Regenerative Medicine
New Approaches to Health Care
Anthony Atala, MD

Regenerative Medicine
Based on the field of cell transplantation (started in 1930s)

First clinical application: engineered skin for burn patients, 1981

Regenerative Medicine/Tissue Engineering
A field of research for over 60 years. Why so few clinical advances?

- Inability to expand cells \textit{in vitro}
- Inadequate biomaterials
- Inadequate vascularity
Wake Forest Institute for Regenerative Medicine

- Growth factor biology
- Cell differentiation
- Molecular mechanisms
- Cell-matrix interactions

Progenitor Cells and Specific Growth Factors: Expansion Potential

1 cm²
Day 1 (5 X 10⁴ cells)

Day 60 (50 X 10⁹ cells)
Enough cells to cover a football field

Tissue Types Grown

- Bladder
- Blood vessel
- Bone
- Cartilage
- Cornea
- Corpora
- Diaphragm
- Ear
- Fascia
- Fat

- Heart
- Heart valve
- Intestine
- Kidney
- Liver
- Nasal Turbinate
- Nerve
- Ovary
- Pancreas
- Salivary Glands

- Skeletal Muscle
- Skin
- Smooth Muscle
- Tendon
- Testis
- Trachea
- Urethra
- Urinary Sphincter
- Uterus
- Vagina
Cell Delivery Vehicles

- Biocompatibility
- Cell attachment
- Cell viability
- Degradation curves
- Inflammatory responses
- Biomechanical properties

The scaffold should replicate the biomechanical and structural properties of the tissue being replaced.

Vascularity: Problem

- Cells cannot be implanted in volumes greater than 3 mm³
- Nutrition to the cells is limited (limited vascularity)
Tissue Formation in Vivo: Strategies for Vascularization

Soker et al, Tissue Engineering

Urethras Reconstructed with ECM with or without Cells

First use of a natural biomaterial implanted inside the body in humans for organ regeneration, 1996

1 cm is the maximum distance for cell regeneration using extracellular matrices without cells
First use of a natural biomaterial implanted inside the body in humans for organ regeneration, 1996

Bio-Material

Normal Tissue

Normal Tissue

Injured Tissue

Regenerated Tissue

F Chen et al., Urology 54:1, 99._
RE DeFilippo et al., J Urol 168:1789, 02.
AW El-Kassaby et al., J Urol 169:170, 03.
T Shwareb et al., J Urol 179: 421, 08.
Patient with Complex Urethral Trauma

Tubularized Urethral Repairs Require Matrices and Cells

Use of a Tubularized Engineered Organ in Patients

March 2011

Tissue-engineered autologous urethras for patients who need reconstruction
Five patients with complex urethral trauma reconstructed with tubularized engineered urethras (scaffolds with cells), 6 years out

Pre-op

Earliest post-op

Latest post-op

Flow Rates

Urethra: Clinical Experience

• Over an 18 year follow-up using ECM scaffolds without cells as onlay (partial circumference) repairs

• Over an 9 year follow-up using scaffolds with cells for tubularized (complex) repairs

Fabrication of a Vascular Substitute

Electrospun nanofiber substrate, with endothelial and smooth muscle cells

Stitziel et al., Biomaterials, 2005.
Peripheral Blood-derived Endothelial Cells for the Creation of Tissue Engineered Blood Vessels

Other Current work:

Toshiharu Shinoka
Laura Niklason
François Auger
Nicolas L’Heureux, PhD
Todd McAllister

Lee DJ et al, 2009
Wake Forest Institute for Regenerative Medicine
Flow-Dependent Endothelialization

<table>
<thead>
<tr>
<th>Flow Rate</th>
<th>Endothelialization Status</th>
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<tbody>
<tr>
<td>1 L/min</td>
<td>Not optimal</td>
</tr>
<tr>
<td>3 L/min</td>
<td>Not optimal</td>
</tr>
<tr>
<td>2 L/min</td>
<td>Optimal</td>
</tr>
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</table>

Optimal endothelialization achieved

Vaginal Epithelial Cells

Vaginal Smooth Muscle Cells

AE1/AE3

Estrogen β

D-Actin

Cells are grown, expanded and seeded on polymer scaffolds

Human tissue harvest

Analyses

Implantation
**In Vivo Cell Implantation Model**

1 Week 2 Weeks

3 Weeks 4 Weeks

Vaginal tissue harvest  Cells are grown, expanded and seeded on polymer scaffolds  Analyses  Total replacement

**Bioreactor Design**

**Vaginograms**

Unseeded  Seeded

1 Mo 1 Mo 3 Mo 6 Mo
### Gross Examination

<table>
<thead>
<tr>
<th>1 Mo</th>
<th>3 Mo</th>
<th>6 Mo</th>
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<tbody>
<tr>
<td>Seeded</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Unseeded</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
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### Molecular Analyses

#### Western Blots
- Collagen I
- Collagen II
- Collagen III
- Elastin

#### RT-PCR
- GAPDH
- TGF-β
- CK2
- vWF

<table>
<thead>
<tr>
<th>Unseeded</th>
<th>Normal</th>
<th>1 Month</th>
<th>6 Months</th>
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<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
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</table>

Trichrome
- AE1/AE3
- α-Actin

Engineered
- x100
- x100
- x40
- x100
- x100

Normal
- x100
- x100
- x40
- x100
- x100

Seeded
- x100
- x100
- x40
- x100
- x100

1 Mo
- x100
- x100
- x40
- x100
- x100

3 Mo
- x100
- x100
- x40
- x100
- x100

6 Mo
- x100
- x100
- x40
- x100
- x100
Tensile Stress

<table>
<thead>
<tr>
<th>Time</th>
<th>Tensile Stress (Mpa)</th>
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<tr>
<td>1 Month</td>
<td>0.2</td>
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<tr>
<td>6 Months</td>
<td>2.4</td>
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Engineered Vaginal Tissue

Tensile Strain

<table>
<thead>
<tr>
<th>Time</th>
<th>Tensile Strain (%)</th>
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<tr>
<td>1 Month</td>
<td>20</td>
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<tr>
<td>6 Months</td>
<td>80</td>
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Engineered Vaginal Tissue

Neurofilament Protein

<table>
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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>1 month</td>
</tr>
<tr>
<td>3 months</td>
</tr>
<tr>
<td>6 months</td>
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</tbody>
</table>

Organ Bath Studies

<table>
<thead>
<tr>
<th>Condition</th>
<th>EFS 30 Hz</th>
<th>Phenylephrine</th>
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<tbody>
<tr>
<td>Unseeded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NL Vagina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Month</td>
<td></td>
<td></td>
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</tbody>
</table>

Clinical Trial

MRI of patient with vaginal agenesis

Same patient with engineered vagina
Female Sexual Function Index Assessment

<table>
<thead>
<tr>
<th>Domain</th>
<th>Maximum Score</th>
<th>Sexual Function Index Patient Score</th>
<th>Avg all patients</th>
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<tbody>
<tr>
<td>P1</td>
<td>4.8</td>
<td>4.8 6 5.4 6 6</td>
<td>5.6</td>
</tr>
<tr>
<td>P2</td>
<td>5.1</td>
<td>6 5.7 5.8 5.6 5.6</td>
<td>5.6</td>
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<tr>
<td>P3</td>
<td>4.8</td>
<td>5.7 5.7 5.7 5.7 5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>P4</td>
<td>5.6</td>
<td>6 5.6 6 6</td>
<td>5.8</td>
</tr>
<tr>
<td>Pain</td>
<td>5.6</td>
<td>5.6 5.6 6 6</td>
<td>5.8</td>
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</table>

Creation of First Engineered Organ Experimentally: Bladder

- Patients with high pressure /low capacity bladders
- All failed medical therapy and were considered candidates for bladder reconstruction
Pre-Op Post-Op

Urodynamic Studies

Pre-Op

Post-Op
Long-Term Follow-Up, Lancet Phase I patients
Compliance increased by 5, 3.1 and 4.5 fold

Biomechanical Stimulation Enhances Regeneration
Translation to Clinical Outcomes

- Cycling post-operatively is required for biomechanical stimulation
- Biomechanical stimulation promotes regeneration and improves outcomes

Pediatric Bladder Capacity (mL, Mean ± SD)

<table>
<thead>
<tr>
<th>Time</th>
<th>Non-Responders</th>
<th>Responders</th>
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<tbody>
<tr>
<td>Screening</td>
<td></td>
<td></td>
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<tr>
<td>Month 6</td>
<td></td>
<td></td>
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<tr>
<td>Month 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 12</td>
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</tr>
</tbody>
</table>

“Normal” range (Kaefer et al)

THE LANCET

“Tissue-engineered autologous bladders for patients needing cystoplasty”

Kaitlyn McNamara and her mother

Clinical Experience:
- Phase 1 completed
- Phase 2, completed
- Phase 3, Starting
- Over 13 year follow-up
- Work still in progress

April 2006

Uterus

Estrogen receptor B

kD  NL  CS

61
49
Fetus in Tissue Engineered Uterus
Near-term

Creation of First Solid Functional Organ

Complex organ composed of skin, muscle, nerves, and blood vessels

Chen et al.; PNAS, 2010

Penile Replacement: Study Overview

Autologous cavernosal cell harvest

Cells are grown and expanded

Cells are seeded on decellularized solid matrices

Corporal tissue penile replacement

Analyses

Retrieval of engineered corporal tissue

Total Corpora Replacement Cavernosography

Native corpora

Matrix with cells

Matrix without cells

Excision only
Cavernosometry

Creation of First Solid Functional Organ

<table>
<thead>
<tr>
<th>Pregnant Vaginal swab +</th>
<th>Pregnancy Rate</th>
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</thead>
<tbody>
<tr>
<td>Experimental (with cells)</td>
<td>83% (10/12)</td>
</tr>
<tr>
<td>Control (without cells)</td>
<td>0% (0/12)</td>
</tr>
</tbody>
</table>

Chen et al., *PNAS*, 2010

Solid Organs - Strategies

Fresh liver

Decellularized liver as a scaffold

Siddiqui M, Baptista P, Soker S et al.

First Demonstration of a Functional Human Liver Organoid

Baptista P et al., 2011
Decellularization of the Kidneys

- Intact vascular tree preserved
- Seeding with endothelium
- Seeding with hepatocytes

Kidney Matrix Seeded with Cells

- vWF (Endothelial)
- GFP (Endothelial)
- Albumin (Hepatocytes)

Printed 3D Heart Constructs

Xu, T. et al.
Printed 3D Heart Constructs

Integrated Organ Printing System

Reverse Engineering
Medical Data → 3D CAD Model → CAD/CAM System

3D Structure
Fabrication System
Code Generation
Cloned Renal Cells

- Tamm-Horsfall Protein
- AQP1
- Von Willebrand factor
- Synaptopodin
- AQP2

Retrieved Renal Units

- Cloned Cells
- Allogeneic Cells
- Unseeded

Renal Device

- Reservoir
- Porous Membrane
- Coated Collagen
Retrieved Renal Tissues

**RT-PCR**

- AQP 1
- AQP 2
- THP
- Synaptopodin
- GAPDH

**Western Blot Analyses**

- AQP 1
- AQP 2
- CD 4
- CD 8

Renal Functional Assessment

- **Erythropoietin Levels**
  - Normoxia
  - Hypoxia

- **1,25-Dihydroxyvitamin D3**
  - Media
  - Fibroblasts
  - Allogenic
  - Clone

![Nature Biotechnology](image)
**Body-on-a-chip XCEL Program**

Bio-printed miniature human organs using micro-chip and bio-sensing technologies for drug screening and discovery

Wake Forest Institute for Regenerative Medicine in collaboration with Harvard, Johns Hopkins, University of Michigan, Morgan State, and Edgewood Chemical Biological Center

Source: Wikimedia
Only 3 retroviral proven clonal populations of stem cells described in the literature up until recently that could give rise to all 3 germ layer derivatives:

- Embryonic and induced pluripotent stem cells
  Pro: very high replicative potential
  Con: tumor potential, (issues with rejection)

- Adult bone marrow stem cells:
  Pro: low tumor potential, can be used without rejection, bone marrow transplantation
  Con: low replicative potential (ecto and endo)

Amniotic Fluid and Placental Derived Stem Cells

- Fresh AF or back-up cytogenetics lab culture
- Select c-Kit^{pos} (CD117^{pos}) cells
- Establish clonal lines- no feeder layers

DeCoppi et al., 2007
Stem Cells were Isolated and Differentiated to:

Bone                Fat              Muscle            endothelium            Liver                  Heart

parent  offspring  differentiated cells (3 germ layers)

Clonality Confirmed by Southern Blot Showing Retroviral Insertion Fingerprint

Wake Forest Institute for Regenerative Medicine

SSEA-4  OCT4

Wake Forest Institute for Regenerative Medicine
AFS Cells Show Higher Similarity to ES and iPS Cells than Bone Marrow MSC

Expression profile analysis of "stemness genes" in embryonic, iPS, BM-MSC and AFS cells

Preservation of Telomere Length

Isolated Cells after 250 Population Doublings

AFS Costimulatory Molecule Expression

*Do not form teratomas when injected in vivo*

AFS cells show little to no expression of MHCII and costimulatory molecules and as a result may be able to avoid alloreognition.
The cells can be used in an autologous manner, thus avoiding rejection. Or, a bank of 100,000 specimens could supply approximately 99% of the US population with a perfect genetic match for transplantation.
Liver Differentiation

Morphology (insert shows higher mag.)

Albumin mRNA

Immunoblot: hepatocyte proteins

HNF4α
Nrf2
MDR
α-feto protein

Liver Differentiation

Urea secretion

Control
day 45

Cartilage Differentiation

Kolambkar et al., J Mol Histol, 2007

Control
TGF-β3
TGF-β3 + dex
TGF-β1 + dex

Carraro, G. et al., Stem Cells, 2008;26:2902-2911

Lung

hAFSC can integrate into the upper airways and express the Clara cell-specific marker CC10

hAFSC can acquire a type II pneumocyte cell-specific marker SPC

Human and Murine Amniotic Fluid Stem Cells Display Hematopoietic Activity

Ditadi et al., Blood

CC10
Clinical Utility of Amniotic Fluid and Placental Stem Cells

- The stem cells, derived from amniotic fluid and placental tissue, can be obtained during pregnancy or at the time of birth from discards.
- The cells are neither embryonic nor adult stem cells, but have properties of both.
- This system avoids the teratoma, tumor potential, and rejection concerns surrounding the use of other stem cells.
- The stem cells can be rapidly expanded to large quantities sufficient for clinical translation, thus avoiding the limitations of adult stem cells.
- The stem cells could be stored at the time of birth for future "self" use, or could be banked in large quantities, thus avoiding rejection.

<table>
<thead>
<tr>
<th></th>
<th>Embryonic</th>
<th>IPS</th>
<th>Amniotic/Placenta</th>
<th>Marrow/Cord</th>
<th>Tissue Specific</th>
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</thead>
<tbody>
<tr>
<td>Growth Potential</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Tumor Free</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Rejection Free</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<td>+++</td>
</tr>
<tr>
<td>Lineage Potential</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

DeCoppi et al., 2007

Image by: C Harrington & LB Oliver
**Tissue Types Grown**

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- Blood vessel
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- Fascia
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- Skeletal Muscle
- Skin
- Smooth Muscle
- Tendon
- Testis
- Trachea
- Urethra
- Urinary Sphincter
- Uterus
- Vagina

**MULTI-DISCIPLINARY TEAM**

- Growth factor biology (molecular biologists)
- Cell growth and expansion (cell biologists)
- Biomaterial production (material scientists)
- Cell-Biomaterial interactions (bio-engineers)
- Small & large animal models (physiologists, biochemists, veterinarians)
- Clinical trials (physicians, epidemiologists, statisticians, regulatory specialists)

*A consortia of 30 universities working together with the US Army, Navy, Air force, and Marines

1. Burns
2. Extremity Injuries
3. Craniofacial
4. CTA
5. GU

Skin cells sprayed onto excised burn
Delivering FDA approval & the first Class I efficacy data w/ long-term outcome data
How to stop a runaway stage

Method 1

Method 2

From the book "Guide to Western Stuff"

Some of the work in this presentation was made possible, in part, by grants from the following institutions:


Luke M, 10 Years After Receiving his Engineered Organ