Update on Cornea Transplantation

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Professor
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University of Wisconsin
Eye Anatomy
Eye Anatomy

Normal cornea
Eye Anatomy
Eye Anatomy
Eye Physiology
Cornea Physiology

Corneal Endothelial Specular Microscopy

Normal Endothelial Cells

Fuch’s Endothelial Corneal Dystrophy
Cornea Swelling

Normal cornea
Cornea Transplantation in the United States 2016

18,500 Full thickness cornea transplant
22,000 Partial thickness cornea transplant
6,500 Cells and membrane only
Trends in Cornea Transplantation

Domestic Surgery Use of U.S. Supplied Intermediate-Term Preserved Tissue

2005 2011 2016
Trends in Cornea Transplantation

Figure 3: Domestic DMEK Trends

2011-2016 Domestic DMEK Trend - U.S. Eye Banks
Trends in Cornea Transplantation
Trends in Cornea Transplantation
Trends in Cornea Transplantation
Eye Donation

A brief look at restoring sight.
Lions Eye Bank of Wisconsin

Non-Profit serving the state of Wisconsin for almost 50 years

150 hospitals in our service area

Fulfill 100% of corneal transplant surgeries in the state
Cornea recovery is ideally done under 12 hours.

Corneas normally transplanted within 7 days of death, but surgeon preference is often 5 days or less.
2016 Metrics

Total Donors: 1026
Corneas Recovered: 2049
Corneas Transplanted: 1552
Transplant rate: 76%
National Transplant rate: 67%
Average Wisconsin Agency Recoveries

- **Organ**: 150
- **Tissue**: 350-500
- **EYE**: 1056

Exact
Donor Eligibility

- Current admission course
- Medical/Social history
- Screening for:
  - Sepsis/Infection
  - Communicable diseases
  - High Risk Behavior
  - Ocular history
Recovery-Dispatch

• Recovery Technicians cover Regions of the state.

• They are expected to be on the road within 30 minutes of dispatch.
Eye Recovery

- Total recovery time is generally 1-1.5 hours

- Eye procurement can be done in the OR, morgue, funeral home or even on the unit- anywhere there is room, a sink and flat surface for equipment

- LEBW will recover as soon as possible upon receiving authorization

- Insure that patient identification remains on the body and access to the complete chart is available to the recovery technician
Recovery-Steps

- Medical Chart Review
- Donor Body Exam/Ocular Penlight Exam
- Blood Sample
- Ocular Prep
- Recovery Procedure
- Restoration and Clean up
DEATH TO PRESERVATION TIME IMPACT ON PROBABILITY OF PLACEMENT

- Tissue – Body Refrigeration within 12 hrs post CTOD
- Tissue – No Refrigeration within 12 hours
- Corneal timelines regardless of cooling

PROBABILITY OF PLACEMENT

8 hours 10 hours 12 hours 15 hours 18 hours 24 hours
Tissue Suitability

Slit Lamp Microscopy

Specular Microscopy
Post Recovery Eligibility Determination

• Follow Up Calls
• Confirm fluid information
• EMS verification
• Sepsis/Systemic infection concerns
DEATH TO TRANSPLANT IMPACT ON PROBABILITY OF PLACEMENT

PROBABILITY OF PLACEMENT

Days from Death

1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days 1-2

Authorization and DRAI obtained

Records requested

Recovery performed & tissue transported to eye bank

Evaluation of tissue performed

Blood sample sent for testing
Days 2-3

- Serology results received
- Chart review performed
- Additional records requested
- Autopsy results obtained
Days 3-5

- Donor released
- Tissue offered to surgeon
- Tissue processed
- Tissue packaged and shipped to surgery location
Days 5-7

Tissue can still be offered if not yet placed

Odds of placement are decreased

Day 5 is last day to offer for international surgeries
Day 8-13

Tissue considered “too old” for sight-restoring surgery

Can be used for patch graft surgeries

Odds of discard outweigh odds of transplant
Tissue is expiring and no longer able to be transplanted
Your Call to Action

• Wait to release to funeral home
• Calling referrals within 1 hour of death
• Have chart available for initial screening
• Ensure correct identification is on decedent
• Perform eye care
  • Elevate head
  • Close eyes and apply moist gauze
  • Apply a light ice pack
• Electronic Medical Record access
Tip of the Iceberg

Small aspect of the process

There is much more to learn about eye donation

Check out our table to find out how we can visit your unit
The Adventures of the Icons of Innovation
(or, to the laboratory... and beyond!!!)

Shawn A. Hunter, Ph.D.
Director, Research & Development

Douglas T. Miller Symposium on Organ Donation and Transplantation
May 18, 2017

Extraordinary people transforming “the gift” to save and enhance lives
My Background

• Graduated from the University of Cincinnati
  – Degrees in Aerospace Engineering/Engineering Mechanics
  – Tissue biomechanics major
  – Worked closely with orthopaedic surgeons

• Primary expertise in orthopaedic biomechanics
  – Tendon, ligament, fibrocartilage
  – Thesis on meniscus allograft development
  – Also conducted studies on cardiovascular tissues, shoulder joint, and skin

• Post-doc at Cincinnati Children’s Hospital/UC
  – Focused on cartilage functional tissue engineering
  – Developed doubly transgenic mouse model to track collagen growth

• Recruited to start R&D @ CTS in Jan 2007
About CTS

- CTS started in 1986 within Community Blood Center
- HQ in Dayton, operations nationwide
- Center for Tissue Innovation & Research opened in Kettering, 2011
- Not-for-profit corporation
- Contract manufacturing & private label biologics
- Distribute allografts domestically & internationally >450,000 grafts in 2016
- Largest not-for-profit supplier of skin to burn victims
- Awarded 2014 Manufacturer of the Year by Dayton Business Journal
About CTS

- Full-service tissue bank
- Recover tissue from cadaveric donors
- Screen for suitability
  - Infectious diseases
  - Microbiological contamination
  - Donor medical/social history
- Process tissue
- More screening & testing!
- Package & distribute
Allograft Use Today

- Allografts are a safe, viable alternative to autografts for bone repair in numerous orthopaedic, dental, spinal and trauma applications.
- Allograft use has risen steadily as they avoid the morbidity site associated with autograft harvest, are readily available, and come in a variety of forms.
Evolving Market

- Traditional tissue banking advancing toward tissue engineering/regenerative medicine (TE/RM) therapies, bioactive products, and cellular technologies
  - Tissue products with viable cells are on the market
  - Demineralized bone matrix products are a decades-old technology, but recent advancements aimed at improving osteoinductive potential and handling characteristics
  - Autologous and/or allogeneic cells being collected, manipulated, and re-infused or implanted on tissues/scaffolds
- Expanded opportunities for combination products
  - Allografts may improve incorporation of synthetic components
- Xenografts
- Industry regulation in transition period
  - HCT/P vs. Biologic vs. Medical Device
CTS R&D Mission

• Generate intellectual property and translate into new product development to better aid patients

• Develop new or improve existing processes to bolster graft safety and efficacy and enable manufacturing scalability

• Evaluate scientific merit of new technologies and collaboration opportunities to advance healthcare
Sterilization Alternatives

- Gamma irradiation primarily used for allograft terminal sterilization
  - Efficient in deactivating microorganisms and most viruses
- Biomechanical integrity of the tissue may be compromised
  - Linked to higher allograft failure rates
  - Dose dependent with greater detrimental effects at high doses
- "Low dose" (15-20 kGy) effects are debatable

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dose</th>
<th>Tissue</th>
<th>Test Protocol</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balsly et al.</td>
<td>18.3–21.8 kGy</td>
<td>BTB &amp; Semi-T</td>
<td>Tension to failure @ strain rate of 10% initial length/sec</td>
<td>Did not significantly reduce the tensile strength or elastic modulus</td>
</tr>
<tr>
<td>(2008)</td>
<td></td>
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<tr>
<td>Gibbons et al.</td>
<td>20 kGy</td>
<td>BTB</td>
<td>Tension to failure @ strain rate of 100% initial length/sec</td>
<td>No significant reductions in any biomechanical parameters</td>
</tr>
<tr>
<td>(1991)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curran et al.</td>
<td>20 kGy</td>
<td>BTBs</td>
<td>Cyclic loading 50-250 N for 1000 cycles, Loaded in tension to failure</td>
<td>Elongated 27% more than non-irradiated controls</td>
</tr>
<tr>
<td>(2004)</td>
<td></td>
<td></td>
<td></td>
<td>20% decrease in tensile strength for irradiated specimens</td>
</tr>
<tr>
<td>Fideler et al.</td>
<td>20 kGy</td>
<td>BTBs</td>
<td>Tension to failure @ strain rate of 100% initial length/sec</td>
<td>Max force, strain energy, modulus &amp; max stress significantly reduced</td>
</tr>
<tr>
<td>(1995)</td>
<td></td>
<td></td>
<td></td>
<td>Stiffness, elongation, &amp; strain reduced but not statistically significant</td>
</tr>
</tbody>
</table>
E-Beam Sterilization

- Widely used for sterilizing medical products & packaging materials
- Accelerated beam of electrons kills bacteria by directly breaking DNA chains & creates reactive compounds that induce further chemical destruction
- Requires only seconds of exposure compared to hours for gamma
  - Tissue damage due to heat and free radicals significantly reduced
- Limited penetrability concerns addressed by increasing beam power
- E-beam currently used to terminally sterilize tissue-based products
  - DBM, xenografts
  - Benefits include reduced tissue degradation, well-controlled dose ranges, and rapid turnaround
- Biomechanical effects on soft tissues uncertain
Study Design

- Paired, bisected BTBs from 10 donors
- Paired anterior & posterior tibialis from 10 donors
- Randomly assigned to treatment group (n=10/group)
  - E-beam High (17.1–21.0 kGy dose)
  - E-beam Low (9.2–12.2 kGy dose)
  - Gamma irradiated (17.1–21.0 kGy dose)
  - Aseptic, non-irradiated control

- Biomechanical Testing
  - Preconditioning: Sinusoidal loading from 0–20 N at 0.5 Hz for 10 cycles
  - Subfailure: Sinusoidal loading from 0–200 N at 2 Hz for 2000 cycles
  - Unloaded and allowed to relax for 5 minutes
  - Failure: Tension to failure at rate of 100% initial length per second
  - Testing conducted at physiologic temperature (37±2°C) with constant saline misting for specimen hydration
Biomechanical Testing

Tibialis

BTB
Subfailure Properties

Cyclic Elongation

<table>
<thead>
<tr>
<th></th>
<th>Tibialis</th>
<th>BTB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong> (Non-Irradiated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>E_{LO}</strong> (E-beam Low)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>E_{HI}</strong> (E-beam High)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>G</strong> (Gamma Irradiated)</td>
<td></td>
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</tbody>
</table>

*Elongation (%)*
Structural Properties

Maximum Load

Load (N)

Tibialis  BTB

N : Non-Irradiated
E_{LO} : E-beam Low
E_{HI} : E-beam High
G : Gamma Irradiated
Structural Properties

Stiffness

- **N**: Non-Irradiated
- **E\textsubscript{LO}**: E-beam Low
- **E\textsubscript{HI}**: E-beam High
- **G**: Gamma Irradiated

<table>
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<tr>
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<th>Tibialis</th>
<th>BTB</th>
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</thead>
<tbody>
<tr>
<td><strong>N</strong> Non-Irradiated</td>
<td><img src="image" alt="Stiffness Bar (N)" /></td>
<td><img src="image" alt="Stiffness Bar (N)" /></td>
</tr>
<tr>
<td><strong>E\textsubscript{LO}</strong>: E-beam Low</td>
<td><img src="image" alt="Stiffness Bar (E\textsubscript{LO})" /></td>
<td><img src="image" alt="Stiffness Bar (E\textsubscript{LO})" /></td>
</tr>
<tr>
<td><strong>E\textsubscript{HI}</strong>: E-beam High</td>
<td><img src="image" alt="Stiffness Bar (E\textsubscript{HI})" /></td>
<td><img src="image" alt="Stiffness Bar (E\textsubscript{HI})" /></td>
</tr>
<tr>
<td><strong>G</strong>: Gamma Irradiated</td>
<td><img src="image" alt="Stiffness Bar (G)" /></td>
<td><img src="image" alt="Stiffness Bar (G)" /></td>
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</table>

* indicates a significant difference.
Material Properties

Failure Stress

<table>
<thead>
<tr>
<th></th>
<th>Stress (MPa)</th>
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<tbody>
<tr>
<td>N</td>
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<tr>
<td>E_{LO}</td>
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<tr>
<td>E_{HI}</td>
<td></td>
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<td>G</td>
<td></td>
</tr>
</tbody>
</table>

N : Non-Irradiated
E_{LO} : E-beam Low
E_{HI} : E-beam High
G : Gamma Irradiated
Material Properties

Failure Strain

- N: Non-Irradiated
- E_{LO}: E-beam Low
- E_{HI}: E-beam High
- G: Gamma Irradiated

![Graph showing strain (mm/mm) for Tibialis and BTB with different irradiation types.](image-url)
Material Properties

**Elastic Modulus**

<table>
<thead>
<tr>
<th></th>
<th>Tibialis</th>
<th>BTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Irradiated (N)</td>
<td>200</td>
<td>180</td>
</tr>
<tr>
<td>E-beam Low (E$_{LO}$)</td>
<td>220</td>
<td>200</td>
</tr>
<tr>
<td>E-beam High (E$_{HI}$)</td>
<td>240</td>
<td>220</td>
</tr>
<tr>
<td>Gamma Irradiated (G)</td>
<td>260</td>
<td>240</td>
</tr>
</tbody>
</table>

N : Non-Irradiated
E$_{LO}$ : E-beam Low
E$_{HI}$ : E-beam High
G : Gamma Irradiated
Conclusions

- E-beam at both doses did not significantly alter biomechanical properties compared to aseptically processed, non-sterilized controls.
- Dose-related difference in tibialis maximum load & failure stress:
  - $E_{LO} > E_{HI}$
  - $E_{HI}$ equivalent to aseptic controls.
- Reduced stiffness of $E_{LO}$ BTBs:
  - Due to differences in size; elastic modulus not different.
- Published: J Bone Joint Surg 96-A (16), 2014.
Brittany

- Brittany is walking well after her knee surgery. The 19-year-old tore her anterior cruciate ligament (ACL) in a skiing accident.
- Brittany’s dad, Mark, is also the reason there is so much more to the story than just a successful surgery.
- About two months after the active Oregon teen’s accident on the slopes, another accident occurred that eventually took Mark’s life.
- “We were on the four-wheeler together and he fell off and hit his head; complications from the fall led to his death”, said Brittany.
- Brittany’s family is thankful for her father—a Pastor, rancher, and donor—who helped his own daughter.
TE/RM Scaffold

Tissue Engineering (TE) - combines principles of engineering and biology to fabricate new generations of reparative tissue constructs

Regenerative Medicine (RM) - a treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cell populations or tissues

Basic Components:
- Cells
- Scaffolds/biomaterials
- Growth factors/cytokines
TE/RM Scaffold

- Are allografts an ideal scaffold?
  - Natural architecture intact
  - ECM components present
  - Necessary biomechanical properties present

- Challenge – promote & control cell interaction
  - Combine with cells? What type?
  - Process to increase recruitment of host cells?
  - Direct cell differentiation?
TE/RM Scaffold

- Cells are known to react to specific surface features
  - Roughness can facilitate cell attachment
  - Surface features in the 5-100 \( \mu m \) scale influence cell behavior
- Can cortical bone surface be engineered at cellular scale to enhance cell interaction and promote osteoinductivity?
- Co-developed a micromachining process to create specific, cellular-scale features on cortical bone
- Goal: Optimize allograft surface for cell interaction and direct the patient’s cells to stimulate new bone growth
- Research Objectives
  - Examine how pluripotential stem cells interact with modified bone surfaces
  - Cell attachment, proliferation, differentiation, protein expression
Methods

Cortical bone specimen
1.0 cm x 1.0 cm x 0.5 cm

Patterned area
0.5 cm x 0.5 cm
Results
Results

48 hrs
C = 9.11 ± 6.68%
P = 66.62 ± 35.62%

72 hrs
C = 11.03 ± 11.65%
P = 96.66 ± 31.29%

96 hrs
C = 6.91 ± 3.89%
P = 87.70 ± 15.11%

7 days
C = 9.00 ± 4.53%
P = 52.18 ± 26.01%
Results

10 days

C = 6.87 ± 5.36%
P = 83.85 ± 36.11%

14 days

C = 3.49 ± 1.68%
P = 35.52 ± 12.53%

17 days

C = 6.57 ± 2.46%
P = 56.10 ± 30.16%

21 days

C = 8.43 ± 5.19%
P = 55.71 ± 33.98%
Results

96 hrs

17 days

21 days
Results

Measured Surface Area (mean ± SD)

- *p = 0.008 compared to Control
- Geometrical area = 2066 µm²
Results

Bone Remodeling

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Bone Area (µm²)</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>0</td>
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<tr>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>96</td>
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<tr>
<td>168</td>
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<tr>
<td>240</td>
<td>0</td>
</tr>
<tr>
<td>336</td>
<td>0</td>
</tr>
<tr>
<td>408</td>
<td>0</td>
</tr>
<tr>
<td>504</td>
<td>0</td>
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</tbody>
</table>

Bone added

Bone removed
Results

SP7 Production (mean ± SD)

Chemiluminescence

Time (hrs)

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>SP7_C</th>
<th>SP7_P</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td></td>
<td></td>
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<tr>
<td>504</td>
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</table>

- p = 0.013
- p = 0.029
- p = 0.044
- p = 0.049
- p = 0.008
Conclusions

- Significant increases in RUNX2 and SP7 production suggests ASC differentiation to bone cell lineage.
- Visual evidence supports this as bone removal and formation was observed.
  - Functions of both osteoclasts and osteoblasts.
- Changes in surface roughness correlated to new bone deposition.
- Patterned surfaces elicit key cellular responses between 7 and 10 days and generally diverge from non-patterned surfaces at later timepoints.
  - Suggests sustained response.
As a young boy Mark found himself like most other children his age, extremely active and always running around. Right around the age of 12, he began to experience pain in his right hip. Over time the pain increased, causing him to compensate by limping. Mark's parents took him to his family doctor, who thought he may be suffering pain due to his femur slipping. The doctor thought that perhaps placing a pin in his hip would help to hold his femur in place. Mark was sent to the hospital to get some x-rays taken before any big decisions were made. What was meant to be a routine trip for x-rays, actually turned out to be an admission to the hospital.

The x-ray revealed a grapefruit sized growth located at the epiphysis on Mark's right femur. He was immediately admitted to the hospital for additional testing, a biopsy and subsequent surgery. After the tumor was removed, the surgeon used a bone graft to repair the femur. In the late 1970s, it was still a very experimental procedure, as the bone was used from a donor that would have to combine to form new bone that was thought to maybe not ever be as strong as the original. Thankfully, the test results on the tumor were benign.
Joyce

“I do not know who my donor family is, but would like to tell them that I am truly grateful for their gift to me and my family.”
Wound Care

- Customer approached CTS w/ lab-scale decellularization technology
- R&D worked with Manufacturing to develop a validated, production-scale process
- Product successfully launched and being used clinically with positive results
Skin Graft Recovery

• Dermatome
  – Obtain graft of set thickness
  – Can yield long strips of 3-4” width
  – Use can be ergonomically and physically challenging
  • Must push while applying downward force
  • Must maintain “sweet spot” angle
  – Anatomical contours can affect thickness consistency

• Amalgatome MD
  – CTS involved in development & assessment
  – Obtain graft of set thickness
  – Can yield long strips of 3-4” width
  – Improved ergonomics
  • Device is pulled, minimal downward force needed
  • “Sweet spot” angle built in
  – Better handling over anatomical contours
Skin Graft Recovery

• Optimization of recovery and graft manufacturing processes
  – Automation
  – Improve task repeatability to reduce technician variation
• New process needed to create larger skin grafts with more uniform thickness
  – Variability arises from donor geometry, recovery device limitations, and technician skill level
• Identified equipment from another industry to ‘plane’ full-thickness skin
• Numerous engineering modifications required
  – Ensure proper function to obtain desired grafts
  – Safety
  – Ability to be used in tissue cleanroom environment
Optimized Skin Grafts

11.5 x 18.0”

8.0 x 23.0”
Pediatric Patients

Bubba the Bear Kit

Shriners Burn Center Clown
I was on my way home from work. A sixteen-year-old girl turned left in front of me on the country road. I swerved, she didn’t stop and forced me off the road. I wound up trapped in a burning jeep for 20 minutes. Next thing I knew I was waking up in a hospital bed it was 5.5 weeks later and I had lost both of my legs as a result of the fire. When I woke up I thought my life was over.

The infections I had were killing me. I would not have survived if they hadn’t had tissue available to graft on to my legs and cover those wounds. I would have died within days because they couldn’t get the infections under control without closing those wounds.

Tissue donors give other people a chance to live full lives and longer lives as their very last act on this earth.
Johnny

Johnny is a severely injured Burn Survivor from Shriners Hospital for Children in Galveston, Texas. He was burned in a house fire in Mexico when he was 4 months old and was transported to Shriners where he began his lifelong care. His injuries left him with one arm and only one eye that he can see from. He is now twenty four years old.
Anyone can be an Everyday Hero!

I am an Everyday Hero
I envision new ways to save lives through organ, tissue and eye donation. I am creative. I think outside the box. Challenge me. Push me to do more. I am an Icon of Innovation.
Through Research, We Can...

- Honor the donor and donor family’s gift
- Maximize the donor’s gift
- Provide the gift to more recipients
- Improve recipient's quality of life

THANK YOU