INTRODUCTION

Broadly, the inability of Endothelial Progenitor Cells (EPCs) to migrate towards and incorporate into sites of trauma/wound leading to multiple types of secondary complications is termed as EPC Dysfunction (EPD) (Bezerin, 2016; Bezerin et al., 2016). EPC-research primarily uses murine bone marrow (m-EPCs) and human peripheral blood-derived EPCs (h-EPCs). Other sources

KEYWORDS: Endothelial progenitor cell, dysfunction, high glucose exposure, murine and human models, assay panel.

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have been explored with varying success resulting in a host of data sets demonstrating the effect of high-glucose (HG) and Diabetes mellitus (DM) upon EPCs resulting in EPD (Creager et al., 2003; Fadini et al., 2007; Kolluru et al., 2011; Robinson et al., 2016). However, for better understanding of these phenomena, it is crucial that a set of parameters be identified that responds similarly to HG/DM exposure in similar fashion, irrespective of systemic variability and be brought onto a common platform.

The two most common characteristics of EPD, viz. lowered EPC numbers and inability to migrate towards wound areas, are common to EPCs derived from both sources (Loomans et al., 2004; Avogaro et al., 2011). These EPD phenomena are assessed using various assays like adhesion, colony formation, viability, tubule formation and migration – with multiple test systems – to understand the inter-assay differences and points of similarities.

Although detection of EPD may be easy, it is difficult to accurately interpret them unless assessed on a common platform. This will make distinguishing of HG-induced EPD from EPD caused due to other diseases such as ankylosing spondylitis, cardiovascular disease, atherosclerosis etc. (Ashahara et al., 1997; Hadi et al., 2005; Verma et al., 2005).

Comparative analysis revealed that while most cellular parameters show system-related differences in detection of EPD, certain parameters faithfully detect EPD irrespective of source, site, and pathophysiology of diabetes, in vitro or in vivo. These findings strengthen our premise that accurate detection of DM-/HG-induced EPD requires a battery of parameters. Consequently, we propose a panel of assays that will not only allow accurate detection of HG-/DM-induced EPD irrespective of test system used, but will also facilitate extrapolation of data across test systems and as platform for development of clinical rescue strategies.

**MATERIALS AND METHODS**

**Chemical Reagents and Materials**
Endothelial growth medium (EGM-2 SingleQuots), fetal bovine serum (FBS), Dulbecco’s modified Eagle’s medium (DMEM) were purchased from Lonza, MD, USA and Invitrogen (CA, USA), respectively. MTT, 2′,7′-dichloro-fluorescein-diacetate (DCFH-DA), Griess reagent, Streptozotocin, Ulex Europeas Agglutinin-1 and Vitronectin.
(VN) were from Sigma-Aldrich, St. Louis, MO, USA. All plastic-ware was from BD Falcon, Bukit Batok Crescent, Singapore.

**Experimental Animals**
Swiss albino female mice, 6–8 week old, weighing at least 22 g or more were housed in an inbred colony at the National Centre for Cell Science (NCCS), India, and were used in the experiments. They were given free access to standard feed and water.

**Induction of Experimental Diabetes in Mice and Estimation of Plasma Glucose**
Randomly selected 8 week old male mice were made diabetic by intra-peritoneal administration of Streptozotocin (STZ; 145 mg/kg body weight). Mice exhibiting fasting plasma glucose concentrations of > 200 mg/dL were considered as frankly diabetic. These mice were then supervised for 2–3 weeks and thereafter used for experimentation.

**Human Peripheral Blood Collection**
Human peripheral blood (h-PB) was collected from age-sex-matched non-Diabetic (ND; n = 20) and Diabetic (D; n = 20) individuals, with written informed consent. Diabetic patients were on Metformin, but not on insulin or statins. All available samples were used for all given experiments. All chosen subjects were frankly diabetic (fasting plasma glucose concentrations > 200 mg/dL) for more than 5 years.

**Ethical Declarations**
All animal procedures in this study were complied with, and approved by the Institutional Animal Ethics Committee (IAEC) of NCCS and the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of NCCS (Approval no. EAF/B-179). All human EPC-related protocols used in this study were approved by the Institutional Ethics Committee (IEC) of NCCS and Institutional Committee for Stem Cell Research (IC-SCR) of NCCS.

**Isolation, Culture and Characterization of EPCs**
The EPC isolation protocol as described by Asahara et al. (1997) was followed with modifications. Briefly, mice were sacrificed, femurs were extracted aseptically, and bones were flushed using plain DMEM. The cellular suspension was loaded onto HiSep LSM (HiMedia, India) and centrifuged (30 min, 1500
rpm, RT). The buffy coat was aspirated and MNCs were seeded (1 × 10^6/plate) onto petri-dishes and cultured for 48 h (DMEM + 10% FBS, 37°C, 5% CO₂). Later, the non-adherent fraction was collected and seeded (1000 MNCs/plate) onto Vitronectin (VN)-coated wells in 24 well plates or 35 mm dishes and cultured in EGM2 + 20% FBS for 14 d (37°C, 5% CO₂) (Tepper et al., 2002). Medium was replenished every 48 h. Counting revealed that hPB (ND/D) as well as mBM (ND/D) yielded similar numbers of EPCs (Supplementary Figure 1A). EPCs were identified by co-positivity of Ac-LDL uptake and Ulex europeaus Agglutinin 1 (UEA-1) binding. On day 14, cells were washed with PBS and incubated with Alexaflour 488-labelled Ac-LDL (Invitrogen, CA, USA) for 3 h at 37°C, fixed with chilled 2% buffered PFA, then counter-stained with TRITC-labelled Ulex europaeus Agglutinin 1 (UEA-1; 10 mg/ml). Dual positive cells were considered as EPCs and counted in ten random non-overlapping fields using an inverted fluorescence microscope and images analyzed using Image-pro software (Leica TCS SP5II, Leica Microsystems, Germany). The EPCs obtained using this protocol were > 95% pure (Supplementary Figure 1B).

**EXPERIMENTAL DESIGN**

**Test Systems**

All isolated EPCs were exposed to varying concentrations of glucose (16.5, 25 and 32 mM). All Glucose concentrations were made in either EGM2 or DMEM as per the experimental design. Glucose concentration in media was considered as the ‘basal’ glucose level, while the higher concentrations were above these basal levels. This was termed as HG-treated group and was considered as indicative of *in vitro* supra-glucose exposure. On the other hand, EPCs derived from non-diabetic and diabetic humans and mice were isolated and used for performing same assays – without further exposure to HG concentrations. This group was called as the *in vivo* group and represented the effects of *in vivo* hyperglycaemia (Diabetes).

EPCs from all groups (HG treated/untreated/diabetic/non-diabetic) were cultured in EGM2 for 14 d and post-sacrifice, the cultures were used to perform various cellular and functional assays including CAA, CFU, detection of proliferation potential and viability, migration and tubule formation. The study design in schematically illustrated in Supplementary Figure 3.
Cellular assays

Determination of proliferation potential
All EPC cultures were sacrificed on day 14 and used for assessment of proliferation potential. The proliferation potential of EPCs was determined by incubating EPCs with MTT (0.5 mg/ml) for 4 h in the dark at 37°C with 5% CO₂. Purple-blue formazan crystals formed were dissolved in acidified iso-propanol and absorbance was measured at 570/660nm. Percent proliferation was calculated and data were graphically represented as mean percent Proliferation ± SD.

Cellular adherence assay (CAA)
All EPCs were sacrificed on day 14 and used for cellular adhesion assay by staining with crystal violet, washed with PBS and the stain was extracted using 2% SDS. Absorbance was measured at 570/660 nm and the resultant values were analyzed. Data were represented graphically as percent cellular adhesion ± SD.

Colony forming unit assay (CFU)
EPCs were trypsinized and allowed to form colonies in the presence/absence of HG after which the colonies were stained with crystal violet. An accumulation of at least 15 cells or more was termed as a colony. Ten non-overlapping fields were counted per well and at least four wells were analyzed/group/experiment. Data are represented as mean ± SD of three independent experiments.

Viability estimation
EPCs cultured with or without HG were assessed for viability by staining cells with trypan blue. Ten non-overlapping independent fields were counted per well and at least four wells were counted/group/experiment. Data are represented as mean percent viability ± SD of three independent experiments.

Functional assays

EPC migration assay
The chemotactic migratory ability of EPCs was assessed using modified Boyden chamber (BD) assay. Briefly, 500 EPCs were placed in the upper chambers of 24-well Trans-well plates with polycarbonate membrane (8 µm pores); while Serum-free DMEM supplemented with VEGF (50 ng/ml) was placed in the lower chamber. Use of EGM2 was avoided as EGM2 contains VEGF. After 2 h, the membrane was washed, fixed, stained with crystal violet and the migrated EPCs were counted. At least 30 random fields/well were counted per experimental group.
Tubule formation assay
After day 14, EGM2 was replaced with DMEM + 10% FBS to facilitate differentiation and plates were imaged after 96 h. At least 10 random fields containing newly formed tubules were counted and expressed as % tubule formation ± SD.

Gene expression studies
mRNA was isolated using mRNA isolation kit (Invitrogen). After quantification using NanoDrop 1000 (Thermo Scientific), reverse transcription was performed using Omniscript kit as directed (Qiagen). Specific primers for CXCR4 (F: TCAGTGGCTGACCTCCTTT; R: CTTGGCCTTGTGACTGTTGGT), VEGFR2 (F: AGATGACAACCAGACGGACA, R: AGCCTTCAGATGCCAGAC) and eNOS (F: CACGAGGACACGTGTTTTCGGACTTCCTGTAAACAT, R: CGGAACC) genes were designed using Gene Runner (3.05) to quantify mRNA expression in the EPCs from various experimental groups. PCR products were separated on 1.5% agarose gel and beta-actin was used for normalization purposes.

Reactive oxygen species (ROS) assay
EPCs were trypsinized and exposed to HG concentrations, following which EPCs from all groups were manually counted and incubated with DCFH-DA (10 µM, 45 min, 37°C in dark) for detection of ROS level of HG-/DM-exposed EPCs. The fluorescence intensity (FI) was measured using Fluoroskan Ascent FL (Thermo Fischer Scientific) at 560/650 nM. Data were represented as Mean FI/1000 EPCs.

Nitric oxide (NO) assay
NO was estimated using Griess reagent. Fifty microliters of EPC conditioned medium was incubated in dark at 37°C with equal volume of Griess reagent (20 min). Optical density (OD) was recorded at 570 nm using a spectrophotometer. The data were represented as mean OD ±SD.

MnSOD activity assay
Manganese superoxide dismutase (MnSOD) activity in lysates of HG-treated or untreated EPCs was measured using MnSOD activity assay kit (ENZO life Sciences) as per instructions of the manufacturer. The OD was recorded at 570 nm using a spectrophotometer. The data were presented as MnSOD activity/500 ng protein.
Data Analysis and Statistical Procedures

At least three independent experiments, with 3–4 replicates each, were analyzed for statistical significance and data were expressed as mean ± SD. Statistical comparisons were made between groups using one-way Analyses of Variance (ANOVA). The significance of difference between groups was determined by the Tukey post-hoc analysis. $P < 0.05$ was considered as significant.

RESULTS

The primary objective of the current study was to device of a set of assays that may faithfully assay HG-/DM-induced EPD irrespective of source and type of HG exposure. For this, we first assessed the cellular responses of human and murine EPCs upon in vitro and in vivo exposure to HG using a set of routine cellular assays.

Cellular Responses of EPCs Exposed to HG In Vitro and to DM-induced Hyperglycaemia In Vivo

In vitro cellular adhesion in h-EPCs and m-EPCs revealed that HG-exposure for initial concentrations (16.5 mM) caused significant decrease in cellular adhesion potential compared to the untreated EPCs while further incrementing glucose concentrations (25 and 32 mM) did not cause further decrease in the cellular adhesion (Figure 1A). On the other hand, DM caused marked decrease in cellular adhesion of EPCs while the adhesion of m-EPCs was not affected by the same (Figure 1B).

HG exposure affected m-EPCs, but not h-EPCs, evidenced by the dose-dependent depletion in viability of HG-exposed EPCs (Figure 1C). However, in vivo glucose exposure (DM) caused marked depletion in viability in m-EPCs as well as h-EPCs (Figure 1D); indicating that this parameter is affected differently on exposure to in vitro exposure, but responds similarly to DM exposure in vivo.

Similar to cellular adhesion, the initial glucose shock (16.5 mM) caused a comparable depletion in proliferation potential of m-EPCs and h-EPCs. Further increase in glucose concentrations (25 and 32 mM) did not affect either murine or human EPCs (Figure 1E). While DM caused depletion in proliferation potential in h-EPCs, m-EPCs were immune to such changes (Figure 1F). Since these changes in parameters are source-specific, they are unsuitable for EPD detection.
Figure 1: Cellular assays performed with EPCs isolated from murine and human sources. Cellular adhesion of murine and human EPCs exposed to high glucose (16.5, 25 and 32 mM) concentrations (A) and of EPCs isolated from diabetic and non-diabetic humans and mice (B). Decrease in viability due to in vitro high glucose exposure (C) and in vivo diabetes (D) in human and murine EPCs was estimated using trypan blue dye exclusion test. Proliferation potential on exposure to in vitro HG (E) and in vivo diabetes (F) was estimated using MTT assay, while in vitro (G) an in vivo (H) colony forming unit was estimated by staining colonies with crystal violet followed by manual counting of at least 10 independent fields per group, per experiment. A group of cells exceeding 15 cells was considered as a colony. Comparative effects of osmolar changes and hyperglycemia on murine (I) and human (J) EPC dysfunction were assessed by exposing EPCs to similar concentrations of Mannitol and Glucose, respectively, followed by the CFU assay. All data are average of at least three independent experiments and represented graphically as mean ± SD. **P < 0.050; *P < 0.001.
Colony forming unit ability of m-EPCs and h-EPCs show identical susceptibility profile upon HG-exposure (Figure 1G and Supplementary Figure 2A). The \textit{in vivo} EPD profile is also markedly similar to \textit{in vitro} profile from both sources (Figure 1H). It is thus clear that of all parameters tested, only the CFU assay faithfully indicates damage profile, irrespective of system variabilities and may be used as an indicator for the detection of EPC dysfunction.

**Observed Effects are due to HG Exposure and not due to Osmolarity**

To assess whether the above effects were due to HG exposure and not due to osmolarity. We used Mannitol at the same concentrations as glucose (16.5, 25 and 32 mM) on murine and human EPCs. EPCs exposed to varying concentrations of Mannitol did not undergo any change in CFU profile in either cell types (Figures 1I–J), while both types of EPCs exposed to glucose concentrations were affected in a dose-dependent fashion. Thus effects observed here were due to HG exposure and not due to changes in osmolarity. Having established this, we continued assessment of differential systemic effects.

**Murine and Human EPCs Exposed to HG- or DM-induced Hyperglycaemia Show Comparable Functionality Loss**

Tubule formation, important for EPC functionality is affected in DM. Exposure to incremental HG caused dose-dependent decrease in the tubule formation ability of EPCs from both sources (Figure 2A). Diabetic human and murine EPCs demonstrated a similar profile (Figure 2B). It must be noted here that percent tubule formation in h-EPCs was higher than m-EPCs, which may be attributed to the difference in the site as it is known that the potential of PB-EPCs to form higher number and longer tubules is greater than BM-EPCs (Versari \textit{et al.}, 2009).

The migratory ability, another crucial factor may lead to dysfunctionality upon HG-/DM-exposure. Study of the effect of HG-/DM-exposure on this cellular parameter from both sources revealed dose-dependent decrease in migratory potential (Figure 2C). Diabetes also caused similar depletion in migratory potential irrespective of source and site, with m-EPCs being more susceptible to depletion compared to their h-EPCs (Figure 2D). These data demonstrate that loss of cellular and functional parameters is an effective predictor of EPC dysfunction.
dysfunction in both test systems.

As both parameters show similar responses to HG/DM irrespective of site, source and treatment, these parameters may be used for accurate determination of EPD. Collectively, we understand that not all cellular and/or functional parameters may be affected in any one system, the \textit{in vitro} and \textit{in vivo} patterns may not necessarily match each other, and differences in chosen parameters introduce inter-assay and inter-species variability.

Therefore only a few parameters reliably predict EPD irrespective of source. So, for further confirmation of the above we used molecular and biochemical parameters.

\textbf{Figure 2:} Functional assays with EPCs isolated from murine and human sources. Percent tubule forming ability of EPCs exposed to control (16.5 mM) and high glucose (25 and 32 mM) concentrations (A) and of EPCs isolated from diabetic and non-diabetic humans and mice (B) was estimated to assess HG-/DM-induced EPC dysfunction. Depletion in migration potential due to \textit{in vitro} high glucose exposure (C) and \textit{in vivo} diabetes (D) in human and murine EPCs was estimated using Boyden’s modified chamber assay and represented as % migration towards VEGF. All data are average of at least three independent experiments and represented graphically as % mean ± SD.

*\(P < 0.001\); **\(P < 0.050\).
Disparity in NO and ROS Levels in HG-exposed m-EPCs and h-EPCs

Since reduced levels of NO and simultaneously enhanced levels of ROS are strong indicators of EPD (Loomans et al., 2005; Gallagher et al., 2007; Wu et al., 2011), we assessed NO and ROS in the cultured EPCs (Tessari et al., 2010). Exposure of m-EPCs to 16.5 and 25 mM glucose caused gradual reduction in NO with 25 mM glucose showing maximal reduction in NO concentrations, but surprisingly, exposure to 32 mM HG caused lower reduction in NO concentrations as compared to the earlier concentrations (Figure 3A). h-EPCs also responded in similar fashion to incremental HG-exposure highlighting...
the commonality of response irrespective of source. DM-exposed m-EPCs also showed reduction in NO levels (Figure 3B). However, contrary to expectations and to previous reports h-EPCs did not show DM-induced decrease in NO concentrations (Imanishi et al., 2008). e-NOS mRNA expression analysis further confirmed these findings (Figure 3C–D). This rules out the possibility of systemic NO saturation. We report here, probably for the first time, such pattern of NO expression in EPCs exposed to HG/DM. However, the current evidence is insufficient to challenge earlier reports demonstrating the depletion of NO in human samples. Detailed investigations upon these observations are required for any further comment.

Assessment of ROS, another contributing factor to EPC dysfunction, revealed dose-dependent increase in cellular ROS in m-EPCs (Figures 4A–B). However, ROS levels in diabetic h-EPCs
showed ROS levels comparable to their ND counterparts, thus corroborating the pattern observed with respect to NO; demonstrating the higher tolerance for stress of human cells perhaps pointing towards a stronger ROS scavenging enzyme repertoire.

**m-EPCs and h-EPCs Show Elevated MnSOD Activity When Exposed to HG In Vitro.**

MnSOD is the primary antioxidant defense mechanism for EPC rescue because of its proximity to the mitochondria; systemic expression of which brings about restoration of dysfunctional EPCs (Walter et al., 2005; Marrotte et al., 2010). Our data reveals that HG-exposure caused a dose-dependent increase in MnSOD activity levels in m-EPCs and h-EPCs (Figure 4C). In contrast, DM caused a marked decrease in activity levels in m-EPCs, while no effect was observed on the h-EPCs (Figure 4D). These findings further corroborate our NO- and ROS-related findings. It is thus clear that although crucially important in EPD, the use of NO and/or ROS levels as indicators of EPD cannot be substantiated.

**Gene Expression Pattern of m-EPCs Closely Mimics h-EPCs in Their Response to HG-Induced or Diabetes-induced EPC Dysfunction**

HG-exposure caused a dose-dependent decrease in mRNA expression of CXCR4 and VEGFR2, the genes closely associated with EPD, in both test systems (Figure 5A), with murine cells being more susceptible compared to h-EPCs. These findings corroborate reports stating that HG-/DM-induced dysfunction causes reduction in CXCR4 and VEGFR2 mRNAs (Ebener et al., 2010; Castilla et al., 2012). We, for the first time, add to the earlier information by demonstrating that HG/DM-exposure causes similar changes in m-EPCs/h-EPCs and are used as accurate indicators of HG-/DM-induced EPD.

Only parameters which show similar and coordinated responses across all investigated test systems have been incorporated in the panel for detection of HG-/DM-induced EPD. CFU, tubule formation, migration, and CXCR4/VEGFR2 expression are the parameters that show consistency and hence are incorporated in the panel. Studies performed using this assay panel may
Figure 5: CXCR4 and VEGFR2 mRNA expression in EPCs isolated from murine and human sources. (A) CXCR4 and VEGFR2 mRNA expression in EPCs exposed to HG concentrations and in vivo diabetes in murine test system (upper panel) while expression of these mRNAs in the human system (lower panel). All images are representative of multiple independent experiments. All expressions are normalized against beta actin. (B) Representative densitometry analysis of CXCR4 (B-a and B-b) and VEGFR2 (B-c and B-d) mRNA expression from human and murine EPCs exposed to high glucose concentration in vitro and in vivo. All images are representative of multiple independent experiments. All expressions are normalized against beta actin. *P < 0.05.

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DISCUSSION

The established models of EPD routinely use m-EPCs and h-EPCs in various types of cellular experiments involving EPCs as well as other cell types (Desai et al., 2009; Kanji et al., 2014). However, these datasets cannot be compared with each other due to limited understanding of respective similarities/dissimilarities between the two systems. A comparative study comprising the two model systems using a set of known parameters for detection of EPC dysfunction is required. For this purpose, we used such a model system to demonstrate that parameters for detection of EPD remain constant irrespective of source, sites and types of diabetes (T1D/T2D). It should be noted that our study deals with EPC dysfunction in a wider sense and incorporates STZ diabetic model as well as diabetes in humans, irrespective of type of diabetes and the method of diabetes induction in mice. Our premise is that our “study panel” will faithfully identify EPD – irrespective of type of diabetes and model of such induction. Also, we have used a composite population of EPCs – which may include late as well as early EPCs. As is evident from our current data, our study panel comprising the parameters for detection of EPD remain the same irrespective of onset, pathophysiology, etiology and type of diabetes.

True h-EPCs have been identified in current study as CD34+ CD133+ VEGFR2+ cells while m-EPCs have been identified using Dil-LDL+ UEA1+ positivity (Desai et al., 2009; Kanji et al., 2014). Researchers use a varying set of cellular assays for detection of EPD, such as colony forming unit assay (Tepper et al., 2002), colony/cellular adhesion assay, proliferation potential (Mai et al., 2009) and viability detection assay (Choi et al., 2004). For functionality detection, tubule formation assay and migration assay are routinely employed (Chen et al., 2007). NO, a strong indicator of EPC-dysfunction and ROS are both used with varying frequency. These parameters are most often used in conjunction with each other, in isolation, or in various permutations and combinations as per the requirement (Tepper et al., 2002; Li et al., 2011; Kanitkar et al., 2013). Admittedly, the freedom of choice lies with the researchers, but these factors increase the
variability in the obtained results, and the interpretations thereof. Apart from the aforesaid, EPCs from varying sources may possibly vary in their responses to HG-exposure and this may reduce the extrapolative values of the data produced using these systems.

In the current study we demonstrate that EPCs differ in their responses depending on the cell sources and thus highlight the need of such a test panel which can identify EPD irrespective of source, site and pathophysiology of diabetes (Figures 1–4). Other groups have demonstrated similar findings w.r.t. tubule formation and systemic dissimilarities in their response to treatment alternatives in DM or other disorders (Mestas et al., 2004; Driver et al., 2011; Seok et al., 2013). Hypoglycaemic agents like metformin are known to enhance EPC function. However, it may be worth noting that in spite of this treatment, the diabetic human EPCs showed significantly compromised functionality in the parameters of our test panel, underscoring the importance of the present study.

The bottom line is that the current study fills the need for a comprehensive study that demonstrates the points of similarities/dissimilarities between human and murine EPCs and highlights their differences/similarities with respect to high glucose exposure or in vivo diabetes.

Our current, and other unpublished data, where we have used higher concentrations for longer time intervals, show that these cellular functions are not very strongly affected by further increase in glucose concentrations. So although useful, our studies counter-recommend them for accurate detection of diabetes-induced EPD.

Decreased production of NO is a definite indicator of EPD (Tabit et al., 2010). We find that NO is affected by HG concentration as well as by DM in m-EPCs, but not in h-EPCs (Figure 3A). This finding is in opposition of many other findings. Our eNOS gene expression data concurs with our NO assay, thus validating the findings of the earlier dataset. It is known that NO has multiple sources in mice as well as humans, and some of the sources – other than eNOS – may be the cause of this NO production.

Although both types of EPCs show uniform increase on exposure to HG (Figure 4A), the in vivo system shows a different story. The ROS levels in h-
derived ND-EPCs were higher than their murine counterparts (Figure 4B). Perhaps h-EPCs are better “buffered” against the increase in ROS levels than their murine counterparts. This aspect needs to be studied.

EPCs may compensate for oxidative stress levels in early diabetes by increasing expression of anti-oxidative enzymes, especially MnSOD (Callaghan et al., 2005; Marrotte et al., 2010; Sukmavati et al., 2015).

In summary, we clearly show that the current assay panel may be employed for accurate detection of HG-/DM-induced EPD unfettered by the concerns of in vitro/in vivo, diabetes type or source and site of sample used. In these circumstances the HG-/DM-EPCs will make an excellent model for universal detection of EPD, or for its experimental manipulation for rescue of the same.

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AUTHOR CONTRIBUTIONS
KD and MK researched data and wrote the manuscript. SK and RD researched data. MK designed the study. VK wrote, reviewed and edited the manuscript, directed the research and obtained financial support.

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