

# The Effect of Age and Gender on the Frequency of Peripheral Blood Endothelial Precursor Cells

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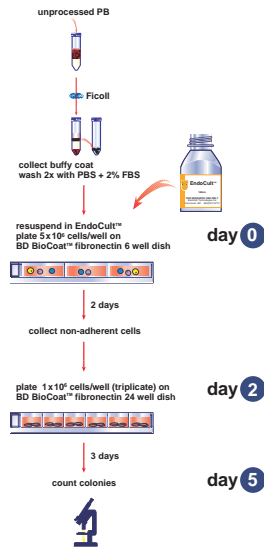
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## Background

Increasing evidence suggest that endothelial precursor cells (EPC) are present in the systemic circulation of adults and can contribute to vascular repair, regeneration and tumour growth. The frequency of circulating EPC may also serve as a biological marker for vascular function and cardiovascular disease risk (Hill *et al.* 2003 NEJM; Vasa *et al.* 2001 Circ Res). For example, Hill *et al.* described an *in vitro* assay that showed an inverse correlation between the number of colony-forming units of EPC and the risk of cardiovascular disease in healthy men. However, there has been no systematic study of the frequency of peripheral blood (PB) EPC in the general population. We have used this assay to monitor the frequency of PB EPC in adult men and women of varying ages to establish the normal ranges and temporal fluctuations. In addition, we have determined whether sample cryopreservation alters the assayed EPC frequency. PB EPC were assayed as described in Figure 1. PB mononuclear cells were suspended in EndoCult<sup>™</sup> liquid medium and plated on fibronectin coated 6 well plates (5 x 10<sup>6</sup> cells/well). After 48 hours, non-adherent cells were plated in triplicate (10<sup>6</sup> cells/well) on 24 well fibronectin coated plates and scanned for endothelial colony growth three days later. An endothelial colony was defined as a central core of round cells with elongated sprouting cells at the periphery.

## Methods

Figure 1. 5 day EPC colony assay



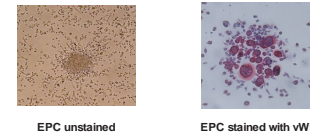
## Results

Table 1  
Frequency of PB EPC in the general population

gender	male (mean ± SD)	female (mean ± SD)
number of donors	15	13
age range	23-54	24-54
EPC/10 <sup>6</sup> cells (range)	21 ± 18 (1-67)	20 ± 17 (2-58)
EPC/mL of blood	35 ± 35	26 ± 23

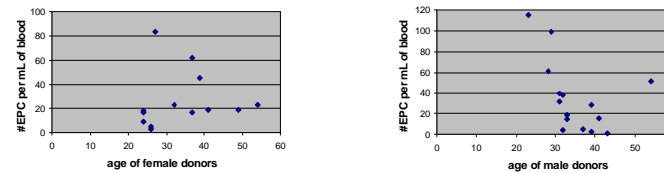
PB samples were obtained with consent from male and female donors within similar age ranges and cultured as described in Figure 1. There was no significant difference between gender and average PB EPC frequency in the general population. However, we did observe variability in the number of EPC per 10<sup>6</sup> cells from different donors.

Figure 2  
Representative colonies after 5 days of culture



All photographs were taken at 125 x magnification on day 5. Photograph 2 shows von Willebrand factor (vWF) staining using the APAAP procedure in which colonies were fixed with 4% paraformaldehyde and stained at 1:50 dilution with vWF (clone 2F2-A9 from BD Pharmingen).

Figure 3  
No correlation in PB frequency with age in both male and female donors



The frequency of EPC per mL of ficolled blood was measured in 15 male and 13 female donors. EPC number did not correlate with age or white blood cell count.

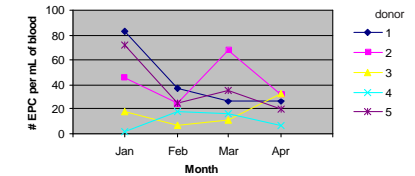
Table 2  
Assessment of the variability within the EPC assay (intra-assay variability)

tube #	total start cell # pre-Ficol (10 <sup>6</sup> )	recovery from Ficol (%)	total cell # post-Ficol (10 <sup>6</sup> )	# EPC/10 <sup>6</sup> cells plated	# EPC/mL Ficolled blood
1	22.3	27.3	6.1	21	80
2	22.3	25.8	5.8	23	74
3	22.3	25.8	5.8	23	74
4	22.3	19.7	4.4	20	52
5	22.3	25.8	5.8	15	48
6	22.3	25.8	5.8	30	96
7	22.3	23.9	5.3	24	67
8	22.3	28.3	6.3	29	104
9	22.3	21.0	4.7	23	60
10	22.3	22.6	5.0	25	70
mean ± SD	22.3 ± 0.0	24.6 ± 2.8	5.5 ± 0.6	23 ± 4	72 ± 18

PB from one male donor was collected and a cell count performed (4.45 x 10<sup>6</sup> cells/mL). Five mL of this sample was aliquoted into ten separate tubes and the EPC assay was performed on the blood sample from each individual tube as

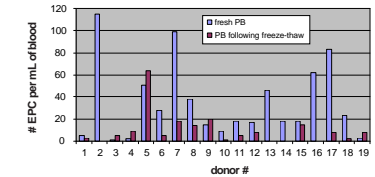
described in Figure 1. The coefficient of variation (CV) for the intra-assay variability was 25%. This variability is much lower than the variability observed in the general population (Table 1).

Figure 4  
Temporal fluctuations in the PB frequency in the general population



The frequency of PB EPC was measured in 5 donors (3 females (1-3) and 2 males (4, 5)) once a month over a four-month period using the assay described in Figure 1. There are fluctuations in the frequency of EPC in the systemic circulation of individuals in the general population.

Figure 5  
Sample cryopreservation alters the EPC frequency



To test the effect of cryopreservation, the frequency of PB EPC was measured in 19 donors using the assay described in Figure 1. For each donor, the EPC frequency from fresh PB was determined. The PB from each donor (10<sup>7</sup> cells) was frozen in a medium containing 90% FBS/10% DMSO in the gaseous phase of liquid nitrogen. One week later, samples were thawed and subjected to the same assay. Cryopreservation reduced the number of assayed EPC in 14 out of 19 samples, and in 12 of these the decrease was more than 50%. This reduction could not be attributed to general cell loss, as the cellular recovery (93 ± 2%) and viability (90 ± .05%) were high.

## Conclusions

- There was no correlation between PB EPC frequency and age or gender
- The range of PB EPC for both males and females was variable
- Cryopreservation reduced the number of EPC in 74% of samples, indicating that improved freeze-thaw methods maybe required if EPC are to be quantitated from frozen samples and used in clinical studies
- Continued monitoring of EPC frequency in a greater number of individuals will establish normal ranges and temporal fluctuations for clinical assessments of risk factors for diseases