MNCs and Their Use in the Generation of Tissue Engineered Vascular Grafts: of Mice, Monocytes and Mendoza

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Denver, Colorado

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Conflict of Interest Statement

Fenwal:  Clinical Device Trials

Pall Corp:  Consultant

Genzyme:  Consultant
Definition of Cell Therapy

Discipline that encompasses the varied use of cellular and non-cellular elements to treat, improve, repair, grow or replace various tissues or organs; and to treat, prevent or cure, various types of diseases.
Regenerative Medicine

• Term first coined in a 1987 Workshop
• Old Term – “Tissue Engineering”

Multidisciplinary technologies used to control cell behavior to allow their implantation in combination with non-biological scaffolds, in place of traditional synthetic prostheses, to effect tissue repair and reconstruction*

• Progression from the inert to the viable

• *Rhodes N, Vox Sang 2004;87:(suppl 2),s161-3
Some Cell Sources for Cell Therapy

- Cord Blood
- Embryonic Stem Cells
- Mesenchymal Cells
- Ex-vivo Expanded cells
- Mononuclear Cells
- Bone Marrow cells
- CTL (cytotoxic T- lymphocytes)
- Peripheral blood (CD34+; dendritic cells)
- Adipose tissue cells (fat)
- Endothelial cells
- UVEC
- iPS
- Amniotic fluid cells
- Wharton’s Jelly cells
- Baby Teeth
WHY IS REGENERATIVE MEDICINE BENEFICIAL

Annals of Internal Medicine
Established in 1927 by the American College of Physicians

ARTICLE

Transmission of Hepatitis C Virus to Several Organ and Tissue Recipients from an Antibody-Negative Donor

Bara D. Tugwell, MD; Priti R. Patel, MD, MPH; Ian T. Williams, PhD, MS; Katrina Hedberg, MD, MPH; Feng Che, PhD; Omara V. Nolte, PhD; Ann R. Thomas, MD, MPH; Judith E. Woll, MD; Beth P. Bell, MD, MPH; and Paul R. Cieslak, MD

1 November 2005 | Volume 143 Issue 9 | Pages 648-654

Background: Although hepatitis C virus (HCV) transmission through tissue transplantation has been rarely reported, a donor with undetected viremia may infect several recipients. A patient developed acute hepatitis C shortly after tissue transplantation. Ninety-one tissues or organs had been recovered from the donor.

Investigation of Rabies Infections in Organ Donor and Transplant Recipients — Alabama, Arkansas, Oklahoma, and Texas, 2004

On July 1, this report was posted as an MMWR Dispatch on the MMWR website (http://www.cdc.gov/mmwr).

On June 30, 2004, CDC confirmed diagnoses of rabies in three recipients of transplanted organs and in their common

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Previous
Volume 350:2564-2571 June 17, 2004 Number 25

Clostridium Infections Associated with Musculoskeletal-Tissue Allografts
Ideal Tissue Engineered Graft

- Non-obstructive
- Fully functional
- Non-thrombogenic
- No calcification
- Non-immunogenic
- Infection resistant
- Chemically inert
- Non-hemolytic
- Durable
- Easy to insert
- Permanent
TE Strategies

• I - Implant fresh or cultured cells – donor or patient-derived w/ or w/o a degradable scaffold

• II - Implant complete “mature” 3-D tissue formed in vitro from donor or patient-derived cells w/a scaffold

• III – In situ tissue regeneration – implants a scaffold into injured tissue - promotes autologous repair

• Scaffolds: Not simply a mechanical support: “informative”

(Rezai et al, Artificial Organs 2004;28:142-151)
Types of Scaffolds

• Collagen – Type I, abundant, easily shaped, xenogenic sources; antigenic receptors removed, concern over xenozoonoses remains

• Fibrin – plentiful

• Alginates – “-” charged algae co-polymer

• Hyaluronic acid

• Synthetic polymers -

• Bioceramics – hydroxyapatite, TCP

• Cadaver or animal bones (xenogenic)
Composition

P(LA/CL) : [Poly (L-lactide-co- ε-caprolactone)]

\[
\text{CH}_3
\]

\[
\text{O} \quad \text{-} \quad \text{C} \quad \text{-} \quad \text{C} \quad \text{m} \quad \text{O} \quad \text{-} \quad \text{(CH}_2\text{)}_5\text{C} \quad \text{n} \\
\text{H} \quad \text{O} \\
\text{O}
\]

Product Variation

Color: Violet and Beige

Size: USP2 to USP6-0 are available.

Composition

PGA : Polyglycolic acid

\[
\text{H} \\
\text{O} \quad \text{-} \quad \text{C} \quad \text{-} \quad \text{C} \quad \text{n} \\
\text{H} \quad \text{O}
\]

Characteristics

- Superior knot stability
- Superior flexibility
- Superior tensile strength

Remaining strength in vitro

- Remaining strength (kgf)
- Immersion time (day)
Fig. 1. Schematic of scaffold fabrication using the dual cylinder chamber system. (A) Flat polyester felts were easily rolled into tubular constructs using a gradually tapered cylinder. (B) P(CL/LA) solution sealed the nonwoven polyester tube by creating an interconnecting porous structure between the felt fibers. (C) Cross-sectional image demonstrating the configuration of the components and removal of solvents by lyophilization. (D) Resultant hybrid scaffold pushed out through inlet after inner needle cylinder removed.
Classic Tissue Engineering Paradigm

(start with a scaffold)
Transplantation of a Tissue-Engineered Pulmonary Artery


Figure 1. The Tissue-Engineering Technique.
Venous-wall cells were isolated and expanded in vitro and seeded on a biodegradable polymer scaffold. The construct of cells and polymer was implanted as autologous tissue.
### Centrifugal Seeding
(Roh et al., Tissue Engineering 2007;13:2743-9)

<table>
<thead>
<tr>
<th>% area occupied by cells in</th>
<th>Static N=6</th>
<th>Centrifugal N=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>First 0.5mm</td>
<td>4.98 ± 1.54</td>
<td>7.38 ± 0.87</td>
</tr>
<tr>
<td>Second 0.5mm</td>
<td>0.41 ± 0.19</td>
<td>4.64 ± 0.65</td>
</tr>
<tr>
<td>Third 0.5mm</td>
<td>0.21 ± 0.02</td>
<td>4.22 ± 0.53</td>
</tr>
</tbody>
</table>
Fig. 3. Biomechanical characterization of scaffolds. (A) Burst pressure. (B) Suture retention strength. (C) Young's modulus. (D) Tensile strength of PGA–P(CL/LA) and PLLA–P(CL/LA) scaffolds over 24-week time course.
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Markers</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial Progenitor Cell</td>
<td>+ CD133/ VEGF-R2</td>
<td>0.057 ± 0.033</td>
</tr>
<tr>
<td></td>
<td>- CD45</td>
<td></td>
</tr>
<tr>
<td>Endothelial Progenitor Cell (Monocyte origin)</td>
<td>+ CD14/ VEGF-R2</td>
<td>2.76 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>- CD45</td>
<td></td>
</tr>
<tr>
<td>Mature Endothelial Cell</td>
<td>+ CD31/ CD146</td>
<td>0.061 ± 0.024</td>
</tr>
<tr>
<td></td>
<td>- CD45</td>
<td></td>
</tr>
<tr>
<td>Mesenchymal Stem Cell</td>
<td>+ CD73/ CD90/ CD105</td>
<td>0.0017 ± 0.0010</td>
</tr>
<tr>
<td></td>
<td>- CD45/ CD34</td>
<td></td>
</tr>
<tr>
<td>Hematopoietic Stem Cell</td>
<td>+ CD133/ CD34</td>
<td>0.17 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>- CD45</td>
<td></td>
</tr>
<tr>
<td>Monocyte</td>
<td>+ CD14/ CD45</td>
<td>7.1 ± 3.2</td>
</tr>
<tr>
<td>CD4 T cell</td>
<td>+ CD3/ CD4/ CD45</td>
<td>6.7 ± 2.7</td>
</tr>
<tr>
<td>CD8 T cell</td>
<td>+ CD3/ CD8/ CD45</td>
<td>7.9 ± 1.2</td>
</tr>
<tr>
<td>B cell</td>
<td>+ CD19/ CD45</td>
<td>6.8 ± 2.8</td>
</tr>
<tr>
<td>NK cell</td>
<td>+ CD56/ CD45</td>
<td>3.5 ± 1.7</td>
</tr>
</tbody>
</table>

\( n = 3 \)
BMC
MCP-1
Mono/Macs
Cytokines
VEGF
Endothelial cell
Mono/Macs
SMC

A

B

1 wk
6 wk
10 wk
Conclusions

• Seeded cells are essential to maintain patency of the TE vascular graft- paracrine effect likely

• The host immune cells play an integral role in neotissue formation

• While seeded cells attach to the matrix and are incorporated into the matrix, ultimately it is the host cells that make up most of the neotissue
Pediatric TEVG Model

- Scaffold is seeded and incubated in a bioreactor supplying, food, oxygen, tension

- The TE tissues grow and remodels; over time, becomes a living tissue = “cure”

- Graft grows with child - a major pediatric benefit
Clinical Utility of Venous/Arterial Conduits

- CHD common congenital abnormality
- Treatment requires surgical reconstruction
- 25,000 cases/yr in US
- Success limited by inadequate vascular conduit
Patient Information


Number of patients: 23

Age at Operation: 6.9 ± 6.6 (1-24) y.o.

Previous Op (+): 21 / 23

Cell Sources: Bone marrow

Hospital Stay: 11 ± 26 days

Follow-up: 4.7 ± 1.3 years

Oper. Procedure: Extracard TCPC (Conduit between IVC & PA)
Not All Scaffolds are Created Equal

Attached Cell # on Scaffold (Based on Histology)

Attached cell# /mm² scaffold

1000 Cells / mm²

Sheep 1
Sheep 2
Sheep 3
Sheep 4
Sheep 5
Sheep 6

**Monocyte Heterogeneity**

**Bone marrow**
- HSC
- GM-CFU
- M-CFU
- Monoblast
- Pro-monocyte

**Peripheral blood**
- GR⁺
- Inflammatory monocyte
- GR⁻
- Resident monocyte

**Tissues**
- Bone: Osteoclast
- CNS: Microglial cell
- Lung: Alveolar macrophage
- Liver: Kupffer cell
- Connective tissue: Histiocyte
- Spleen: White-pulp macrophage, Red-pulp macrophage, Marginal-zone macrophage, Metallophilic macrophage
Closed vs. Open System

**Closed System**
- A system developed for aseptic collection and separation of blood and blood components, manufactured under clean conditions, sealed to the external environment, sterilized by a validated and approved method. (WHO guidelines on cGMP for blood establishments, 2011)

**Open System**
- Any system that is not validated to be a closed system.
Current Ficoll Open Cell-Seeding System: Disadvantages

- Inter-operator variability
- Intra-operator variability
- Variable quality of cell seeded grafts
- Requirement of ISO class 7 facility
- Large equipment infrastructure and resources
- Risk of contamination
- Limits ability to translate technique from bench to bedside
- TIME and DISTANCE
- Anesthesia risk to child
Current Ficoll Open Cell-Seeding System: Advantages

- It Works
Comparison of Human Bone Marrow Mononuclear Cell Isolation Methods for Creating Tissue-Engineered Vascular Grafts: Novel Filter System Versus Traditional Density Centrifugation Method

Narutoshi Hibino, M.D., Ph.D., Ani Nalbandian, B.S., Lesley Devine, B.S., Rajendra Sawh Martinez, M.D., Edward McGillicuddy, M.D., Tai Yi, M.D., Safa Karandish, B.S., Girolamo A. Ortolano, B.S., Toshiharu Shin’oka, M.D., Ph.D., Edward Snyder, M.D., and Christopher K. Breuer, M.D.
Closed Disposable System for Vacuum Seeding MNC on Scaffolds

US Pat. filed May 17, 2010

LRF
Vacuum Set up Diagram

- Hood
- Vac device
- Cell suspension (Pink)
- Graft (White)
- Regulator
- Specimen Traps
- Sterile Tray
- Vacuum
Decreasing Time of Manufacture

6 Weeks

Endothelial Cell Culture

6 Hours

Ficol BM

3 Hours

Filter BM

30 Minutes

Filter BM - No Incubation
## RESULTS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Seeded, Pall Filter</th>
<th>Seeded, Ficoll</th>
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</thead>
<tbody>
<tr>
<td>Peri-operative mortality</td>
<td>33%</td>
<td>0%</td>
<td>17%</td>
</tr>
<tr>
<td>Premature mortality</td>
<td>17%</td>
<td>0%</td>
<td>33%</td>
</tr>
<tr>
<td>Overall survival</td>
<td>50%</td>
<td>100%</td>
<td>50%</td>
</tr>
</tbody>
</table>
### Holy Grail

**Macrophase quantification as a biomarker and measure of biological effect**

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<table>
<thead>
<tr>
<th>Marker</th>
<th>Function</th>
<th>Expression</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Classically activated macrophages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td>Induces $T_{h}^{1}$-cell development</td>
<td>Induced by IFNγ</td>
<td>107</td>
</tr>
<tr>
<td>iNOS</td>
<td>Produces NO and citrulline from arginine to kill microorganisms</td>
<td>Depends on IFNγ</td>
<td>108</td>
</tr>
<tr>
<td>CCL15</td>
<td>Attracts monocytes, lymphocytes and eosinophils</td>
<td>Upregulated by IFNγ</td>
<td>109</td>
</tr>
<tr>
<td>CCL20</td>
<td>Chemoattractant for DC and T cells</td>
<td>Upregulated by IFNγ</td>
<td>109</td>
</tr>
<tr>
<td>CXCL9</td>
<td>Involved in T-cell trafficking</td>
<td>Induced by IFNγ</td>
<td>9</td>
</tr>
<tr>
<td>CXCL10</td>
<td>Attracts NK and T cells; signals through CXCR3</td>
<td>Induced by IFNγ</td>
<td>9</td>
</tr>
<tr>
<td>CXCL11</td>
<td>Attracts NK and T cells; signals through CXCR3</td>
<td>Induced by IFNγ</td>
<td>9</td>
</tr>
<tr>
<td><strong>Wound-healing macrophages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL18</td>
<td>Attracts lymphocytes, immature DCs and monocytes</td>
<td>Induced by IL-4</td>
<td>110</td>
</tr>
<tr>
<td>YM1</td>
<td>Chitinase-like protein that can bind to extracellular matrix</td>
<td>Strongly induced by IL-4</td>
<td>111</td>
</tr>
<tr>
<td>RELMα</td>
<td>Can promote deposition of extracellular matrix</td>
<td>Strongly induced by IL-4</td>
<td>111</td>
</tr>
<tr>
<td>CCL17</td>
<td>Attracts T cells and macrophages</td>
<td>Induced by IL-4 and suppressed by IFNγ</td>
<td>112</td>
</tr>
<tr>
<td>IL-27Rα</td>
<td>Inhibits pro-inflammatory cytokine production</td>
<td>Upregulated by IL-4</td>
<td>113</td>
</tr>
<tr>
<td>IGF1</td>
<td>Stimulates fibroblast proliferation and survival</td>
<td>Induced by IL-4</td>
<td>114</td>
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<tr>
<td>CCL22</td>
<td>Attracts $T_{h}^{2}$ cells and other CCR4-expressing cells</td>
<td>Induced by IL-4</td>
<td>115</td>
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<tr>
<td>DCIR</td>
<td>C-type lectin containing an ITIM motif</td>
<td>Induced by IL-4</td>
<td>109</td>
</tr>
<tr>
<td>Stabilin</td>
<td>Endocytic receptor that may be involved in lysosomal sorting</td>
<td>Induced by IL-4</td>
<td>116</td>
</tr>
<tr>
<td>Factor XIII A</td>
<td>Can bind to extracellular matrix proteins and contribute to wound healing</td>
<td>Induced by IL-4 and suppressed by IFNγ</td>
<td>117</td>
</tr>
<tr>
<td><strong>Regulatory macrophages</strong></td>
<td></td>
<td></td>
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<tr>
<td>IL-10</td>
<td>Potent anti-inflammatory cytokine</td>
<td>Induced by TLRs in combination with other stimuli</td>
<td>61</td>
</tr>
<tr>
<td>SPHK1</td>
<td>Catalyses the conversion of sphingosine to sphingosine-1 phosphate</td>
<td>Induced by TLRs and immune complexes</td>
<td>10</td>
</tr>
<tr>
<td>LIGHT</td>
<td>Provides co-stimulatory signals for $T$ cells through HVEM</td>
<td>Induced by TLRs and immune complexes</td>
<td>10</td>
</tr>
<tr>
<td>CCL1</td>
<td>Attracts eosinophils and $T_{h}^{1}$ cells; binds CCR8</td>
<td>Induced by TLRs in combination with several other stimuli</td>
<td>118</td>
</tr>
</tbody>
</table>

CCL, CC-chemokine ligand; CCR8, CC-chemokine receptor 8; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor; DC, dendritic cell; DCIR, DC immunoceptor; HVEM, herpesvirus entry mediator; IFNγ, interferon-γ; IGF1, insulin-like growth factor 1; IL, interleukin; IL-27Rα, IL-27 receptor α-chain; iNOS, inducible nitric-oxide synthase; ITIM, immunoceptor tyrosine-based inhibiting motif; NK, natural killer; NO, nitric oxide; RELMα, resistin-like molecule α; SPHK1, sphingosine kinase 1; $T_{h}^{1}$, T helper; TLR, Tol-like receptor.
The distribution of the labeled cells suggests that EC and SMC migrate onto the lumen surface of the scaffold from the neighboring blood vessel.
TE- Just a Passing Fad?

Public wants:

• Freedom from tissue-transmitted diseases
• “Indefinite” life span in vivo for implanted devices and tissues
• Pediatric valves and vessels that will grow with the child – one time surgery
• Freedom from immunosuppressive regimens
• Increased use of autologous tissues
• Replacements for any damaged body part
• Limitless and/or renewable sources of tissue
• High tech approach to modern medical care
Yale Faculty

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• Stuart Seropian
• Erin Medoff

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Jennifer Tassey