Regenerative Medicine Approaches for the Treatment of Growth Plate Injuries

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How I got here

Montreal, CANADA
- High School (biology, chemistry)
- BS in Physiology (McGill University, 2000)
  - Artificial cells, tissue engineering
- MS in Biomedical Engineering (University of Montreal, 2002)
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Chitosan-DNA nanoparticles

- chitosan (+)
- DNA (-)
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2011-2012: Research Assistant Professor in Orthopedics
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University of Colorado Anschutz Medical Campus
2012-present: Associate Professor, Orthopedics
  Faculty Member, Gates Center for Regenerative Medicine
Regenerative Medicine

Khademhosseini A et al. PNAS 2006;103:2480-2487

- Ex vivo
- In situ
Why are we interested in regenerative medicine in orthopedics?

- Critical bone defects and non-unions
- Spinal fusion
  - Bone is the second most common transplant tissue after blood
Why are we interested in regenerative medicine in orthopedics?

- Critical bone defects and non-unions
- Spinal fusion
- Articular cartilage injuries

osteoarthritis
Why are we interested in regenerative medicine in orthopedics?

- Critical bone defects and non-unions
- Spinal fusion
- Articular cartilage injuries
- Ligament and tendon injuries
Regenerative Orthopedics

**Bone formation for spinal fusion**
- Stem cells + bone allografts
- Biological agents

**Articular Cartilage**
- Prevention of post-traumatic osteoarthritis in skeletally immature animals

**Growth Plate Cartilage**
- Growth plate cartilage biology
- Growth plate repair strategies
Growth Plate (Physis)

- **Resting Zone**
- **Proliferative Zone**
- **Hypertrophic Zone**
- **Calcification Zone**
Growth plate injuries can result in growth deformities

- Approximately 1 in 2 boys and 1 in 3 girls will sustain a fracture during childhood
- 18-30% of pediatric fractures involve the growth plate

Growth plate injuries can result in growth deformities

- Approximately 1 in 2 boys and 1 in 3 girls will sustain a fracture during childhood\(^1\)
- 18-30% of pediatric fractures involve the growth plate\(^2\)

Growth Plate Injuries: Current Treatments

- bony bar spans <50% of growth plate volume
- 2 years or 2 cm of growth remaining
Growth Plate Injuries: Current Treatments

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**BONY BAR RESECTION**
18-30% poor outcome
Growth Plate Injuries: Current Treatments

- Bony bar spans <50% of growth plate volume
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**BONY BAR RESECTION**
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- Bony bar spans >50% of growth plate volume

**ARTIFICIAL CLOSURE OF GROWTH PLATE**
Prone to infections, multiple hospitalizations
Growth Plate Injuries: Current Treatments

- **BONY BAR RESECTION**
  - Bony bar spans <50% of growth plate volume
  - 2 years or 2 cm of growth remaining
  - 18-30% poor outcome

- **ARTIFICIAL CLOSURE OF GROWTH PLATE**
  - Bony bar spans >50% of growth plate volume
  - Prone to infections, multiple hospitalizations

**NO treatment is attempting to regenerate the growth plate cartilage**
Developing functional regenerative medicine approaches to treat growth plate injuries.

Prevent Bony Bar Formation

Regenerate Growth Plate Cartilage

Restore Normal Bone Elongation
Rat proximal tibial growth plate drill-hole defect reproducibly creates a bony bar
Rat proximal tibial growth plate drill-hole defect reproducibly creates a bony bar.
Research Projects

Drug Delivery System
- Block angiogenesis
- Recruit endogenous stem cells & promote cartilage formation
- Block osteogenesis (stiffness, siRNA)

Cartilage Biomimetic Hydrogel
- Block bony bar formation
- Promote cartilage formation

3D Printed Implant
- Engineering a biomimetic of growth plate cartilage

Human growth plate characterization and studying the clinical incidence of growth plate injuries
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**Human growth plate characterization and studying the clinical incidence of growth plate injuries**
Project #1: Determine whether local delivery of an anti-angiogenic factor after growth plate injury will prevent bony bar formation

- Vascular endothelial growth factor (VEGF) influences bony bar formation.  

Systemic anti-VEGF antibody reduces bony bar

Systemic anti-VEGF antibody reduces bony bar but also reduces limb lengthening

Hypothesis: Local delivery of anti-VEGF after growth plate injury in rats will reduce bony bar formation without affecting limb lengthening
Alginate mixed with chitosan forms a polyelectrolyte complexed hydrogel

Chitosan:
Cationic polysaccharide
Used extensively for cartilage regeneration

Alginate:
Anionic polysaccharide
Used extensively for drug delivery

Collaboration with Melissa Krebs, PhD – Colorado School of Mines
Alginate mixed with chitosan forms a polyelectrolyte complexed hydrogel

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Varying alginate:chitosan ratio and calcium crosslinking can fine-tune biomaterial properties
Release of antibodies can be modulated in alginate-chitosan hydrogels

Release of antibodies can be modulated in alginate-chitosan hydrogels


Quick Release = Alginate:chitosan 90:10

Slow Release = Alginate:chitosan 50:50
Study Design

Alginate:Chitosan Hydrogel

Anti-VEGF Antibody ~7ug anti-VEGF

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Hydrogel name</th>
<th>α-VEGF</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Intact</td>
<td></td>
<td>-</td>
<td>MicroCT, histology</td>
</tr>
<tr>
<td>2 Untreated</td>
<td></td>
<td>-</td>
<td>Perfusion/Blood vessels</td>
</tr>
<tr>
<td>3 Alginate:chitosan 90:10</td>
<td>Quick Release</td>
<td>-</td>
<td>Limb growth</td>
</tr>
<tr>
<td>4 Alginate:chitosan 90:10 + anti-VEGF antibody</td>
<td>Quick Release + α-VEGF</td>
<td>+</td>
<td>N = 8 limbs total (4 male, 4 female) per time point per outcome</td>
</tr>
<tr>
<td>5 Alginate:chitosan 50:50</td>
<td>Slow Release</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6 Alginate:chitosan 50:50 + anti-VEGF antibody</td>
<td>Slow Release + α-VEGF</td>
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</table>
Local delivery of α-VEGF reduces bony bar formation

Mean +/- SD, one-way ANOVA, N = 8
*P<0.05 vs. Untreated same time point
Quick delivery of α-VEGF increases cartilaginous repair tissue

Mean +/- SD, one-way ANOVA, N = 8
*P<0.05 vs. Untreated same time point
#P<0.05 vs. QR+α-VEGF same time point

Blue = cartilage
Local delivery of α-VEGF reduces vessel formation at injury site

Mean ± SD, one-way ANOVA, N = 8
*P<0.05 vs. Untreated same time point
$P<0.05$ vs. QR same time point
Local delivery of α-VEGF does not affect average physeal height
Local delivery of α-VEGF does not affect limb lengthening

Mean +/- SD, Repeated measured 2-way ANOVA, n=8

1. Untreated < all groups at 2 weeks
2. Intact & Quick Release + α-VEGF > Untreated all times
3. Intact > Slow Release + α-VEGF at 16, 20, 24 weeks
Conclusion and Future Directions

**Conclusions**
- Local delivery of α-VEGF reduces bony bar formation
- Quick delivery of α-VEGF increases cartilaginous tissue formation
- Local delivery of α-VEGF does not affect limb lengthening, or adjacent physis
- There are differences between Quick Release and Slow Release hydrogels

**Future directions**
- Understand which cells are being affected by the anti-VEGF, and how that is leading to decreased bony bar, decreased vessels, increased cartilage
- Reevaluating the growth plate injury model in male and female rats
- Combining α-VEGF with pro-chondrogenic factor (TGF, IGF) to promote chondrogenesis
Cartilage Biomimetic Hydrogel
- Block bony bar formation
- Promote cartilage formation

3D Printed Implant
- Engineering a biomimetic of growth plate cartilage

Problem: Growth Plate Injuries
- 10-30% of growth plate fractures heal with a bony bar

Solution: 3-D Printed Personalized Implant
- Mechanical support
- Biodegradable
Multidisciplinary Team

- Virginia Ferguson, PhD
  - Bone and cartilage tissue characterization
  - 3D printing

- Nancy Hadley Miller, MD
  - Clinical experience

- Karin Payne, PhD
  - Animal models of growth plate injury
  - Cartilage tissue engineering

- Stephanie Bryant, PhD
  - Cartilage mimetic hydrogel
  - 3D printing

- Virginia Ferguson, PhD
  - Bone and cartilage tissue characterization
  - 3D printing
Cartilage Mimetic Hydrogel Induces Chondrogenesis of MSCs

Photopolymerizable cartilage mimetic hydrogel

Rat MSCs in a Cartilage Biomimetic and Biodegradable Hydrogel

Week 3
- Collagen II
- sGAGs

Week 6

Week 9

Elizabeth Aisenbrey, PhD
Testing Cartilage Mimetic Hydrogel in a Rat Model of Growth Plate Injury

Healthy rat femoral growth plate

28 days post-injury
Cartilage Mimetic Hydrogel with TGFβ3 Reduced Bony Bar Formation

N=4-6, * vs. Intact, # vs. Untreated, + vs. Hydrogel
Cartilage Mimetic Hydrogel with TGFβ3 Formed New Cartilage Tissue

Blue = cartilage
Red = Bone
Combining Hydrogel and 3D Printing

3D Printed structure is infilled with hydrogel

3D printing technology

Scanning electron microscopy showing individual pillars
Mechanical Properties Across the Growth Plate

Microindentation maps two gradients in mechanical properties across the zones of the growth plate

Representative heatmaps of tensile modulus, $E_t$, compressive modulus, $E_c$, and permeability, $k$.

Gradients in stiffness found within individual zones of physeal cartilage. Sharp decline in stiffness in hypertrophic region.↑
3D Printing Technology

- Layer-by-layer 3D printing by stereolithography (SLA)

Variable properties in 3D printed structures by SLA

Developed methods to achieve uniform properties in 3D printed structures by SLA

Integration of Cartilage Mimetic Hydrogel with Stiff Structure

Red = stiff pillars
Green = cartilage mimetic hydrogel

High conversion in pillars
- No integration

Shell of low conversion
around pillars
- Integration
Testing the 3D printed construct in vivo

Rabbit model of proximal tibia physeal injury

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total number of rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>10</td>
</tr>
<tr>
<td>Fat Graft</td>
<td>9</td>
</tr>
<tr>
<td>Cartilage mimetic hydrogel</td>
<td>10</td>
</tr>
<tr>
<td>3D structure infilled with cartilage mimetic hydrogel</td>
<td>10</td>
</tr>
</tbody>
</table>

Right tibia: injured
Left tibia: intact

6 mm x 6 mm x 2 mm
3D Printed Implant Led to Decreased Limb Length Discrepancy

Limb length discrepancy = Left tibia (Intact) – right tibia (Injured)

- Untreated
- Fat Graft
- Hydrogel
- 3D Printed Implant + Hydrogel

Mean +/- SEM
*P<0.05
#P<0.05 vs. all groups
No Treatment Led to an Improvement in Tibial Angle

Change in tibial angle =
- Tibia angle (day 0)
- Tibia angle (8 weeks post-treatment)
MicroCT and Histology 8 weeks post-implantation
Mineralization within 3D Printed Implant
Effect of mechanical stiffness of the implant

![Chart showing limb length discrepancy with different stiffness levels and time points.](chart.png)

- **Legend**:
  - Low stiffness
  - Medium stiffness
  - High stiffness

- **Mean +/- SD**: N=8/group
- **Untreated - 8 weeks post-treatment**: (13.1 ± 2.3 mm)
- **Time post-treatment**: 8 weeks, 12 weeks
- **Stiffness Levels**: 100 kPa, 500 kPa, 1 MPa

* vs. 8 weeks
Discussion

- Able to characterize the mechanical properties of rabbit growth plate
- Able to 3D print highly tunable structures of graded mechanical properties
- Established a rabbit model of growth plate injury
- 3D printed structure infilled with hydrogel leads to
  - Increased tibial lengthening
  - Evidence of cartilage tissue formation
  - Evidence of mineralized tissue around pillars
Future Directions

- Fine-tune mechanical properties of structure to mimic the rabbit growth plate
- Study addition of stem cells - endogenous and exogenous
- Long-term study (16 weeks and 1 year)
- Characterizing human growth plate cartilage
Characterization of human growth plate

- Growth plate size across sex and age groups
- Mechanical properties across sex and age groups

Clinical images (epidemiology study at Children’s Hospital Colorado)
- 2008-2018
- 14,436 long bone fractures of the tibia or femur
- Approx. 11.6% involve the growth plate (1,675)

- 2 sources of tissue
  - Discarded surgical tissue from Children’s Hospital Colorado
  - Donor tissue from AlloSource

Provides input for 3D printing/scale-up
Conclusion/Clinical Translation

Prevent Bony Bar Formation Block angiogenesis

Regenerate Growth Plate Cartilage
- Hydrogel with chondrogenic factors
- 3D printed implant

Restore Normal Bone Elongation
Acknowledgements

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- Benjamin Cornelius (Masters Student)
- Joseph Fuchs (Medical student)
- Katie Yamamura, MD
- Francisco Rodriguez-Fontan, MD

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Colorado School of Mines
- Melissa Krebs, PhD
- Nathan Fletcher, PhD
- Jake Newsom
- Matt Osmond, PhD
- Mike Reiderer

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- Stephanie Bryant, PhD
- Elizabeth Aisenbrey, PhD
- Camila Uzcategui (PhD candidate)
- Archish Muralidaran (PhD candidate)
- Sarah Schoonraad (PhD candidate)

- Virginia Ferguson, PhD
- Kevin Eckstein (PhD candidate)
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Thank You!

University of Colorado Anschutz Medical Campus