Stem Cell Transplantation in Diabetic Kidney Disease (DKD)

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Chronic kidney disease (CKD): Gradual loss of kidney function over time

• 15% of US adults—37 million people—are estimated to have chronic kidney disease (CKD)

• Diabetes is one of the major causes of CKD in adults.

Diabetic kidney Disease (DKD)

• DKD is associated with sclerotic glomerulus and poor perfusion.
Control high blood pressure
- Manage high blood sugar
- Lower high cholesterol
- Control protein in urine.

### Treatments

<table>
<thead>
<tr>
<th>STAGES OF CHRONIC KIDNEY DISEASE</th>
<th>GFR*</th>
<th>% OF KIDNEY FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1  Kidney damage with normal kidney function</td>
<td>90 or Higher</td>
<td>50–100%</td>
</tr>
<tr>
<td>Stage 2  Kidney damage with mild loss of kidney function</td>
<td>89 to 60</td>
<td>59–60%</td>
</tr>
<tr>
<td>Stage 3a Mild to moderate loss of kidney function</td>
<td>59 to 45</td>
<td>50–65%</td>
</tr>
<tr>
<td>Stage 3b Moderate to severe loss of kidney function</td>
<td>44 to 30</td>
<td>44–30%</td>
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</table>

### Treatment for advanced diabetic kidney disease

- Kidney dialysis
- Transplant

### Future treatment

**regenerative medicine**: These techniques may help reverse or slow kidney damage
- Pancreas islet cell transplant
- Stem cell therapy

**MSC**  **EPC**
Two Major Types of Adult Stem Cells from BM

• Short term such as Endothelial Progenitor Cells- (part of hemopoetic stem cells) necessary for **acute repair** of ischemic injury,
  • CD34+
  • KDR+ve
  • Mostly in marrow
  • 1% in periphery

• Long term such as Mesenchymal Stem Cells-for body structural tissue repair, or **chronic repair**
  • CD73, CD105, CD90 +ve
  • Source could be any mesenchymal tissue
Endothelial progenitor cells (EPC)

- Endothelial progenitor cells augments neovascularization of tissue after ischemia endothelial injury
- Provide a novel therapeutic option
Our Aim Was To Note…

• If EPCs or modified endothelial progenitor cells augments neovascularization

• Compare EPC therapy with another possible stem cell therapy such as MSC as a therapy in DKD
EPCs have regenerative property in heart

5~6-week old S-D rats

Take peripheral blood (1000 μl) 

EPCs expansion

Intramyocardial injection (5 sites)

Saline or EPC

1 week

12 weeks

Echocardiography Immunohistochemical analysis

Autologous transplantation of EPCs Post-MI

Sen et al, HGT 2010
Comparison of Myocardial Infarction with or without EPC

Trichrome Staining

Representative findings of elastic tissue trichrome staining of the left ventricle samples
Human trials using EPCs


- Other forms of cell therapy in cardiac ischemia has also been used such as iPSc and cardiomyocytes stem cells (Anaversa et al)

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Cell therapy, n/N (%)</th>
<th>Control, n/N (%)</th>
<th>MACCE</th>
<th>Haz. Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td></td>
<td></td>
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<tr>
<td>≤57</td>
<td>40/556 (11.2)</td>
<td>35/237 (14.8)</td>
<td>0.82</td>
<td>(0.52, 1.29)</td>
<td>.73</td>
</tr>
<tr>
<td>&gt;57</td>
<td>67/411 (16.3)</td>
<td>44/048 (17.7)</td>
<td>0.91</td>
<td>(0.62, 1.33)</td>
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<tr>
<td><strong>Ejection Fraction (%)</strong></td>
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<td></td>
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<tr>
<td>≤45</td>
<td>65/467 (13.9)</td>
<td>47/257 (18.3)</td>
<td>0.72</td>
<td>(0.59, 1.05)</td>
<td>.15</td>
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<tr>
<td>&gt;45</td>
<td>42/300 (14.0)</td>
<td>32/228 (14.0)</td>
<td>1.10</td>
<td>(0.70, 1.75)</td>
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<tr>
<td><strong>Baseline EDV (ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>≤130</td>
<td>64/367 (17.4)</td>
<td>33/205 (16.1)</td>
<td>1.10</td>
<td>(0.72, 1.68)</td>
<td>.12</td>
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<tr>
<td>&gt;130</td>
<td>43/400 (10.8)</td>
<td>46/201 (16.4)</td>
<td>0.89</td>
<td>(0.46, 1.32)</td>
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<td><strong>Anterior AMI</strong></td>
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<tr>
<td>no</td>
<td>13/105 (12.4)</td>
<td>11/70 (15.7)</td>
<td>0.79</td>
<td>(0.35, 1.77)</td>
<td>.78</td>
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<tr>
<td>yes</td>
<td>94/662 (14.2)</td>
<td>68/415 (16.4)</td>
<td>0.89</td>
<td>(0.65, 1.22)</td>
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<tr>
<td><strong>Maximal CK (U/L)</strong></td>
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<tr>
<td>≤3450</td>
<td>69/539 (12.8)</td>
<td>57/385 (15.6)</td>
<td>0.85</td>
<td>(0.60, 1.21)</td>
<td>.73</td>
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<tr>
<td>&gt;3450</td>
<td>38/226 (16.7)</td>
<td>22/120 (18.3)</td>
<td>0.95</td>
<td>(0.56, 1.61)</td>
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<tr>
<td><strong>Gender</strong></td>
<td></td>
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<tr>
<td>female</td>
<td>24/153 (15.7)</td>
<td>10/61 (16.4)</td>
<td>0.95</td>
<td>(0.59, 1.53)</td>
<td>.81</td>
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<tr>
<td>male</td>
<td>59/314 (12.3)</td>
<td>63/340 (15.6)</td>
<td>0.87</td>
<td>(0.58, 1.30)</td>
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<td><strong>Diabetes</strong></td>
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<td>no</td>
<td>89/656 (13.6)</td>
<td>65/405 (16.0)</td>
<td>0.84</td>
<td>(0.61, 1.16)</td>
<td>.32</td>
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<td>yes</td>
<td>19/111 (16.2)</td>
<td>14/79 (17.7)</td>
<td>1.24</td>
<td>(0.62, 2.51)</td>
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<td><strong>Hypertension</strong></td>
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<tr>
<td>no</td>
<td>53/386 (11.3)</td>
<td>52/304 (13.0)</td>
<td>1.00</td>
<td>(0.79, 1.29)</td>
<td>.16</td>
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<tr>
<td>yes</td>
<td>54/384 (11.4)</td>
<td>50/244 (20.5)</td>
<td>0.74</td>
<td>(0.51, 1.09)</td>
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<tr>
<td><strong>Hyperlipidaemia</strong></td>
<td></td>
<td></td>
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<tr>
<td>no</td>
<td>40/229 (12.2)</td>
<td>31/207 (15.0)</td>
<td>0.79</td>
<td>(0.49, 1.26)</td>
<td>.34</td>
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<tr>
<td>yes</td>
<td>55/037 (14.2)</td>
<td>35/229 (15.4)</td>
<td>1.07</td>
<td>(0.70, 1.63)</td>
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<tr>
<td><strong>Smoking</strong></td>
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<tr>
<td>no</td>
<td>41/098 (13.3)</td>
<td>31/179 (13.7)</td>
<td>0.88</td>
<td>(0.55, 1.31)</td>
<td>.91</td>
</tr>
<tr>
<td>yes</td>
<td>55/096 (13.9)</td>
<td>38/245 (15.6)</td>
<td>0.89</td>
<td>(0.56, 1.36)</td>
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<td><strong>MRI</strong></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>no</td>
<td>40/225 (14.5)</td>
<td>42/228 (18.4)</td>
<td>0.89</td>
<td>(0.58, 1.38)</td>
<td>.88</td>
</tr>
<tr>
<td>yes</td>
<td>67/492 (13.6)</td>
<td>37/257 (14.4)</td>
<td>0.93</td>
<td>(0.62, 1.39)</td>
<td></td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td>107/767 (14.0)</td>
<td>79/485 (16.3)</td>
<td>0.88</td>
<td>(0.66, 1.18)</td>
<td></td>
</tr>
</tbody>
</table>

FACS Analysis of Human EPCs on exposure to HG

Human EPC, Post 48hrs (2 days) of High Glucose Exposure by FACS Analysis

Within 48hrs there is significant apoptosis and death of EPCs in high glucose
Genes Responsible for the Apoptosis in Hyperglycemia

Relative mRNA Expression in Folds Normal: High Glucose
P53 Gene is activated by multiple factors:

- Super-oxide Accumulation
- Inflammation

Diagram:

- p53
- Bax
- PUMA
- Bcl-2
- Smac
- Cyt c
- Apaf-1
- Caspase-9
- Caspase-3
- IAPs
- Apoptosis
- Cellular targets

Knocking out or silencing p53 may prevent apoptosis and result in EPC survival.
Increased survivability in p53 knockout EPC

- Permanent Knocking down p53 could be translationally detrimental
- **Transient silencing of p53 by adenovirus**

Use of p53-Silenced Endothelial Progenitor Cells to Treat Ischemia in Diabetic Peripheral Vascular Disease

Nabarita Kundu, PhD; Clayton C. Domingues, PhD; Cyril Chou, BS; Nae-Ki Ahn-Madi, BS; Sara Houston, PhD; D. Joseph Jerry, PhD; Satyayudha Sen, MD

**Background**—Peripheral vascular disease is a major diabetes mellitus-related complication. In this study, we noted that expressions of proangiogenic p53 gene and its downstream cascade gene such as p21 are upregulated in hyperglycemia. Therefore, we investigated whether p53- and p21-silenced endothelial progenitor cells (EPCs) were able to survive in hyperglycemic milieu, and whether transplantation of either p53 knockout (KO) or p21KO or p53- and p21-silenced EPCs could improve collateral vessel formation and blood flow in diabetic vaso-occlusive peripheral vascular disease mouse models.

**Methods and Results**—We transplanted p53 and p21KO mouse EPCs (mEPCs) into streptozotocin-induced diabetic (type 1 diabetes mellitus model) C57BL/6J and db/db (diabetes model) post-femoral artery occlusion. Similarly, p53-silenced and Ad-p21-silenced human EPCs (CD34+) cells were transplanted into streptozotocin-induced diabetic NOD.CB17-Prkdcsng/scid/j mice. We measured blood flow at 3, 7, and 10 days and hindlimb muscles were obtained post sacrifice for mRNA estimation and CD31 staining. Enhanced blood flow was noted with delivery of p53 and p21KO mEPCs in streptozotocin-induced diabetic C57BL/6J mice. Similar results were obtained when human Ad-p53shEPCs (CD34+) and Ad-p21shEPCs (CD34+) were transplanted into streptozotocin-induced obese diabetic severe combined immunodeficiency mice. Gene expression analysis of p53 and p21KO EPCs transplanted hindlimb muscles showed increased expression of endothelial markers such as endothelial nitric oxide synthase, vascular endothelial growth factor A, and platelet endothelial cell adhesion molecule 1. Similarly, quantitative reverse transcriptase polymerase chain reaction of human Ad-p53shEPCs (CD34+) and Ad-p21shEPCs (CD34+)–transplanted hindlimb muscles also showed increased expression of endothelial markers such as vascular endothelial growth factor A, noted primarily in the p53-silenced EPCs group. However, such beneficial effects was not noted in the db/db type 2 diabetic mouse.

**Conclusions**—Transient silencing of p53 using adenoviral vector in EPCs may have a therapeutic role in diabetic peripheral vascular disease. (*J Am Heart Assoc.* 2017;6:e005146. DOI: 10.1161/JAHA.116.005146.)

Key Words: adenovirus vector • apoptosis • diabetes mellitus • endothelial progenitor cells • gene therapy

Diabetes mellitus affects more than 11% of US adults and is projected to nearly double by 2025. The presence of moderate hyperglycemia in addition to mild and moderate obesity may confer significant cardiovascular risk. The American Diabetes Association has reported that coronary artery disease and stroke are 3 times more common in prediabetic compared with nondiabetic patients, and overt diabetes mellitus increases this risk 5- to 10-fold. Many patients with prediabetes are either overweight or obese. Both diabetes mellitus and obesity are associated with cardiovascular complications such as endothelial dysfunction, oxidative stress, endothelial cell inflammation, and cardiovascular prothrombotic states. These complications are primary reasons of cardiovascular disease. Cardiovascular disease includes cerebral, cardiac, and peripheral vascular diseases (PVDs). In this study, our main focus will remain on PVD as a consequence of diabetes mellitus. One of the major consequences of diabetes mellitus is PVD. PVD is an inadequate perfusion of blood in the peripheral arteries often triggered by atherosclerosis, one of the major consequences of diabetes mellitus. Every day, 230 patients undergo diabetes mellitus-related amputation due to...
Model: Type 1 Diabetes
The Promise of Mesenchymal Stem Cell Therapy for Diabetic Kidney Disease: Current Diabetes Reports: May 2016, 16:42

Results from animal models suggest distinct potential for systemic MSC infusion to favourably modulate DKD progression.

However, only a few early phase clinical trials have been initiated and efficacy in humans remains to be proven.

Key knowledge gaps and research opportunities exist in this field.

Recent PubMed search shows 21 publications on MSC transplant with only 1 on PBMNC ell therapy in DKD in last 3 years.
Use of Mesenchymal Stromal Cells (MSC) as a therapy

Mesenchymal stromal cells (MSCs) has multipotent differentiation capacity
However, epithelial to mesenchymal cell transformation is of concern post MSC therapy
Transplantation of P53-Silenced Endothelial Progenitor Cells (EPCs) Under Renal Capsule

Type 1 DM mouse. Blood glucose >250mg/dL

Developed proteinuria

STZ

Sub capsular transplantation of Ad-p53sh-EPC/ mMSc

Urine collection for creatinine and protein concentration: week 1, 2, 3, 4

Laser doppler, Ultrasound

Sacrificed mice and collect blood and kidney
Results

Area under the curve (AUC) of urine protein/creatinine concentration ratio decreased with p53sh EPC transplantation.
Blood flow (perfusion) in transplanted kidney increased with p53sh EPCs.

Results

Ultrasound analysis (Visual Sonics)

Measurements of blood pressure and heart rate

Blood flow (perfusion) in transplanted kidney increased with p53sh EPCs.
Blood flow (perfusion) in transplanted kidney increased with p53sh EPCs

Laser doppler analysis
Gene expression analysis showed markers of angiogenesis upregulated with p53sh EPC transplantation.
Renal Histology: H&E AND Isolectin-β4 staining showing improved glomerular architecture, with p53sh EPC delivery
• Transplanting p53 silenced mouse EPCs under renal capsule improves proteinuria, renal blood flow and improves renal histology.

• Transplanting p53 silenced mouse EPCs is more beneficial compared to MSC transplantation, taking all outcome measurements into consideration.
Conclusion

- Modified EPC transplantation may have a prominent cell therapy role in Diabetic Vascular Complications such as DKD.
Stem Cells - Is it the Holy Grail (Chalise of Life) for Kidney Regeneration?

*The Damsel of the Sanct Grael* by Dante Gabriel Rossetti
Some Progress but Lots more to achieve in order to understand stem cell behavior in Hyperglycemia

The process of scientific discovery is, in effect, a continual flight... from wonder. ...........Albert Einstein
Team
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Kristina Rother, NIDDK
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Paul Marvar, Pharmacology-Physiology, GW
Vivek Jain, Pulmonary Medicine, GW

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