Tissue Engineering and Regenerative Medicine

Anthony Atala, MD
Wake Forest Institute for Regenerative Medicine
Wake Forest University School of Medicine
Winston-Salem, NC
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YOU ARE HERE
Combat Trauma

Blunt, penetrating and blast injuries may lead to soft and solid tissue and organ damage.

Trauma, and its subsequent infection and inflammation all lead to tissue loss.

Challenge: Replacement Tissues and Organs
1954, First organ transplant, Boston

Today, Increasing problem: tissue and organ shortage and rejection
Regenerative Medicine / Tissue Engineering

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

First clinical application: engineered skin for burn patients, 1981
A field of research for over 60 years. Why so few clinical advances?

Inability to expand cells in vitro

Inadequate biomaterials

Inadequate vascularity
Wake Forest Institute for Regenerative Medicine

Growth factor biology

Cell Differentiation

Molecular mechanisms

Cell-matrix interactions
Targeted Committed Progenitor Cells
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
<table>
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<td>Heart</td>
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<tr>
<td>Kidney</td>
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<td>Esophagus</td>
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<td>Bladder</td>
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<td>Sm/Sk Muscle</td>
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<tr>
<td>Cartilage</td>
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<tr>
<td>Urethra</td>
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<td>Vessels</td>
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<tr>
<td>Salivary glands</td>
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<tr>
<td>Trachea</td>
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<tr>
<td>Bone</td>
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<td>Breast</td>
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<td>Nerve</td>
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<tr>
<td>Liver</td>
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<td>Pancreas</td>
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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 mm$^3$ (the size of a pencil eraser)

Nutrition to the cells is limited (limited vascularity)
**Tissue Formation in Vivo**

- **muscle only**
- **m + ec**
- **m + VEGF**
- **m + VEGF+ec**

WT Godbey et al, *Gene Ther*, 03.
RC Smith et al, *Hum Gene Ther* 13:697, 02
Building Blocks for the Engineering of Tissues and organs

Muscle | Heart mm | Endothelium | Bone | Cartilage
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.
Urethra: Clinical Experience

Over 100 patients treated to date

Over a 5 year follow-up

80% Success rate
Fabrication of a vascular substitute

Electrospun nanofiber substrate, with endothelial and smooth muscle cells

Peripheral Blood-derived Endothelial Cells for the Creation of Tissue Engineered Blood Vessels

FLK1  CD31

BS1  F VIII

CD14  Control

Engineered Artery

Native Artery
S. Kaushal et al
Engineered Trachea
Vaginal Epithelial Cells

AE1/AE3

Estrogen β

α-Actin

Estrogen β

Vaginal Smooth Muscle Cells
Gross Examination

1 Mo  

3 Mo  

6 Mo
Organ Bath Studies

EFS
30 Hz 60 Hz

AR Stimulation
Phenylephrine

Unseeded

NL Vagina

6 Months

3 Months

1 Month
Clinical Experience- 3 year follow-up in patients with engineered vaginas
Creation of the First Engineered Organ: Bladder
Clinical Studies

Patients with high pressure / low capacity bladders

All failed medical therapy and were considered candidates for bladder reconstruction
Urodynamic Studies

Pre-Op.

Clinical Experience
3 protocols
5 year follow-up

The Lancet, April 06
UTERUS

Estrogen receptor B

$kD$  NL  CS

61  
49
Fetus in Tissue Engineered Uterus

Near-term
**Scaffold Configuration for Digits**

- Cartilage
- Muscle
- Front
- Side

**Scaffold Composition**

- PGA
- Collagen Matrix

**Functional Components**

- Maintain structure
- Contraction and relaxation

**Muscle Function and Digit Movement**

- Contraction
- Movement
- Relaxation

1. Contraction
2. Movement
3. Relaxation
Engineered Digit
Cartilage and muscle composite tissue
Steve Badylak, MD, PhD, DVM
Material-Induced Regeneration

Commercially available product
FDA approved
Fingertip Regeneration in 78-year-old man
Fingertip Regeneration in 78-year-old man
Engineering of Ears
RIGHT KIDNEY SECTIONED IN SEVERAL PLANES, EXPOSING PARENCHYMA AND RENAL SINUS
Renal Cells

- Tamm-Horsfall Protein
- AQP1
- Von Willebrand factor
- Synaptopodin
- AQP2
Retrieved Renal Units

Cloned Cells

Allogeneic Cells

Unseeded

3 months

3 months

3 months
Retrieved Renal Tissues

Control

H & E

H & E

H & E

vWf

Glomerulus

Tubule

Membrane
Successful transplantation of cloned cells

Genome shuffling for industrial microbes

Ribozymes throw light on drug targets

Lactobacilli fight tooth decay
CELL THERAPY
Injectable Cells for Therapy

Cartilage cells: FDA phase II and III multicenter clinical trials, 110 patients, 10 centers, 5 year follow-up

Muscle cells: Phase 1 FDA trial, 32 patients, single Injection, 80% success at 3 and 12 months, 5 year follow-up
Encapsulated Cells

Empty Microcapsules

Nutrients

Alginate capsule

Cells

Proteins, hormones
Applications for Engineered Cells

Tumor therapy
- Endostatin
- Others

Excretion of proteins/hormones
- Menopause (estrogen)
- Diabetes (insulin)
- Parkinson’s (L-Dopa)
- Testosterone
**Stem Cells**

A stem cell can become any cell and it can create any tissue or organ.

Only 2 pluripotent stem cell types described to date:

- Embryonic stem cells
- Adult bone marrow stem cells
Pluripotent stem cells: only 2 identified to date

Embryonic stem cells
Pro: very high replicative potential
Con: Malignant potential, issues with rejection, ethical issues

Adult bone marrow stem cells:
Pro: low malignant potential, can be used without rejection
Con: very low replicative potential
Amniocentesis: amniotic fluid that bathes the fetus in the womb during pregnancy

Placenta: the tissue in the womb that houses the baby

Amniotic fluid and placental tissue: Possible source for stem cells?
Conclusions

A new cell class is described, derived from amniotic fluid and placental tissue obtained during pregnancy or at the time of birth.

This system avoids the malignant potential and ethical concerns surrounding the use of embryonic stem cells.

The stem cells can be rapidly expanded to large quantities sufficient for clinical translation, thus avoiding the limitations of adult bone marrow stem cells.

The stem cells could be stored at the time of birth for future “self” use, thus avoiding rejection.
Stem Cells were Isolated and Differentiated to:

- Bone
- Fat
- Muscle
- Capillaries
- Nerves
- Liver
- Pancreas

400 Human Amniotic Fluid and Placental Samples (10 - 40 wks)
Pancreatic islets repaired after stem cell injection: insulin immuno staining

A: Normal pancreas
B: After STZ injection (28 d)
C: After cell injection (28 d)
Presence of MAFC (FITC-HA)
Control of Glucose Levels after Stem Cell Injection Long Term

![Graph showing glucose level changes over time with and without STZ treatment. The graph displays glucose levels (mg/dl) against days. The y-axis represents glucose levels from 0 to 650 mg/dl, and the x-axis represents days from 30 to 390. Two lines are shown: one for + STZ and another for + cells. The + STZ line shows a rapid increase in glucose levels, while the + cells line shows a more controlled fluctuation.]
Amniotic fluid and placental tissue obtained during pregnancy may be an alternate source for obtaining human stem cells.

This system would avoid the rejection, malignancy, and cell expansion concerns surrounding the use of current stem cells.

The stem cells could be stored for future “self” use.

Forget about transplants. In the not too distant future, people with failing tissues or organs may have new ones fabricated in a laboratory. The tools are already in hand.

Replacement Parts

Y

BY GEOFFREY COWLEY

The Human Body Shop

Bone

Brain

Skin

Heart

Liver

Kidney

Pancreas

Lung

Eye

Hand

Foot

The human body shop is already in business. In the United States, 100 hospitals perform organ transplants each year, mostly kidneys, and another 2000 do bone marrow transplants. Some patients survive for years. By the year 2000, says Mark A. Peterson of the University of Colorado in Denver, the best transplant surgeons will be able to do a bone marrow transplant that works fine in all cases.

There are thousands of patients waiting for new kidneys. But there are no new kidneys for transplant. A new technique for fabricating kidneys has already been demonstrated. It is called tissue engineering because it uses living cells to build tissue—all you have to do is find the right combination of genetic instructions. This week, William M. Shafritz of the University of Wisconsin and his colleagues in Madison used genetically engineered muscle cells and blood vessels to build a kidney that lasted 10 days in a patient. The cells were grown in a beaker in a bath of embryonic stem cells, a technique that Shafritz invented.
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<td>Trachea, Bone, Breast, Lung, Retina, Uterus, Nerve, Liver, Pancreas</td>
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Engineering of Tissues and Organs

Hollow tubes -> Hollow organs -> Solid Organs

Urology: Bladder - 9 years; Urethra – 7 years; Penis- in progress

Gynecology: Uterus - 7 years; Vagina - 5 years

Vascular: Blood Vessels - 5 years; Heart Valves – in progress

Respiratory: Trachea – in progress

Orthopedic: Cartilage, Bone, Skeletal Muscle, Digits

Nephrology: Kidney-in progress

--All Required Integration--
Some of the work in this presentation was performed by over 300 researchers across a 16 year time span:

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)

**********MULTI-DISCIPLINARY TEAM**********
How to stop a runaway stage

Method 1

Method 2

From the book Guide to Western Stuff
The medical means to achieve full tissue and organ restoration in those suffering combat casualties are within our reach.

Additional effort and resources are needed to expand the current state of the field of regenerative medicine so all tissues and organs can be created and delivered to soldiers with combat casualties.

The Army Institute for Regenerative will be formed in 2007 order to accelerate clinical translation by the U.S. Army Medical Research and Materiel Command.

Colonel Robert Vandre
Dr. Frazier Glenn
General Eric Schoomaker
Wake Forest Institute for Regenerative Medicine

Ben Harrison  Cesar Santos  Jason Hipp  Robert Knutson
Colin Bishop  Chanda Turner  Jennifer Hipp  Regina Myers
George Christ  Chris Sullivan  Jian-Ming Zhu  Robyn Shaffer
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Shay Soker  Diane Mann  Koudy Williams  So-Young Chun
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