Maturing EPCs into endothelial cells: may the force be with the EPCs.
Focus on “Fluid shear stress induces differentiation of circulating phenotype endothelial progenitor cells”

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ENDOTHELIAL PROGENITOR CELLS (EPCs) are a unique cell type found circulating in the peripheral blood with the capacity to become mature endothelial cells. EPCs can be released from many sources including the bone marrow, adipose tissue, the vessel wall, as well as potentially the spleen, liver, and intestine (4). Currently, there are two main methods of isolating EPCs: 1) separation during in vitro cell culture by morphological properties/differences, such as time of outgrowth, or 2) cell sorting with specific markers (9). More specifically, cell sorting relies on key markers, such as CD133, CD34, vascular endothelial growth factor (VEGF) receptor 2 (VEGF-R2), or a combination of these markers to isolate and purify an EPC population (12). There still remains debate with respect to the optimal isolation and culturing procedures; however, there is a consensus regarding the therapeutic potential of EPCs as a cell source for regenerative medicine, including for both replacement and endogenous repair.

Evaluation of EPCs as an endothelial cell source for prosthetic vascular grafts shows that these cells (2, 3, 5, 7, 13) can 1) be isolated easily and quickly, 2) grow well and in large numbers in vitro, 3) respond similarly, although not identically, to shear stresses ex vivo and in vitro as other endothelial cells, and 4) provide a bioactive endothelial cell barrier limiting neointimal thickening and increasing patency in the area of implantation. Endogenously, EPCs home to the site of endothelial injury through the following three steps: 1) activation, where the EPCs are deployed from their resident site as a result of EPC-activation factors, such as hypoxia-inducible factor 1α, VEGF, chemokine (C-X-C motif) ligand 12, and erythropoietin; 2) targeting to the area of interest; and 3) exertion of their influence at the desired site (12). Each of these steps is an area of intense study. Previous studies primarily investigated the behavior of adherent EPCs (3, 13); the unanswered question has been how circulating EPCs change in response to their shear environment into a more endothelial phenotype as they home to the site of endothelial injury. Obi et al. (10) seek to answer this question in their study in this issue of American Journal of Physiology-Cell Physiology.

Fig. 1. Immature, circulating endothelial progenitor cells (EPCs) are primed by shear to become more endothelial-like. Cues from sites of vascular injury initiate EPC mobilization from the bone marrow, among other sources. As EPCs circulate, their shear stress environment stimulates differentiation, as noted by changes in many cellular functions and endothelial cell (EC) markers, into endothelial-like cells for vascular repair. SMCs, smooth muscle cells.
Researchers have recently begun to investigate the role of shear on the adherence of circulating EPCs. For example, Angelos et al. (1) investigated the optimal shear magnitude (1 dyn/cm²) for late outgrowth circulating EPCs to bind to fibronectin (1). Xiao et al. (15) determined that CD133 antibody was the most effective in capturing circulating rat EPCs, that shear increases nitric oxide production, cell proliferation, and retention, and that the optimal shear stress magnitude for these effects is 15 dyn/cm². Stellos et al. (14) showed that adhesion of CD34+ cells to immobilized platelets is attributable to the interaction between the junctional adhesion molecule (JAM-C) and Mac-1 integrin. Moreover, Kawahara et al. (8) demonstrated that fibronectin captures more EPCs than VEGF and VEGF-R2 (Flk1/KDR) when tested over a range of shear stress conditions (0–200 dyn/cm²). Finally, Sekiguchi et al. (11) showed that slow adherent bone marrow mononuclear cells have increased endothelial potential compared with fast adherent cells and that this phenomenon is increased by exposure to shear, as determined by expression of angiogenic cytokines and growth factors (11).

While these studies have provided an understanding as to how EPCs adhere and of the optimal conditions for this adhesion, no studies have investigated how circulating, nonadherent EPCs may differentiate as a result of their shear environment. In their study, Obi et al. examined how shear induces differentiation of immature progenitor cells derived from human cord blood. They exposed CD133+ human EPCs to a flow environment using a rotating-cone bioreactor and then assessed a variety of cellular functions and markers. The results indicate that exposure of circulating EPCs to this flow environment increases adhesion in a phosphatidylinositol 3-kinase (PI3K)- and mammalian target of rapamycin (mTOR)-dependent manner, tube formation, and colony formation in a PI3K-, mTOR-, extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun NH2-terminal kinases (JNK), and p38-dependent manner as well as proliferation by PI3K, mTOR, ERK1/2 signaling pathways as illustrated in Fig. 1. Moreover, exposure to this flow environment decreased apoptosis while inducing EPC maturation, as indicated by decreased DNA fragmentation and increased expression of shear-induced endothelial cell markers and phosphorylation of VEGF-R2 (Flk-1/KDR), respectively.

Obi et al. exposed EPCs to a flow environment that had a steady, unidirectional shear (0.25–2.5 dyn/cm²). As EPCs circulate in vivo, however, they are exposed to a wide range of shear environments similar to circulating leukocytes whose shear stresses span from a few dyn/cm² to upwards of 300 dyn/cm² (6). Furthermore, circulating EPCs experience a variety of flow environments including unsteady, multidirectional shear. It is unclear which aspect of flow (i.e., shear stress or shear rate) influences EPC behavior. Obi et al.’s results show that shear primes the EPC to become a mature endothelial cell in transit to its worksite. These results provide insight into how immature, circulating EPCs differentiate in response to flow and could participate in vascular regeneration and repair (both natural and engineered).

There are several possible directions for future research in the EPC field. As an example, at this time it is difficult to work with a pure EPC population due to limitations of cell sorting and culture; however, the field will benefit from experiments on a pure population. More specifically, Obi et al. found that only a portion of circulating EPCs attach. Is this preferential attachment due to the shear stress gradient of the bioreactor or is it due to a subpopulation of EPCs? Further investigation into different well-defined, single-magnitude stress conditions including higher shear magnitudes, as well as oscillating shear, could provide additional insight into this shear-dependent differentiation mechanism. Additionally, an understanding of the cellular deformation of the EPCs as they pass through the microcirculation may provide additional information as to how mechanical cues influence the EPCs.

In summary, Obi et al. investigate how circulating, immature, nonadherent cells are primed by the flow environment to become more endothelial-like. This study represents an initial step in our understanding of how circulating EPCs mature and are differentiated by their flow environment. Together with the insight that will be provided by the future studies, such as those suggested above, critical knowledge about how the flow environment participates in the preconditioning of EPCs for regeneration and repair should be achieved.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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