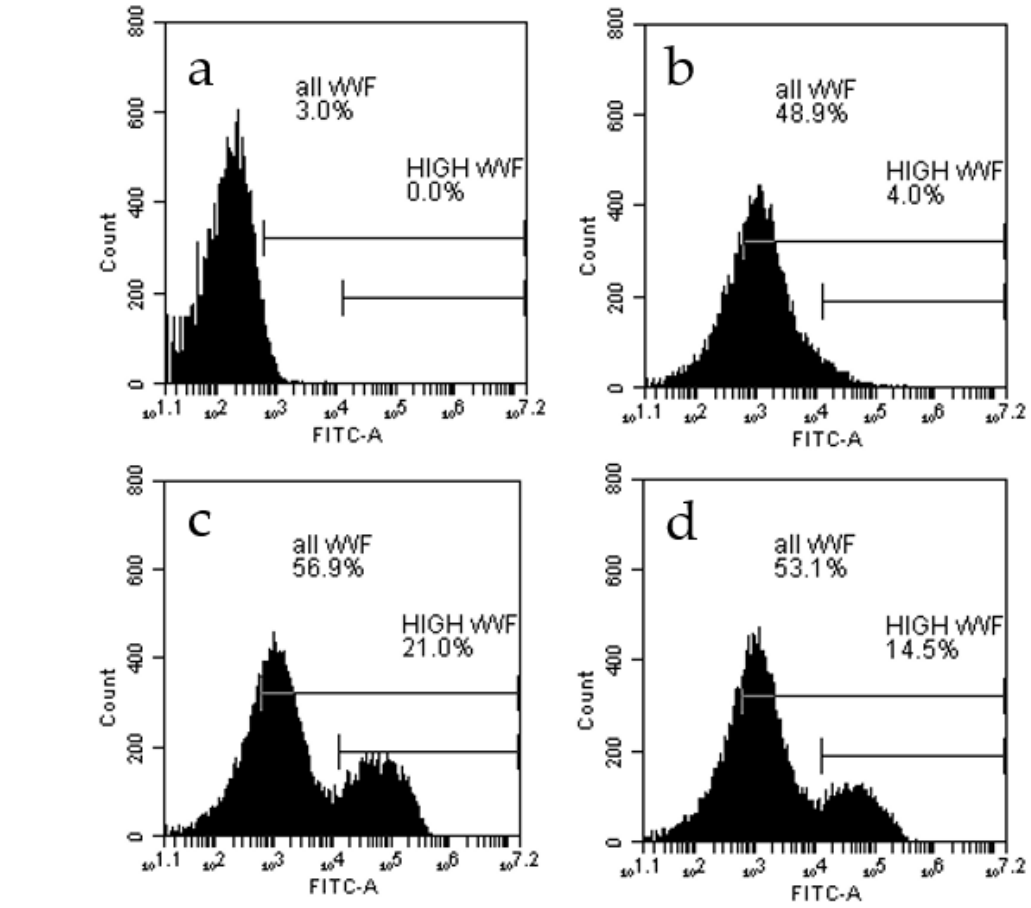
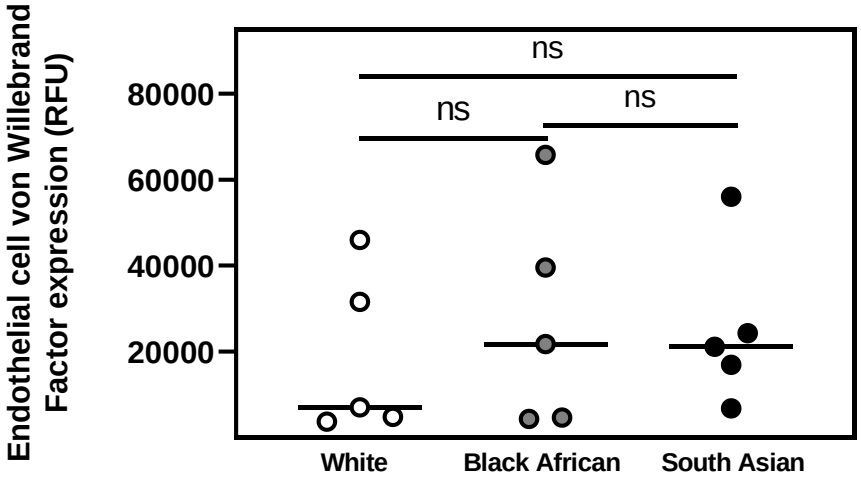


Figure 5. Effects of MP from White (open circles, n=5), Black African (grey circles, n=5), or South Asian (closed circles, n=5) individuals on the phenotype of endothelial cells were determined (Figure 5). An endothelial cell line EA.hy926 was seeded, and incubated overnight with MP, cells were then harvested, stained with an antibody against tissue factor (TF) and analysed using flow cytometry. TF expression is represented as median (range,) and significance was set at P<0.05. Abbreviations: MP, microparticles; TF, tissue factor; RFU, relative fluorescence units. Statistics: Kruskal-Wallis one-way analysis of variance, post hoc Dunn's multiple comparison test.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)





(e)

Figure 6. Effects of MP from White (open circles, n=5), Black African (grey circles, n=5), or South Asian (closed circles, n=5) individuals on the pro-thrombotic phenotype of endothelial cells were determined (Figure 6). An endothelial cell line EA.hy926 was seeded and incubated

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)

overnight with MP; cells were then harvested, stained with an antibody against von Willebrand Factor (vWF) and analysed using flow cytometry. Typical vWF fluorescence histograms are shown for unstained endothelial cells (Figure 6a), those incubated with White MP (Figure 6b), Black African MP (Figure 6c), or South Asian MP (Figure 6d). Two distinct populations of vWF staining were observed in 70% of ethnic minority samples. However, these were seen in only 40% of samples incubated with White MP. vWF expression is represented as median (range) and significance was set at $P<0.05$ (Figure 6e). Abbreviations: MP, microparticles; vWF, von Willebrand Factor; RFU, relative fluorescence units. Statistics: Kruskal-Wallis one-way analysis of variance, post hoc Dunn's multiple comparison test.

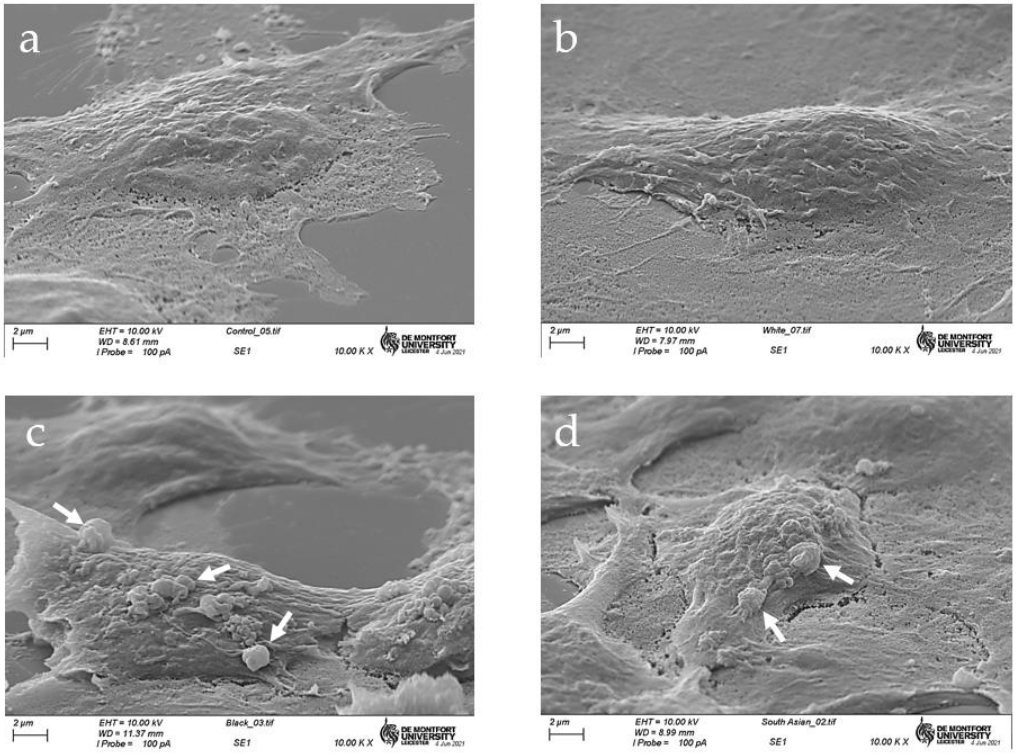


Figure 7. Effects of MP on the gross morphology of endothelial cells were assessed. An endothelial cell line EA.hy926 was seeded, incubated overnight with MP, fixed and dehydrated, and imaged using scanning electron microscopy. Typical representative images are shown. Endothelial cells incubated with media alone (Figure 7a) and MP from White individuals (Figure 7b) have very similar gross morphology, whereas those incubated with MP from Black African (Figure 7c) and South Asian (Figure 7d) individuals had uneven surfaces, membrane ruffling and possible interactions or adherence of MP on the cell surface (white arrows). (all at 10KX magnification, space bar 2 µm). Abbreviations: MP, microparticles.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)
www.abjournals.org

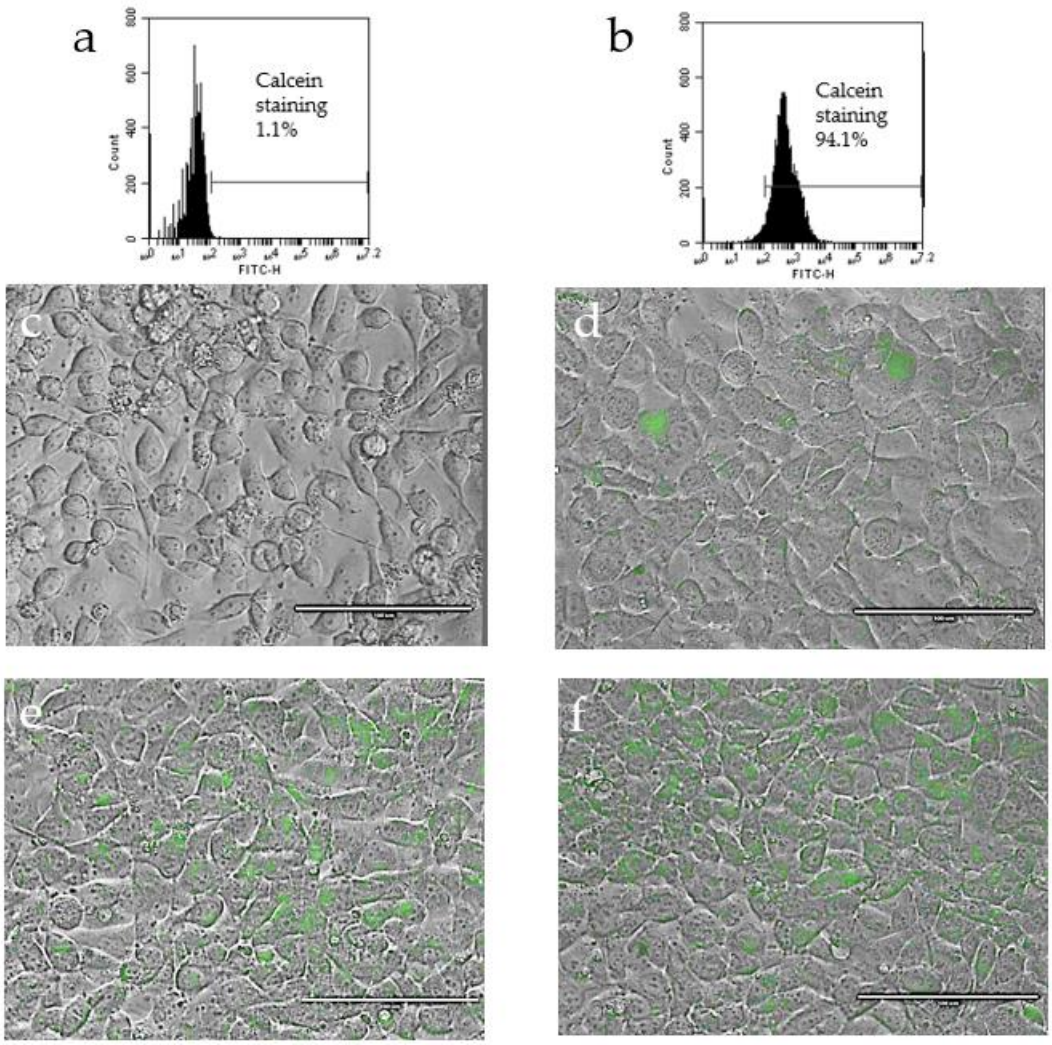


Figure 8. Interactions of MP with endothelial cells were assessed. An endothelial cell line EA.hy926 was seeded, and incubated overnight with MP, which had been labelled with a fluorescent membrane integrator, and interactions were determined using fluorescence microscopy. Typical representative images obtained from flow cytometry are shown for unstained MP (Figure 8a) and those incubated with Calcein (Figure 8b). No fluorescence was observed on cells incubated with media alone (Figure 8c), whilst staining was seen on cells incubated with fluorescent MP from White individuals (Figure 8d), Black African (Figure 8e), and South Asian (Figure 8f) individuals. Endothelial cells incubated with MP from both Black Africans and South Asians showed significant fluorescence. Abbreviations: MP, microparticles. (all at 40X magnification, space bar 100 µm).

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)

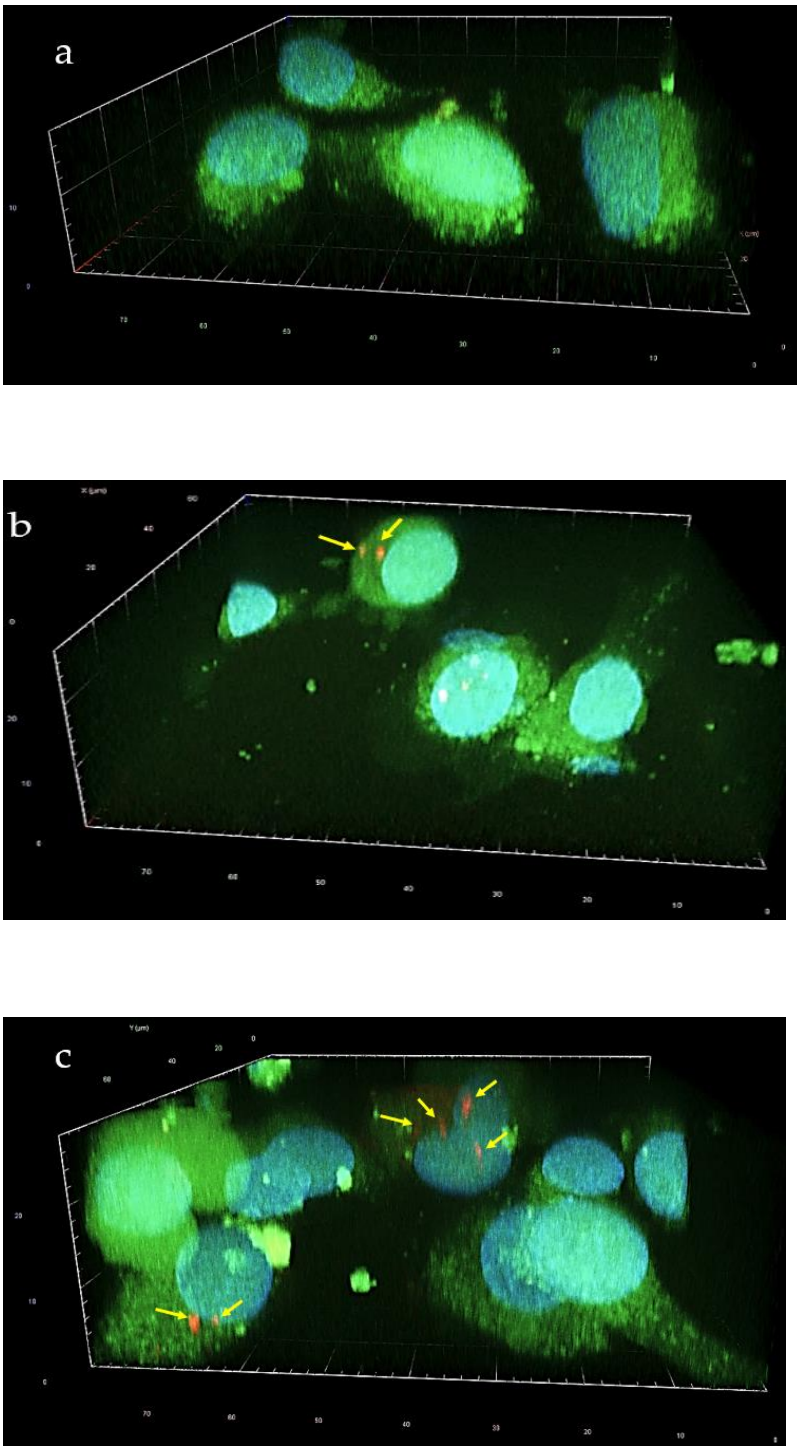


Figure 9. Integration of MP from White (Figure 9a), Black African (Figure 9b), and South Asian (Figure 9c) individuals into endothelial cells was assessed using confocal microscopy. Endothelial cells were stained with CellTracker™ (CT) Green dye, MP with CT Red, and nuclei with blue Hoechst 33342. Typical representative images are shown. Integration of ethnic minority MP was observed (yellow arrows). Abbreviations: MP, microparticles. (all at 40X magnification, space bar 100 µm).

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

TABLES

Table 1. The pro-oxidative phenotype of MP from White, Black African, or South Asian individuals.

Microparticles	Reactive oxygen species signal (DCFH-DA RFU)
White	55.28 (18.08-175.30)
Black African	208.90 (148.40-267.70) (vs White, P<0.007)
South Asian	193.30 (87.62-218.90)

Table 1. The pro-oxidative phenotype of MP from White (n=7), Black African (n=7), or South Asian (n=7) individuals was determined. MP was assessed for reactive oxygen species (ROS) content with DCFH-DA and analysed using flow cytometry. MP ROS is represented as the median (range), and significance was set at P<0.05. Abbreviations: MP, microparticles; ROS, reactive oxygen species; DCFH-DA, 2',7'-Dichlorofluorescin diacetate; RFU, relative fluorescence units. Statistics: Kruskal-Wallis one-way analysis of variance, post hoc Dunn's multiple comparison test.

Table 2. Interactions of MP from White, Black African and South Asian individuals with endothelial cells.

Microparticles	Fluorescent endothelial cells (%)
White	8.40 (7.85-9.63)
Black African	10.43 (9.06-14.30)
South Asian	12.42 (10.13-14.24) (vs. White, P<0.03)

Table 2. Interactions of MP White, Black African, and South Asian (all n=5) individuals with endothelial cells were assessed. An endothelial cell line EA.hy926 was seeded and incubated overnight with MP which had been labelled with a fluorescent membrane integrator, and interactions with cells were determined using flow cytometry. Fluorescent endothelial cells are represented as median (range), and significance was set at P<0.05. Abbreviations: MP, microparticles. Statistics: Kruskal-Wallis one-way analysis of variance, post hoc Dunn's multiple comparison test.

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 6, Issue 2, 2023 (pp. 43-70)

www.abjournals.org

APPENDIX 1

Study	White	Black African	South Asian
Figure 1	13a 4a 17a 12a 22a A E I Y AAA AAE AAI AAP AAY AAQ	35a 54a 44a 14a B C D F J R T AAB AAC AAF AAJ AAR AAT AAZ	10a 30a 8a H M S W AAM AAO AAS AAU
Figure 2c	4a 12a A E I P Y AAA 41 13 AAG 22 12 17	35a 54a 44a B C D R T 54 F R Z	10a 30a H M W 8a 30a S
Figure 2d	4a 12a A E I P Y AAA 41 13 AAG 22 12 17	35a 54a 44a B C D R T 54 F R Z	10a 30a H M W 8a 30a S
Figure 3a	4a 41a 12a AAA AAE AAG AAI AAP AAQ AAY 22	35a 54a 44a 14a AAC AAF AAR AAT	10a 30a 8a H M O S U W V
Figure 3b	4a 41a 12a AAA AAE AAG AAI AAP AAQ AAY 22	35a 54a 44a 14a AAC AAF AAR AAT	10a 30a 8a H M O S U W V
Figure 4a	A E I G 56 22 WI 4 17 12 13 42 58	J B C D Z R 44a 35a 14a 62 54a 66 Z R	P15 V H W S P16 P7 P12 75 10 73 30 78
Figure 4b	A E I G 56 22 WI 4 17 12 13 42 58	J B C D Z R 44a 35a 14a 62 54a 66 Z R	P15 V H W S P16 P7 P12 75 10 73 30 78
Table 1	A E G I P Q Y	B C D F J R T	H M O S U W V
Figure 5	A E I 17a 12a	AAJ B Z 44a 35a	V H W 10a 30a
Figure 6e	A E I 17a 12a	AAJ B Z 44a 35a	V H W 10a 30a
Table 2	A E I 17a 12a	AAJ B Z 44a 35a	V H W 10a 30a

Article DOI: 10.52589/AJBMR-YTFDLV0G
DOI URL: https://doi.org/10.52589/AJBMR-YTFDLV0G