Human Endothelial Progenitor Cells
What is Endothelial Progenitor Cell (EPC)?

• A primitive cell type in the endothelial lineage

• A bone marrow derived cell with properties similar to those of an embryonic angioblast

• Migrate into the blood stream and is able to differentiate into a VARIETY of mature vascular endothelial cells
EPC generation, mobilization, recruitment, and differentiation

EPC can be distinguished from other blood cells by certain surface markers

<table>
<thead>
<tr>
<th>DNA</th>
<th>RNA</th>
<th>CD45</th>
<th>CD133</th>
<th>CD34</th>
<th>CD105</th>
<th>CD146</th>
<th>CD144</th>
<th>CD31</th>
<th>CD13</th>
<th>CD117</th>
<th>VEGFR1</th>
<th>VEGFR2</th>
<th>VEGFR3</th>
</tr>
</thead>
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Nature Reviews Cancer

EPC has a variety of applications

A. Cancer drug discovery:
   - EPC make great contribution to endothelial outgrowth
     (Lin et al., 2000, J Clin Invest 105:71; over 75% at late stages)
   - EPC incorporate into developing tumour blood vessels
     (Asahara et al., 1999, Circ Res., 85:221-228)
   - EPC specifically invade towards human ovarian cancer cells
     (Bagley et al., 2003, Cancer Res.)
   - EPC rescue impaired tumor angiogenesis in transgenic mice
     (Carmeliet et al., 2001, Nature Medicine 7:575)
   - EPC play a very important role in robust angiogenesis during tumor re-growth (VDAs)
     (Shaked et al. 2006, Science 313: 1785)

B. Cancer drug delivery:
C. Cardiovascular disease diagnostics and therapeutics

### Table 1 Conditions, drugs and cytokines that may affect number and function of human EPCs.

<table>
<thead>
<tr>
<th>Condition or factor</th>
<th>Changes in number/function of EPCs or CD34+ cells</th>
<th>Investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physiological:</strong></td>
<td></td>
<td></td>
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<tr>
<td>Gender (eg, estrogens)</td>
<td>↑ CD34+/VEGFR2+ cells</td>
<td>Strehlow et al., <em>Circulation</em> (2003)</td>
</tr>
<tr>
<td>Physical training</td>
<td>↑ EPC number</td>
<td>Adams et al., <em>ATVB</em> (2004)</td>
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<td><strong>Pathological:</strong></td>
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<tr>
<td>Coronary artery disease/number of risk factors</td>
<td>↓ EPC number and migration</td>
<td>Vasa et al., <em>Circ Res</em> (2001)</td>
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<tr>
<td>Smoking</td>
<td>↓ CD34+/KDR+ cells</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>↓ EPCs or CD34+/KDR+ cells</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>↓ EPC migration</td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>↑ CD34+ cells</td>
<td>Shintani et al., <em>Circulation</em> (2001)</td>
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<tr>
<td></td>
<td>↑ CD34+/AC133+/VEGFR2+ cells</td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure (class I-II)</td>
<td>↑ CD34+ cells</td>
<td>Valgimigli et al., <em>Circulation</em> (2004)</td>
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<tr>
<td></td>
<td>↑ CD34+/AC133+/VEGFR2+ cells</td>
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<tr>
<td></td>
<td>↑ EPC CFUs</td>
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<tr>
<td>Congestive heart failure (class III-IV)</td>
<td>↓ CD34+ cells</td>
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<tr>
<td></td>
<td>↓ CD34+/AC133+/VEGFR2+ cells</td>
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<tr>
<td></td>
<td>↓ EPC CFUs</td>
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</tr>
<tr>
<td>In-stent restenosis</td>
<td>↓ EPC CFUs</td>
<td>George et al., <em>ATVB</em> (2003)</td>
</tr>
<tr>
<td></td>
<td>↓ EPC adhesion</td>
<td></td>
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<tr>
<td><strong>Drugs and cytokines:</strong></td>
<td></td>
<td></td>
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<tr>
<td>HMG-CoA reductase inhibitors</td>
<td>↑ EPC number</td>
<td>Dimmeler et al., <em>J Clin Invest</em> (2001)</td>
</tr>
</tbody>
</table>

D. Diabetes Mellitus drug discovery

*In vivo* alterations in EPC number of diabetic patients
*In vitro* reduction in EPC function in type 1 and type 2 diabetic patients
ApproCell’s EPC have typical endothelial cobblestone morphology in culture

EPC at low density

EPC at high density
ApproCell’s EPC express CD34 and CD45

CD34 is expressed in EPC (P4), but not in HUVEC (P4)

CD45 is expressed in EPC (P4), but not in HUVEC (P4).
ApproCell’s EPC are double-positive for LDL uptake and and Lectin binding

EPC passage 6

HUVEC passage 5

Red: Dil-Ac-LDL
Green: FITC-Ulex-Lectin
Blue: DAPI nuclear staining
ApproCell’s EPC express vWF

EPC, passage 3  HUVEC, passage 4  Saos-2 cell line
ApproCell’s EPC form typical capillaries in the sprouting assay
ApproCell’s EPC migrate in response to VEGF induction
QC assays for ApproCell’s EPC

A. Morphological Analysis

B. FACS analysis of certain surface markers

C. qRT-PCR analysis of EPC related gene expression

D. Immunocytochemical analysis of EPC related protein expression

E. Functional analysis

F. Bio-safety tests
   Sterility
   Mycoplasma
   Hepatitis B
   Hepatitis C
   HIV-1
EPC products from ApproCell

A. EPC cells
   - Fresh EPC in T25
   - Fresh EPC in T75
   - Fresh EPC in T225
   - Frozen EPC in Cryovial

B. EPC Kits
   - EPC Proliferation kit (96-well format)
   - EPC migration kit (24-well format)
   - EPC tumor invasion kit (24-well format)
   - EPC tube formation kit (48-well format)

C. EPC medium
   - EPC growth medium
   - EPC freezing medium
   - EPC growth supplement

D. EPC related
   - EPC protein lysates
   - EPC RNA
   - EPC cDNA
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