Regenerative Medicine Approaches for the Treatment of Growth Plate Injuries

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August 20, 2020

Children's Hospital Colorado

Orthopedics
UNIVERSITY OF COLORADO

How I got here

Montreal, CANADA

- High School (biology, chemistry)
- BS in Physiology (McGill University, 2000)
  - Artificial cells, tissue engineering
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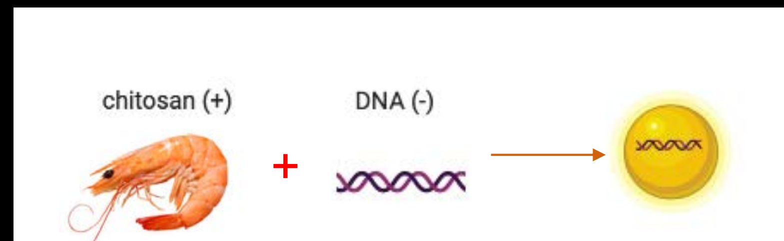
Chitosan-DNA nanoparticles



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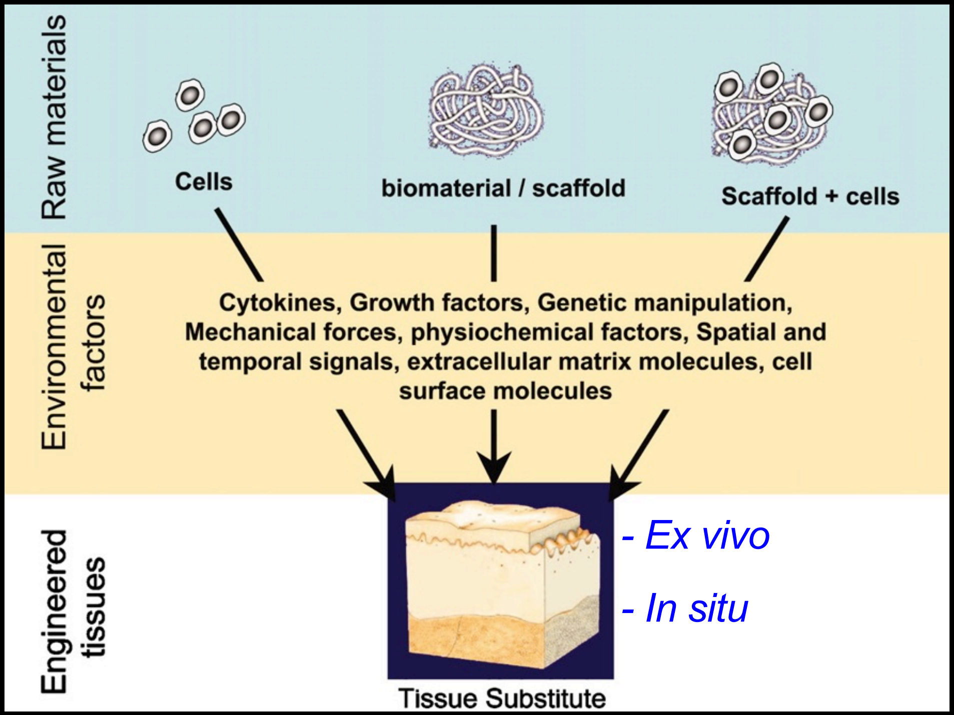
University of Colorado Anschutz Medical Campus

2012-present: Associate Professor, Orthopedics
Faculty Member, Gates Center for Regenerative Medicine

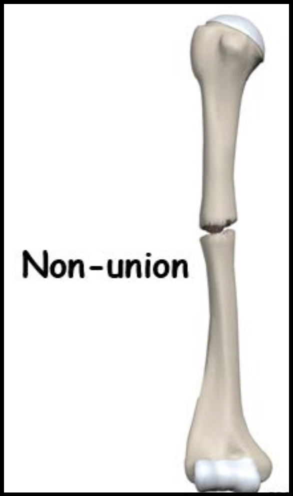
Regenerative Medicine

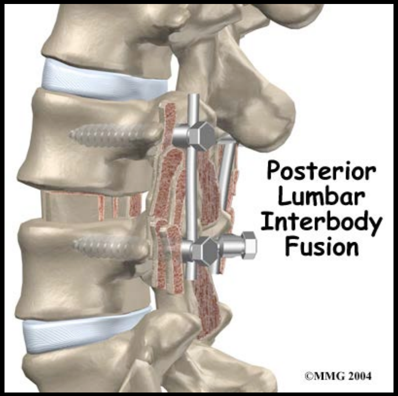


Khademhosseini A et al. PNAS 2006;103:2480-2487

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





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- Articular cartilage injuries

osteoarthritis

Why are we interested in regenerative medicine in orthopedics?

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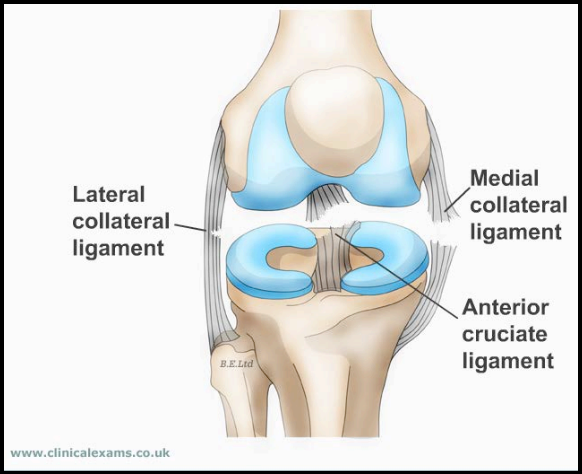
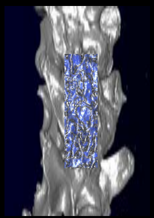
- Spinal fusion
- Articular cartilage injuries
- Ligament and tendon injuries



Regenerative Orthopedics

Bone formation for spinal fusion

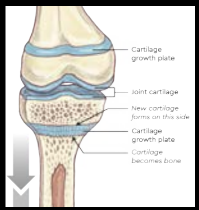


- Stem cells + bone allografts
- Biological agents

Articular Cartilage

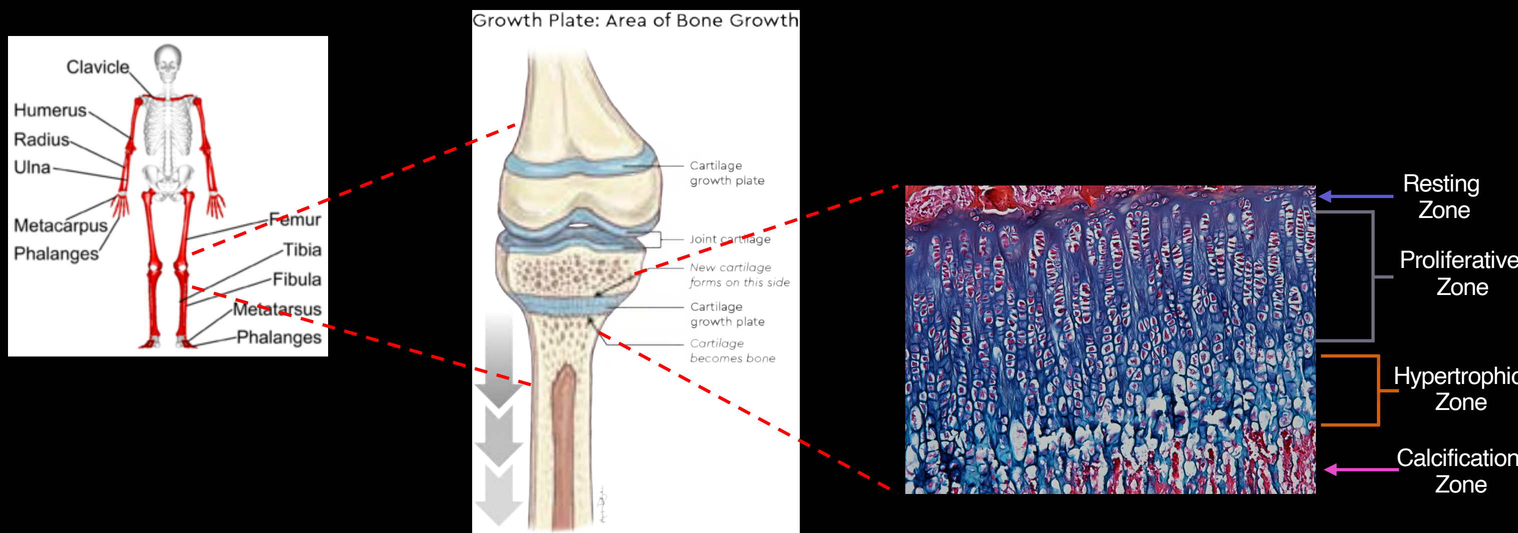
- Prevention of post-traumatic osteoarthritis in skeletally immature animals

Growth Plate Cartilage



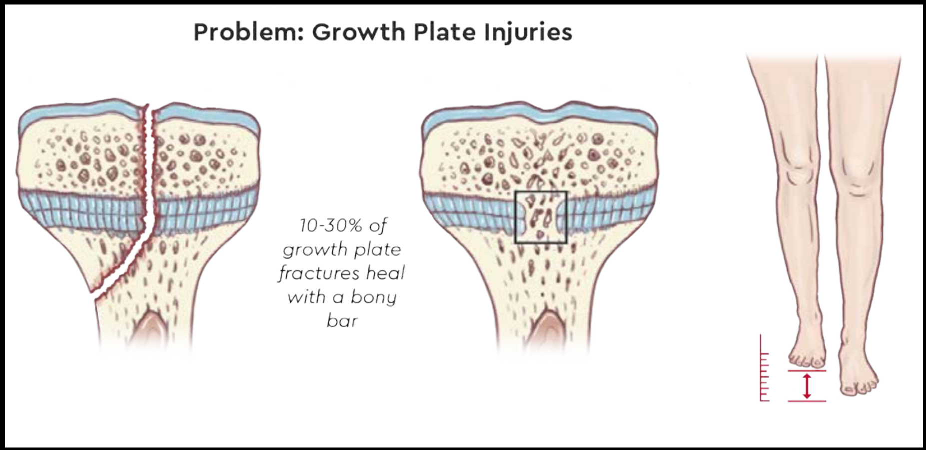
- Growth plate cartilage biology
- Growth plate repair strategies

Growth Plate (Physis)



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]



1. Mäyränpää, M.K., et al., J Bone Miner Res **25**, 2752, 2010.
2. Mann, D.C., et al. J Pediatr Orthop **10**, 713, 1990.

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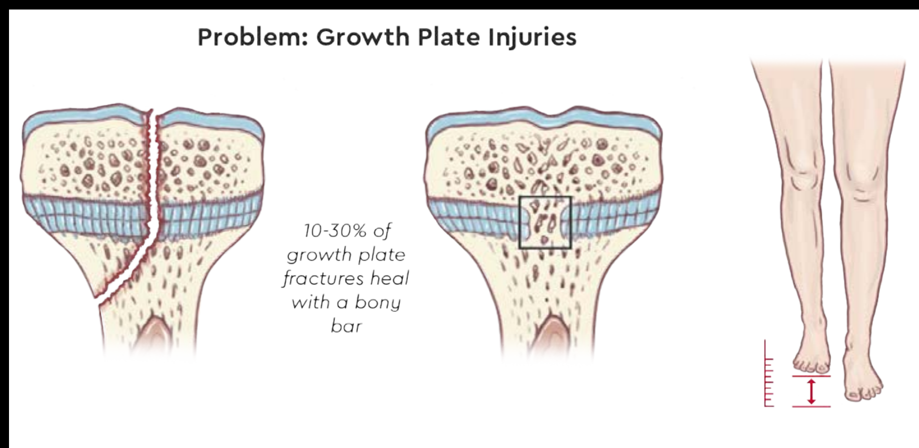

www.paleyinstitute.org

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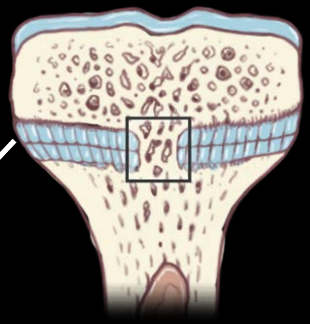
Growth Plate Injuries: Current Treatments



- 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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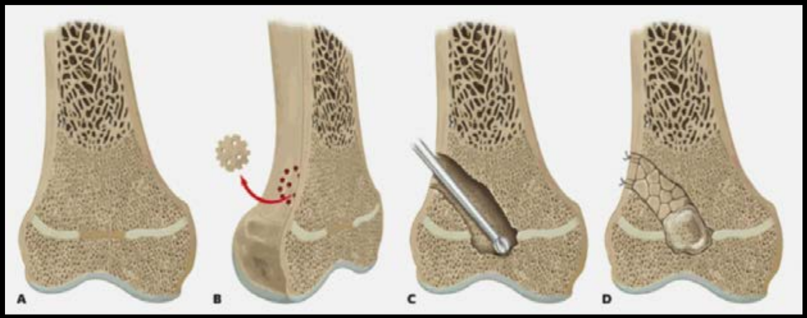
BONY BAR RESECTION

18-30% poor outcome



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Growth Plate Injuries: Current Treatments

- bony bar spans <50% of growth plate volume
- 2 years or 2 cm of growth remaining

BONY BAR RESECTION

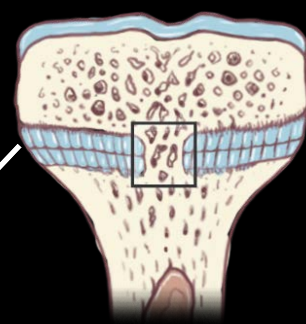
18-30% poor outcome

- 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 spans <50% of growth plate volume
- 2 years or 2 cm of growth remaining

BONY BAR RESECTION

18-30% poor outcome

- bony bar spans >50% of growth plate volume

ARTIFICIAL CLOSURE OF GROWTH PLATE

Prone to infections, multiple hospitalizations

NO treatment is attempting to regenerate the growth plate cartilage

Research Program Focus

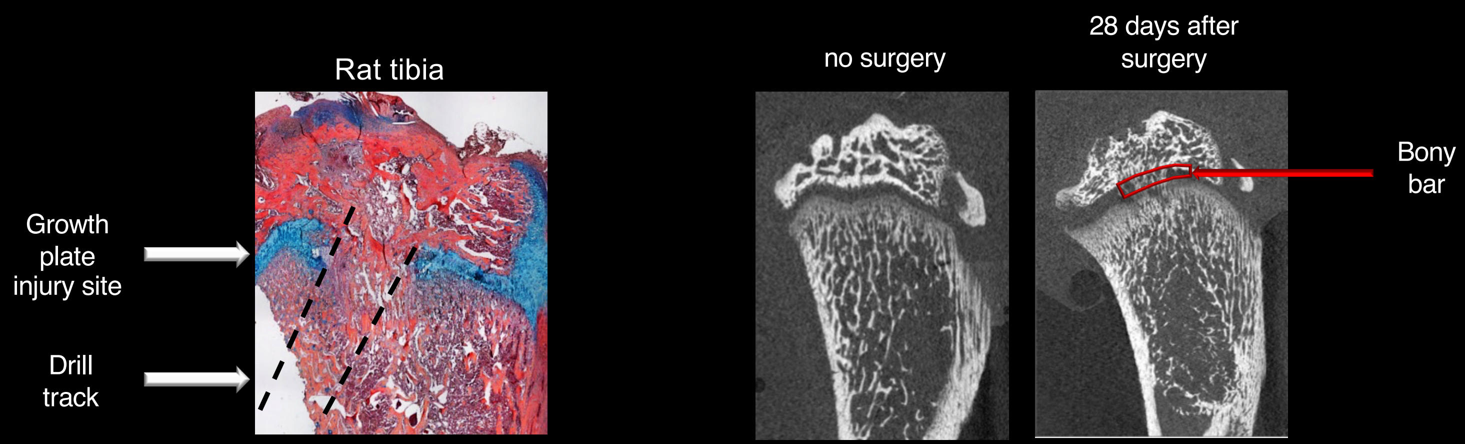
Developing functional regenerative medicine approaches to treat growth plate injuries.

Prevent Bony Bar Formation

Regenerate Growth Plate Cartilage

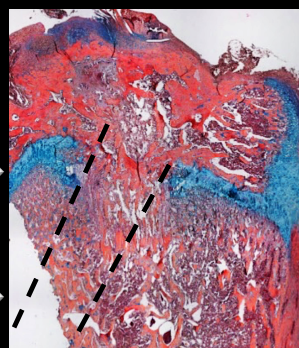
Restore Normal Bone Elongation

Rat proximal tibial growth plate drill-hole defect reproducibly creates a bony bar



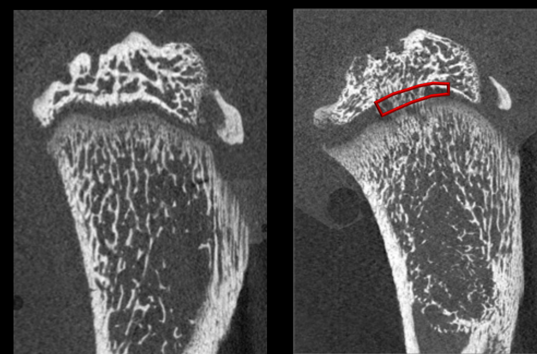
Rat proximal tibial growth plate drill-hole defect reproducibly creates a bony bar

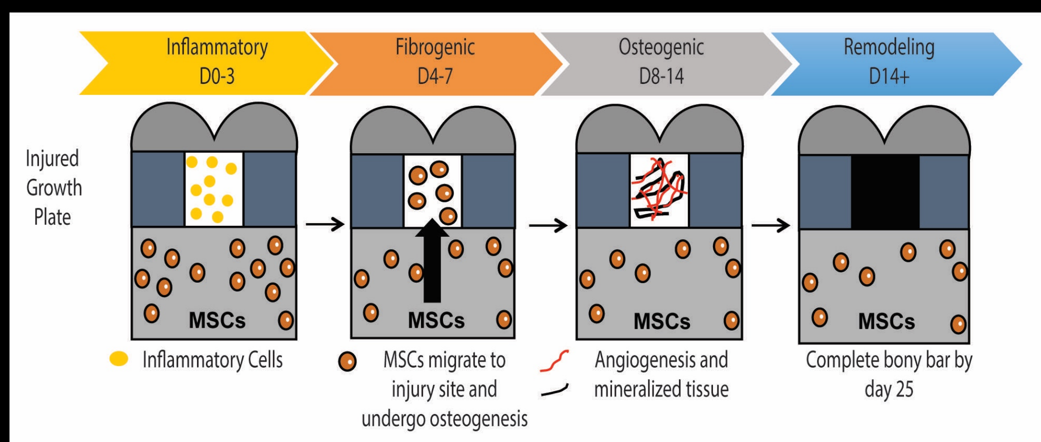
Rat tibia



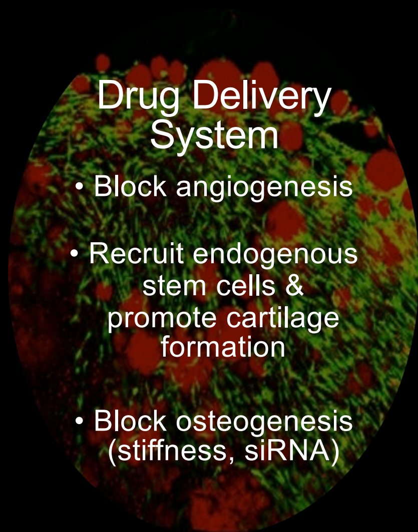
no surgery

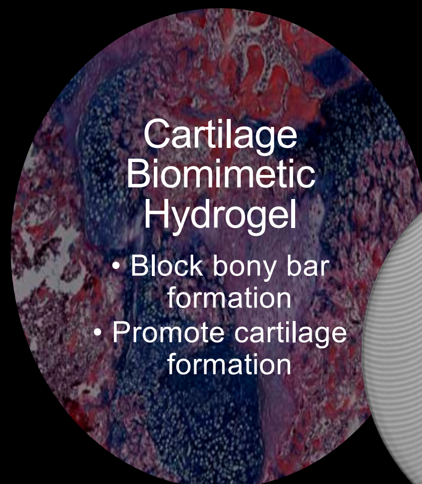
28 days after surgery

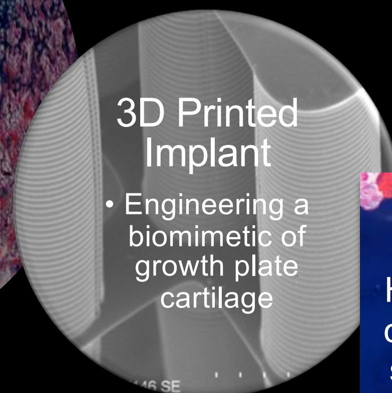


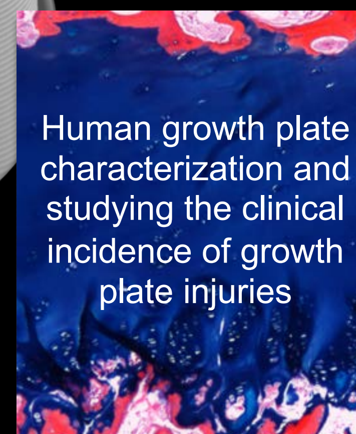


Research Projects









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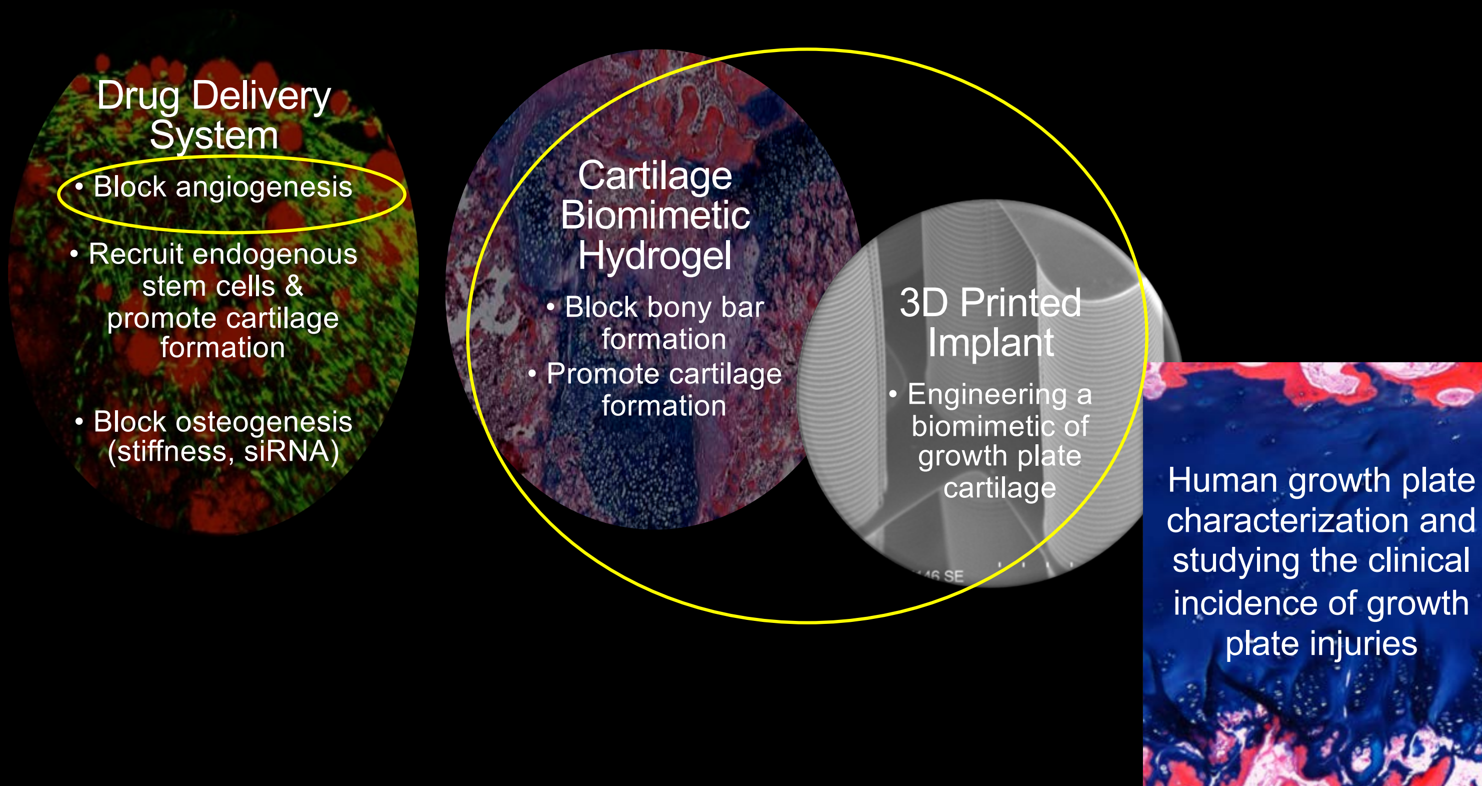
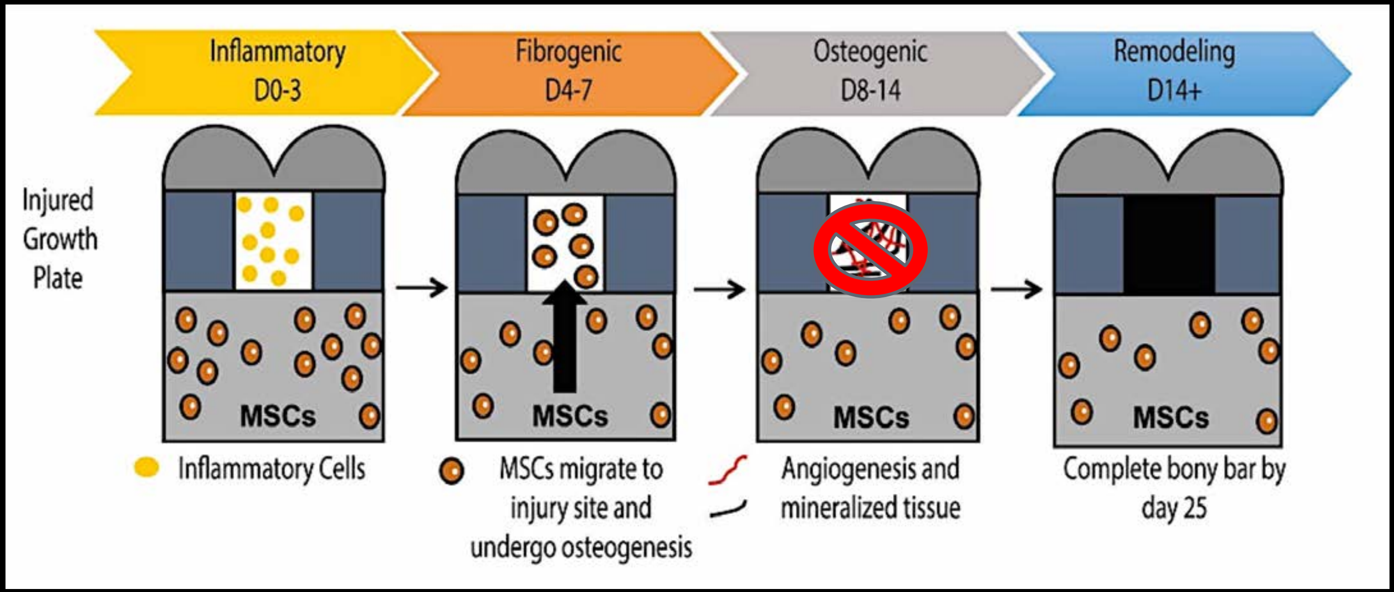


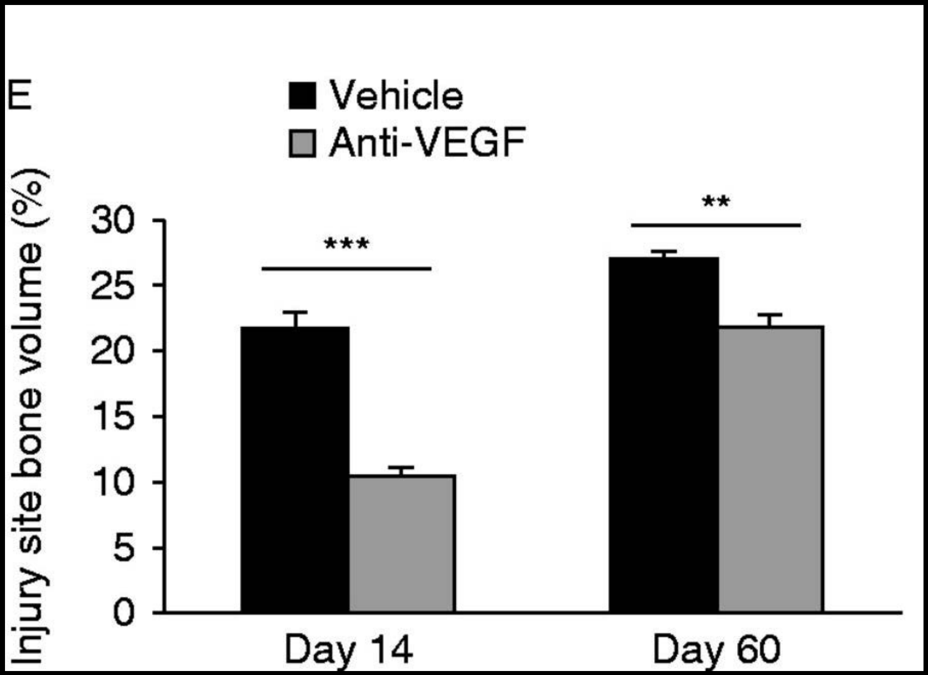
Project #1: Determine whether local delivery of an anti-angiogenic factor after growth plate injury will prevent bony bar formation

- Vascular endothelial growth factor (VEGF) influences bony bar formation[1].



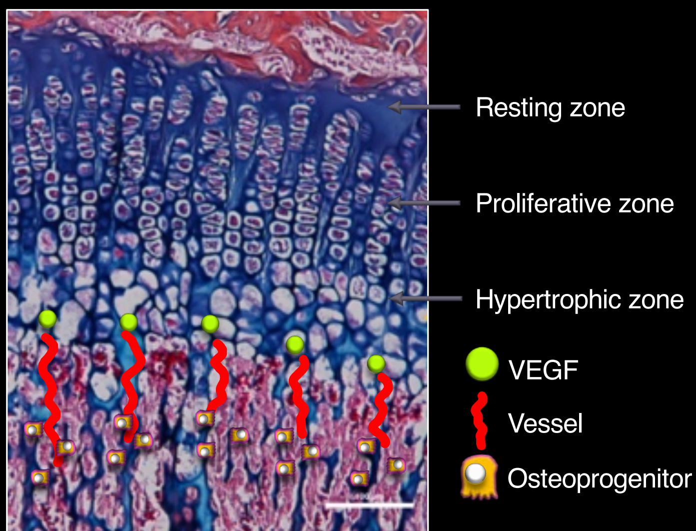
[1]Fischerauer, E., *et. al.* J Mol Hist (2011) 42:513–522

Systemic anti-VEGF antibody reduces bony bar

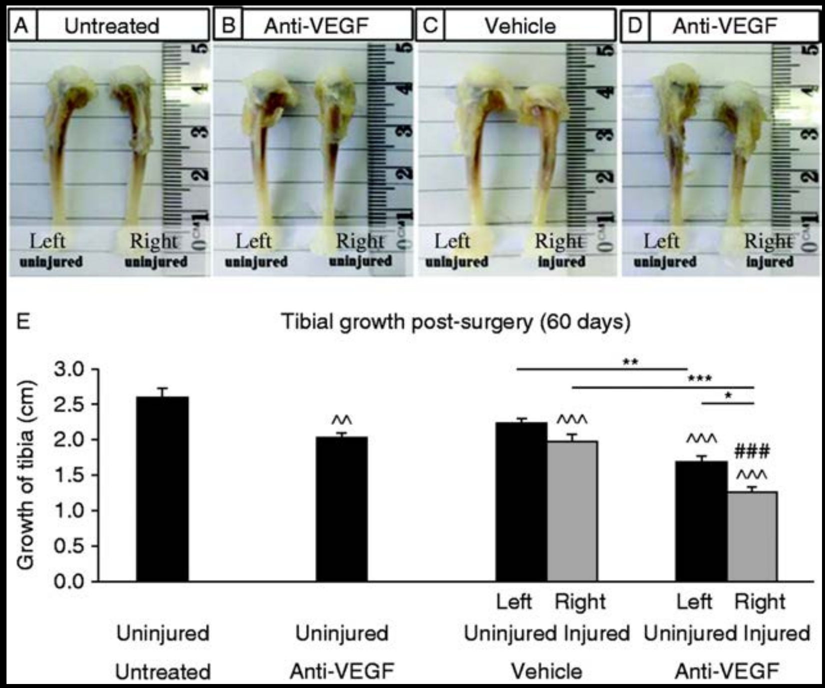


Chung R., et al. J Endocrinol 2014; 221:63-75.

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



Hall *et. al.* 2006 Toxic Pathol



^^ or ^^^ comparison to the untreated control
comparison to uninjured anti-VEGF-treated group
(*P<0.05, ^^, **P<0.01 and ^^^, ###, ***P<0.001).

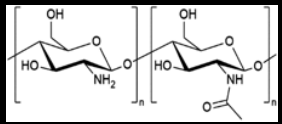
Chung R., et al. J Endocrinol 2014; 221:63-75.

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

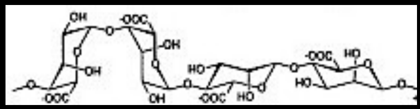
Chitosan:
Cationic polysaccharide

Used extensively for cartilage regeneration



Alginate:
Anionic polysaccharide

Used extensively for drug delivery

Collaboration with Melissa Krebs, PhD – Colorado School of Mines

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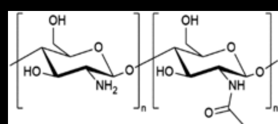

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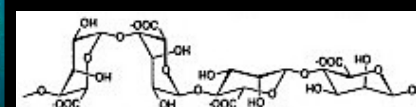
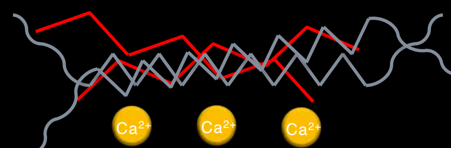
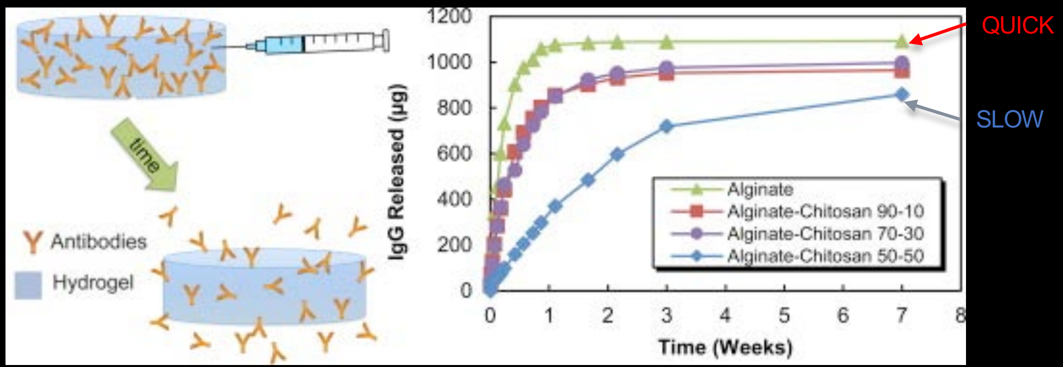




Varying alginate:chitosan ratio and calcium crosslinking can fine-tune biomaterial properties

Release of antibodies can be modulated in alginate-chitosan hydrogels



Fletcher N. et al. Mater. Sci. and Eng. C. 2016; 801-806.

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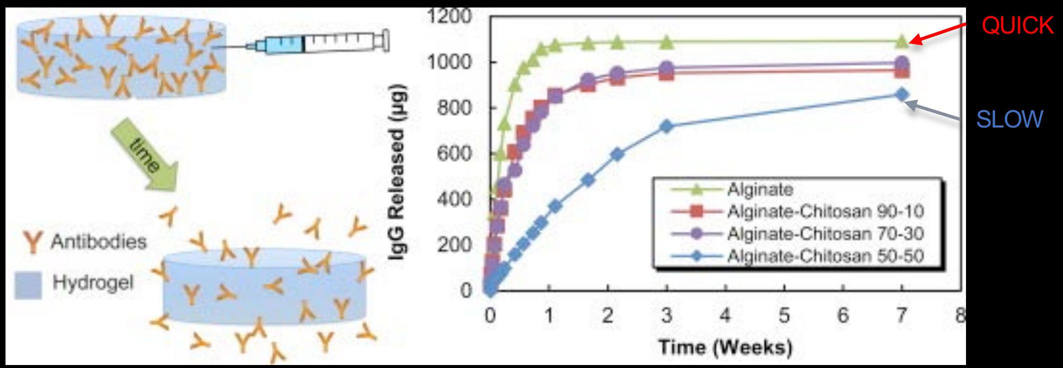

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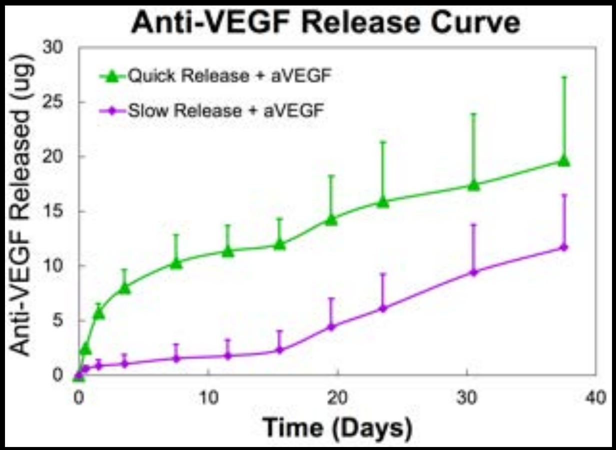
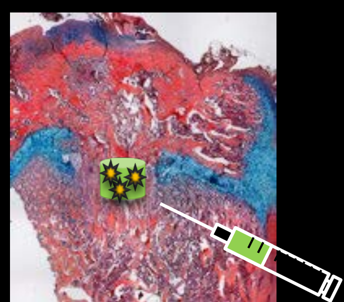


Quick Release = Alginate:chitosan 90:10

Slow Release = Alginate:chitosan 50:50

Study Design


Chris Erickson, PhD



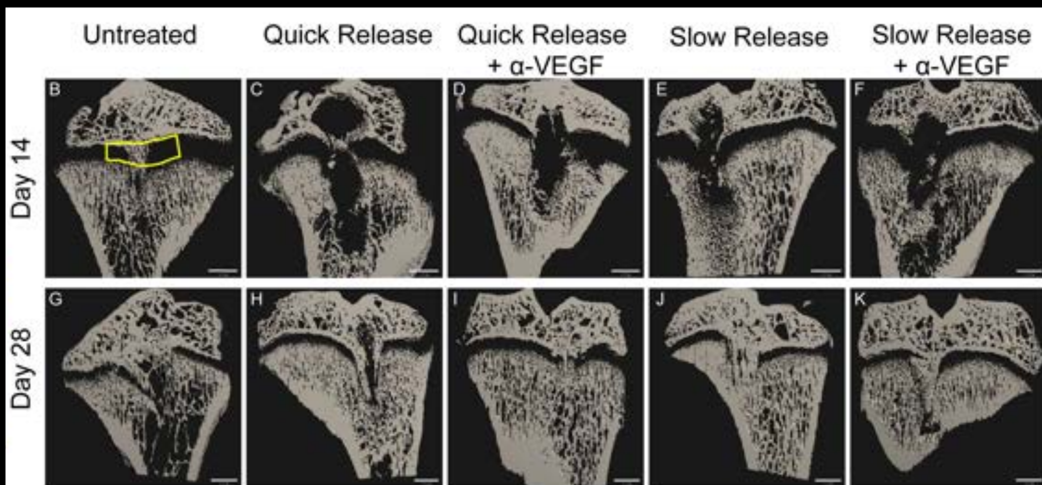
Alginate:Chitosan Hydrogel

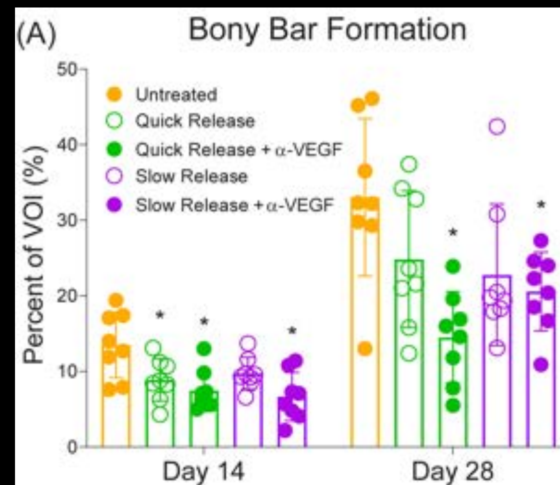
Anti-VEGF Antibody

~7ug anti-$VEGF_{165}$

	Treatment groups	Hydrogel name	α-VEGF	Outcomes
1	Intact	-	-	• MicroCT, histology • Perfusion/Blood vessels • Limb growth • N = 8 limbs total (4 male, 4 female) per time point per outcome
2	Untreated	-	-	
3	Alginate:chitosan 90:10	Quick Release	-	
4	Alginate:chitosan 90:10 + anti-VEGF antibody	Quick Release + α-VEGF	+	
5	Alginate:chitosan 50:50	Slow Release	-	
6	Alginate:chitosan 50:50 + anti-VEGF antibody	Slow Release + α-VEGF	+	

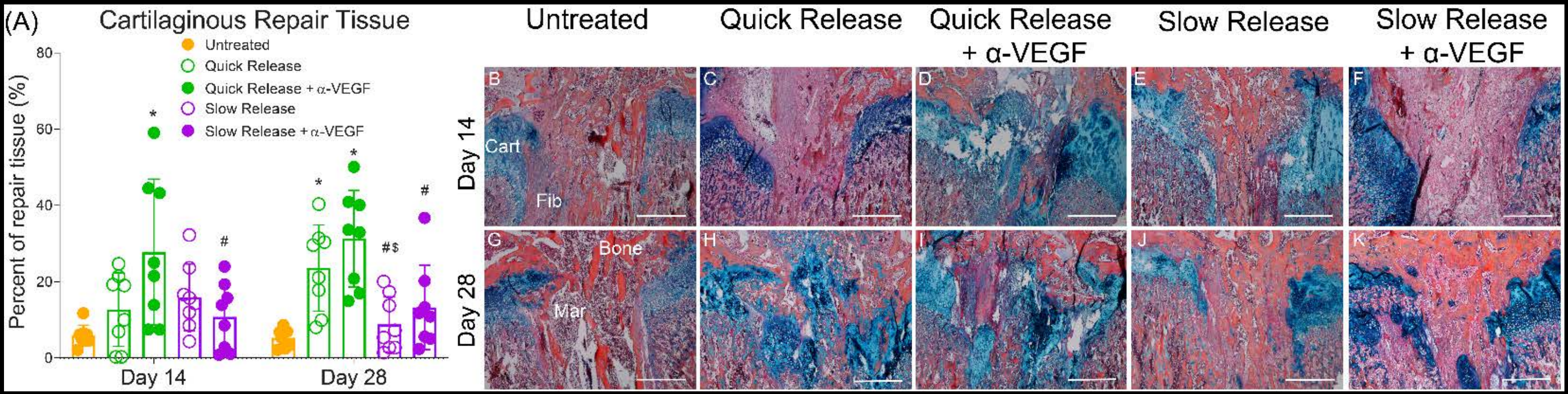
Local delivery of α-VEGF reduces bony bar formation





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

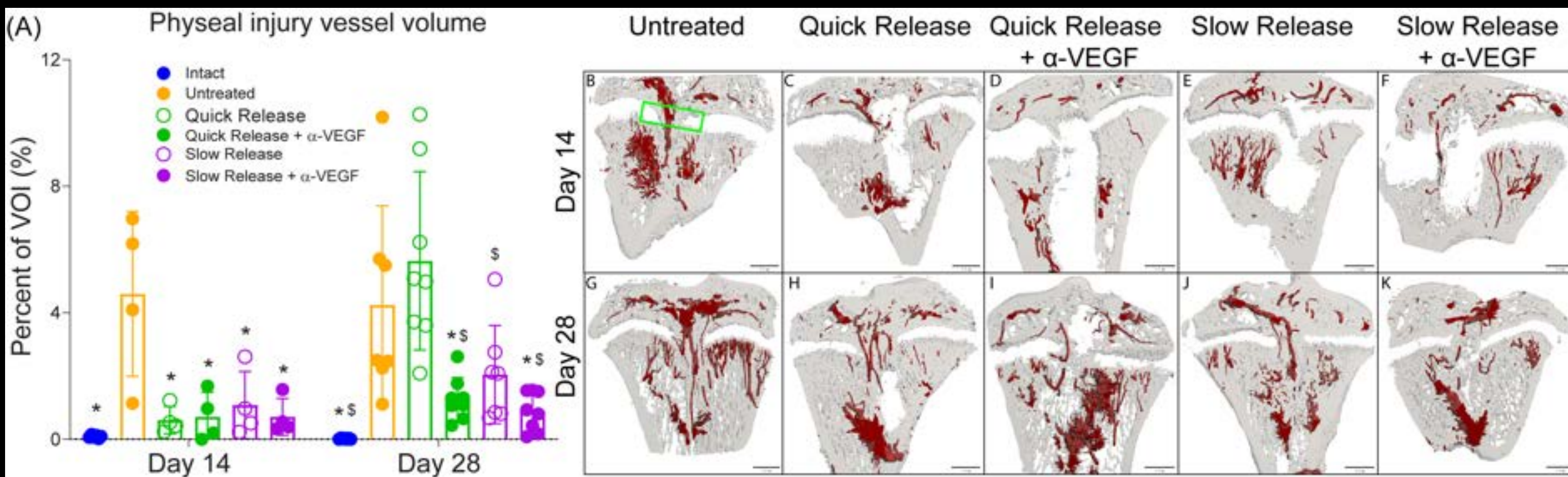
Quick delivery of α-VEGF increases cartilaginous repair tissue



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

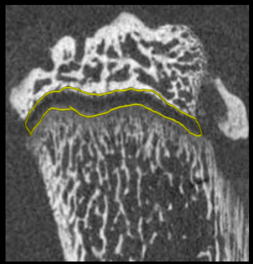
Blue = cartilage

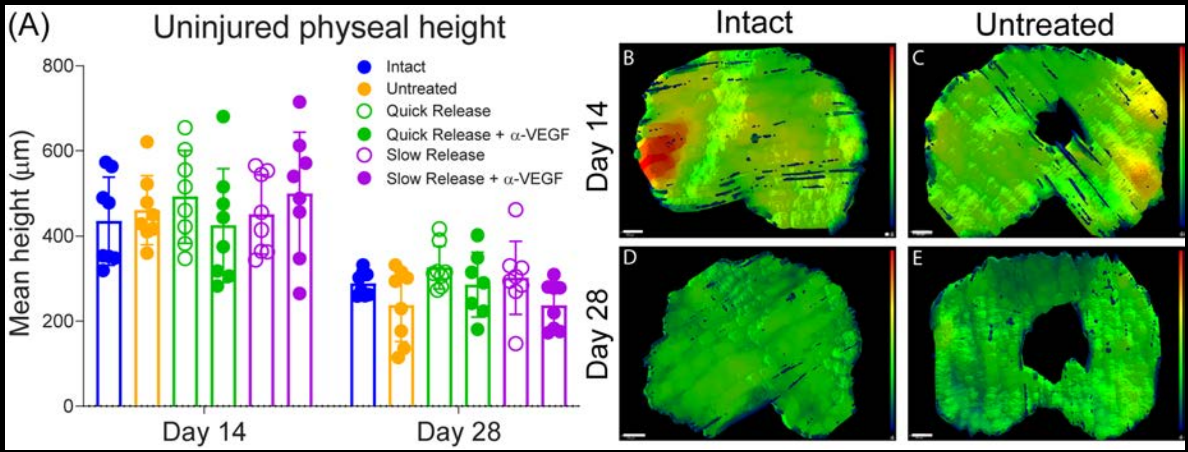
Local delivery of α-VEGF reduces vessel formation at injury site



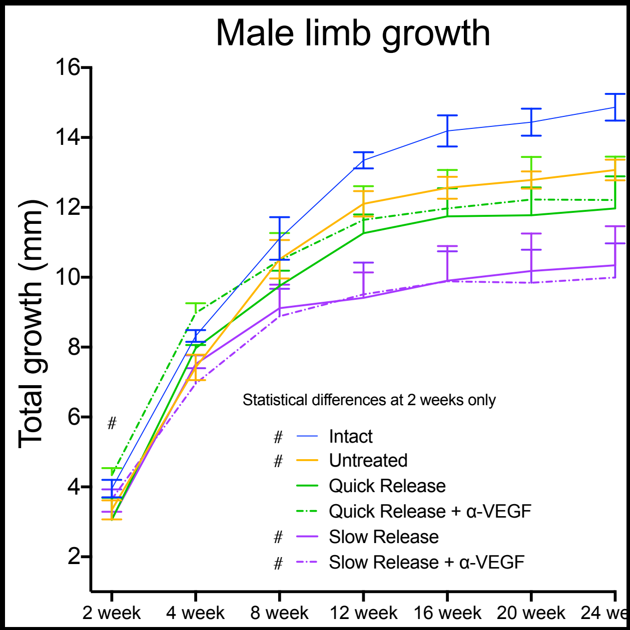
Mean +/- SD, one-way ANOVA, N = 8
*P<0.05 vs. Untreated same time point
$P<0.05 vs. QR same time point

Local delivery of α-VEGF does not affect average physeal height

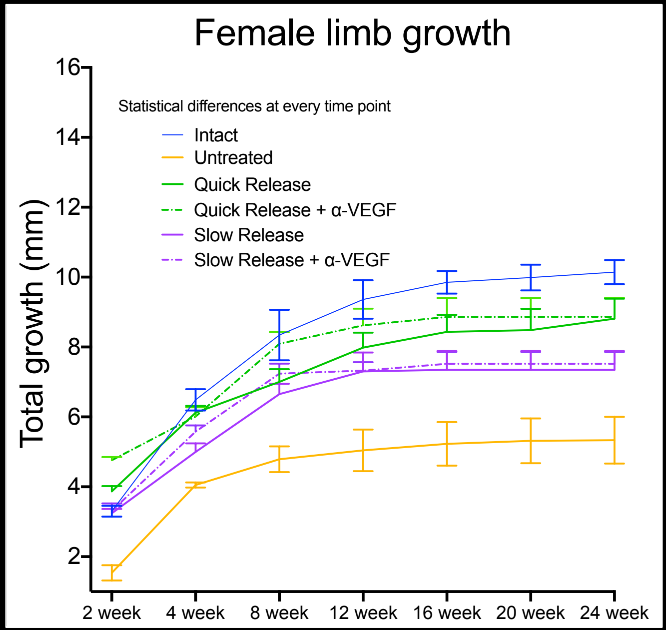




Local delivery of α-VEGF does not affect limb lengthening



# P<0.05 vs Quick Release + α-VEGF



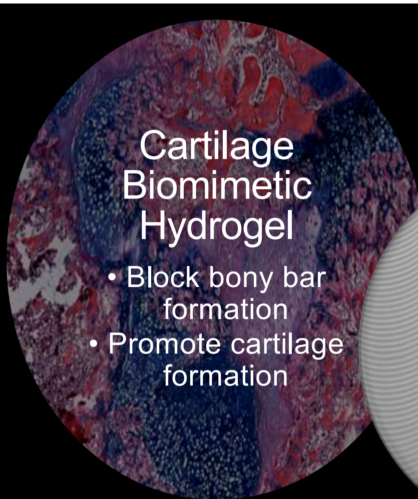
1. Untreated < all groups at 2 weeks
2. Intact & Quick Release + α-VEGF > Untreated all times
3. Intact > Slow Release + α-VEGF at 16, 20, 24 weeks

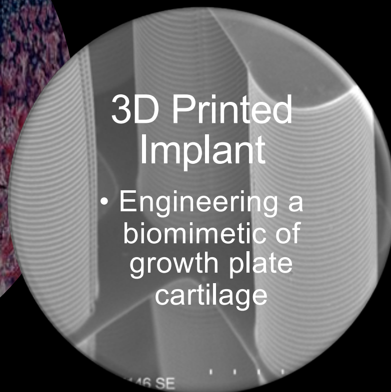
Mean +/- SD, Repeated measured 2-way ANOVA, n=8

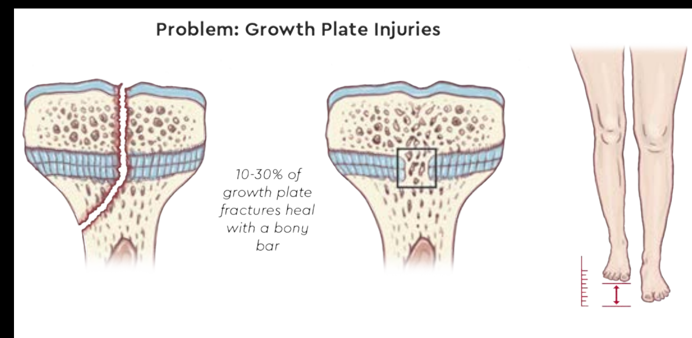
Conclusion and Future Directions

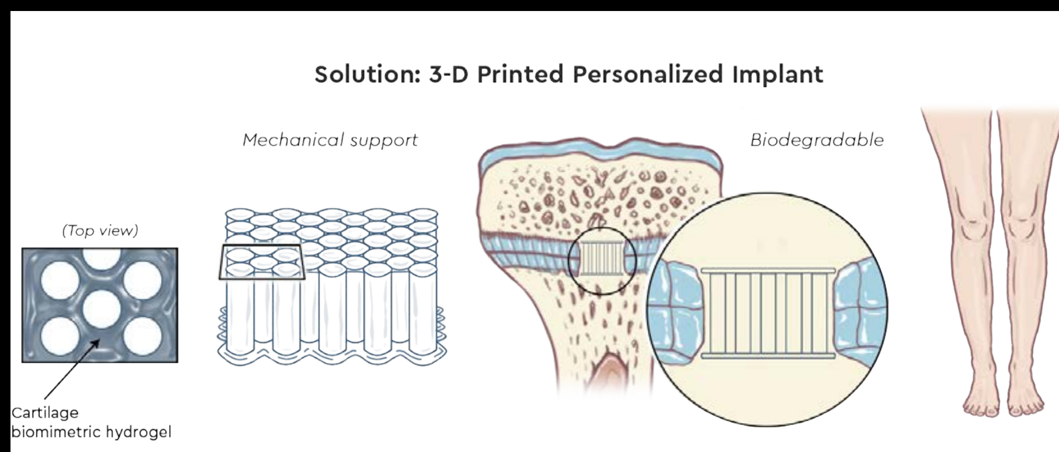
- **Conclusions**
  - Local delivery of α-VEGF reduces bony bar formation
  - Quick delivery of α-VEGF increases cartilaginous tissue formation
  - Local delivery of α-VEGF does not affect limb lengthening, or adjacent physis
  - There are differences between Quick Release and Slow Release hydrogels

- **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
(Top view)
Cartilage biomimetic hydrogel

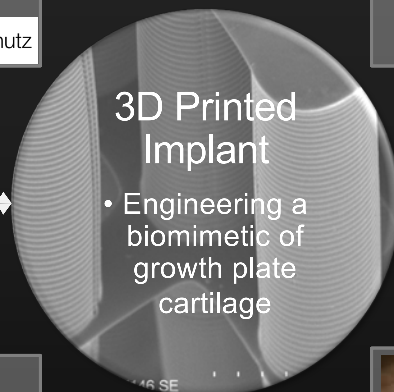
Multidisciplinary Team

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

Anschutz

Nancy Hadley Miller, MD
- Clinical experience

Children's Hospital Colorado



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

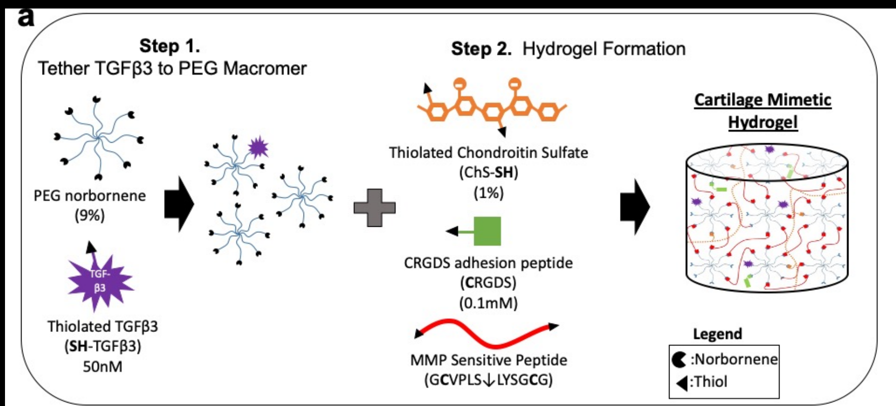
Boulder

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

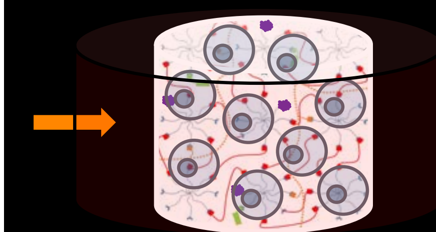
Boulder

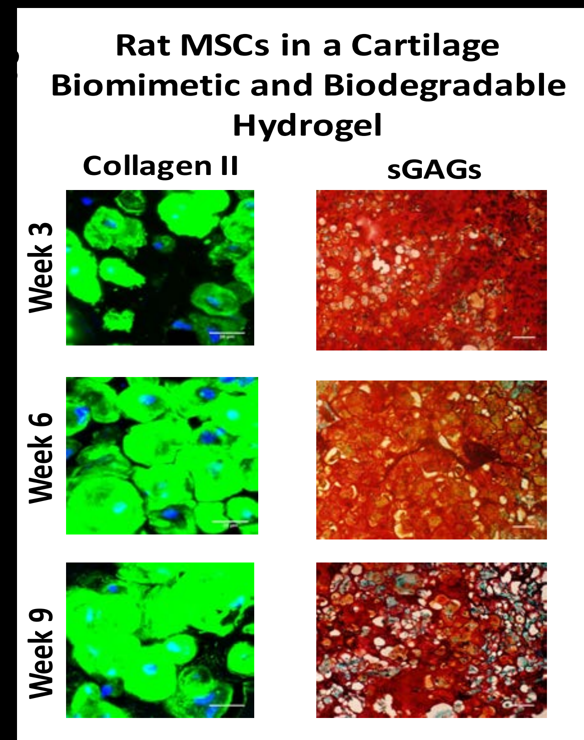
Cartilage Mimetic Hydrogel Induces Chondrogenesis of MSCs


Elizabeth Aisenbrey, PhD

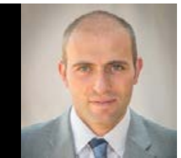


Photopolymerizable cartilage mimetic hydrogel



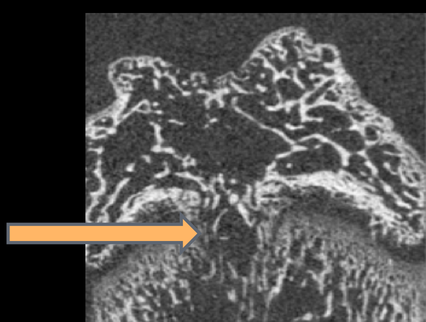


Testing Cartilage Mimetic Hydrogel in a Rat Model of Growth Plate Injury

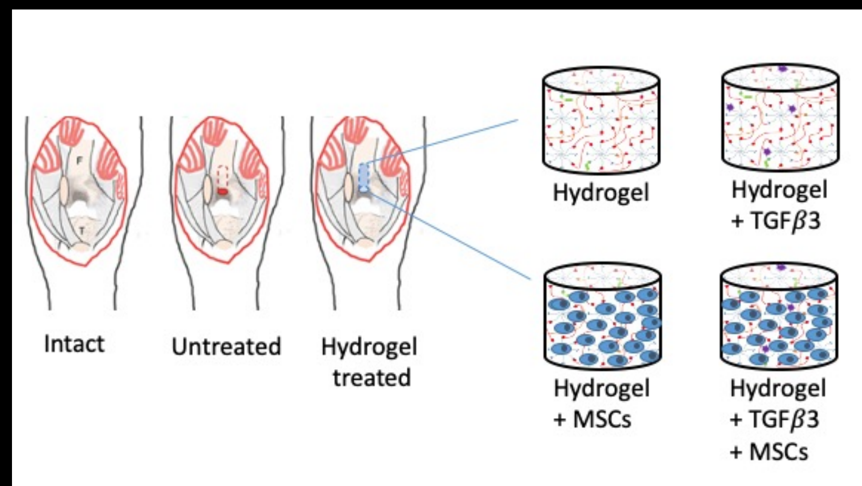


Francisco Rodriguez Fontan, MD

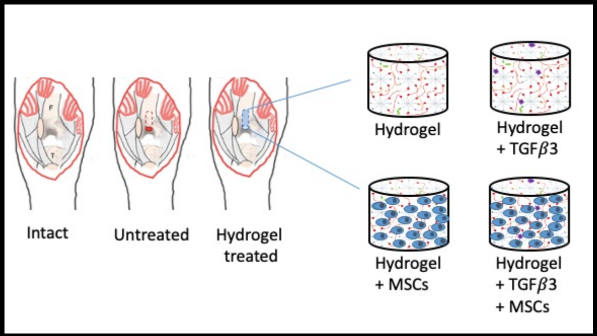
Healthy rat femoral growth plate

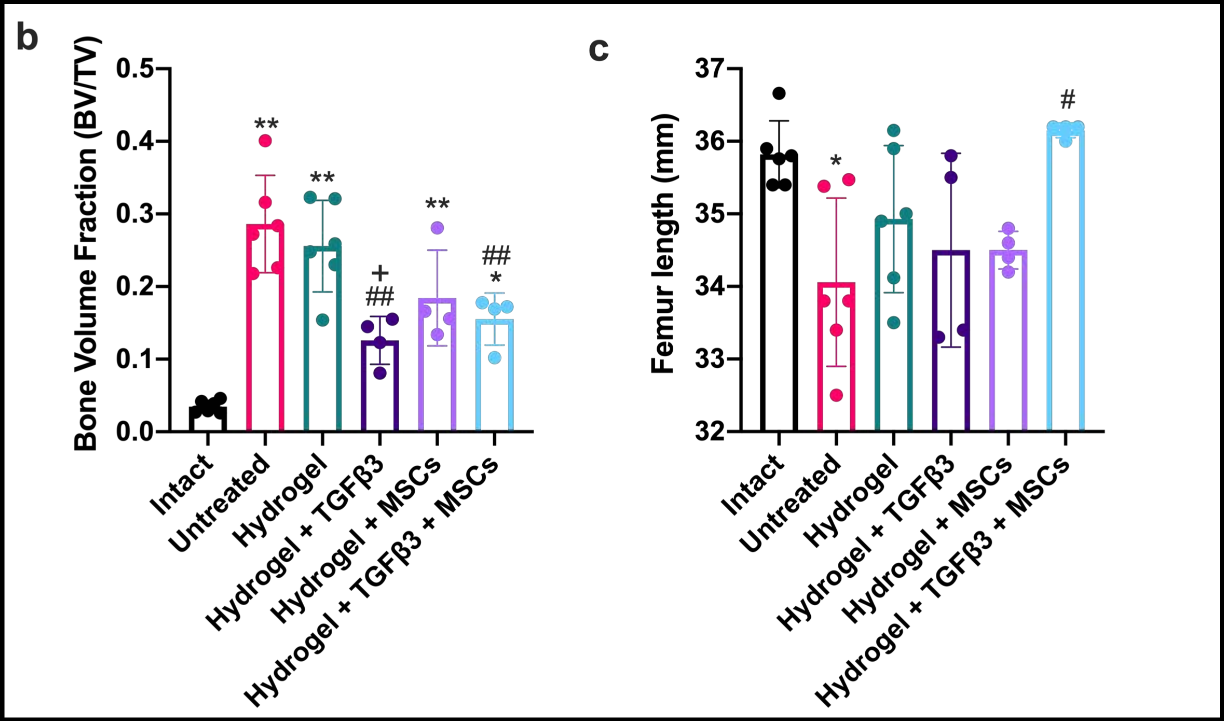


28 days post-injury



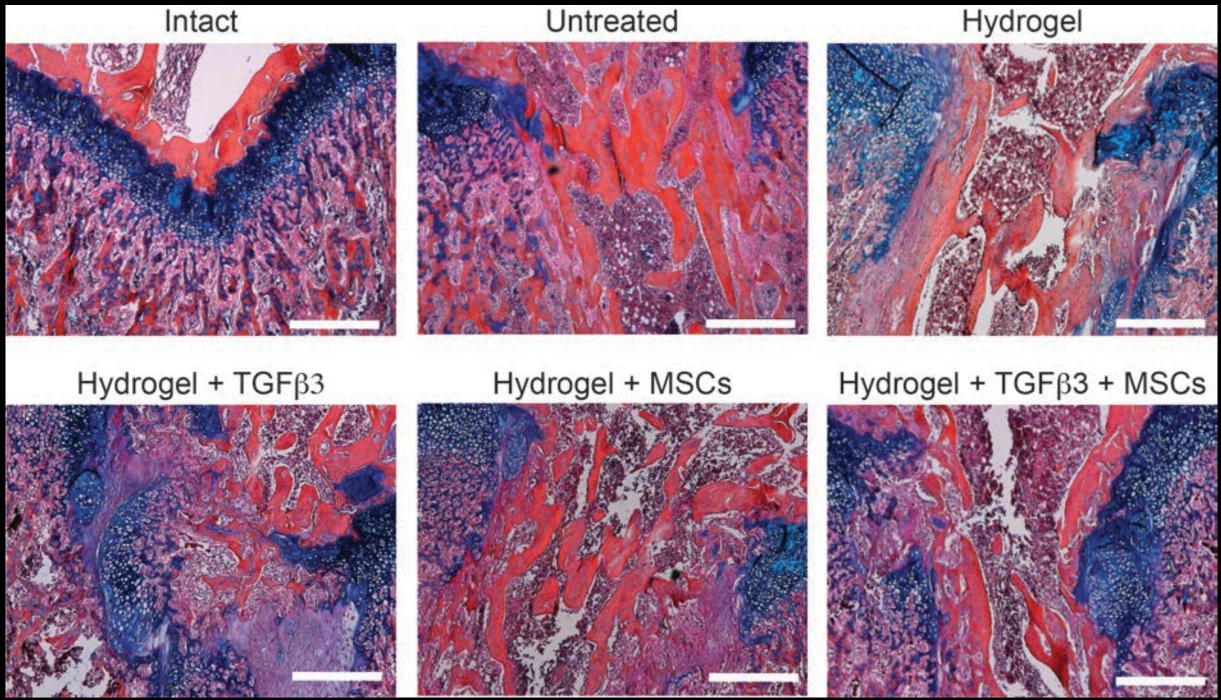
Cartilage Mimetic Hydrogel with TGFβ3 Reduced Bony Bar Formation





N=4-6, * vs. Intact, # vs. Untreated, + vs. Hydrogel

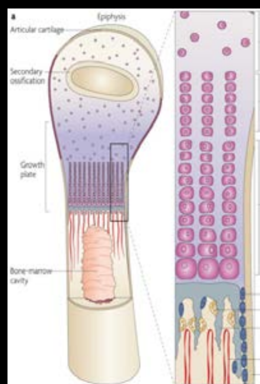
Cartilage Mimetic Hydrogel with TGFβ3 Formed New Cartilage Tissue



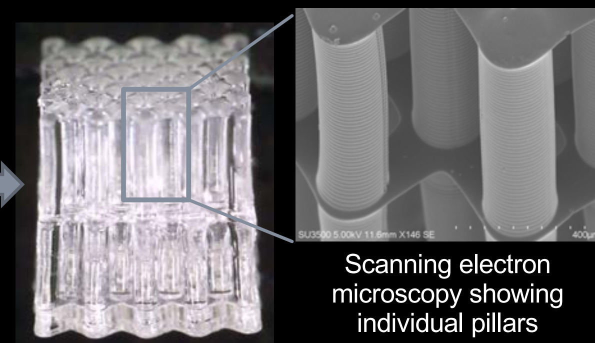
Blue = cartilage
Red = Bone

Combining Hydrogel and 3D Printing

3D printing technology



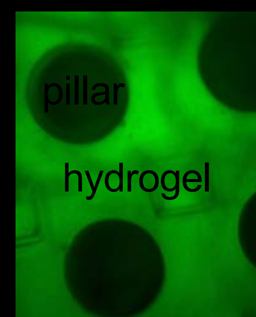
Characterize zonal properties and morphology of the rabbit growth plate



3D Printed structure

Scanning electron microscopy showing individual pillars

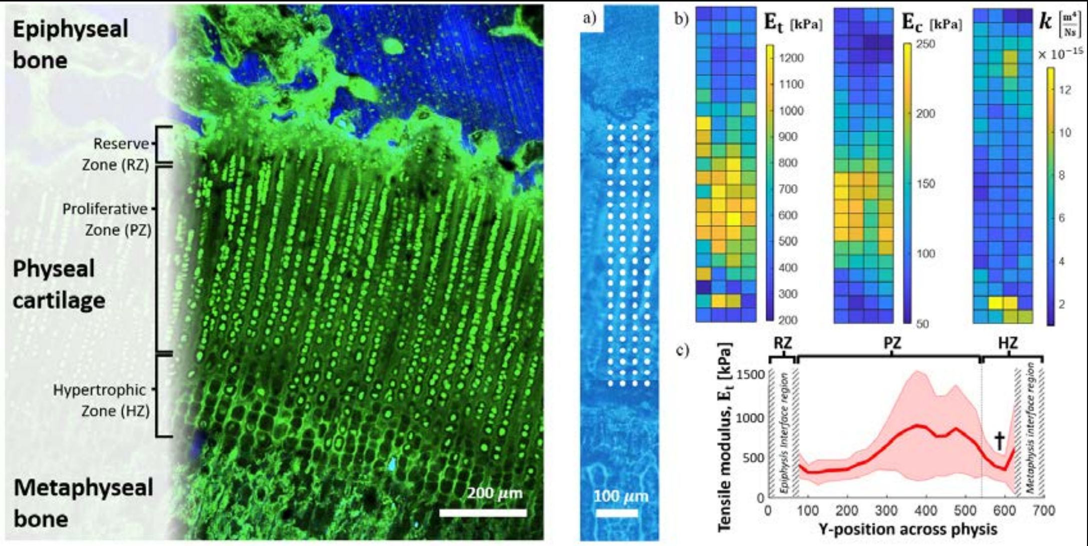
3D Printed structure is infilled with hydrogel



Kevin Eckstein, MS

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.

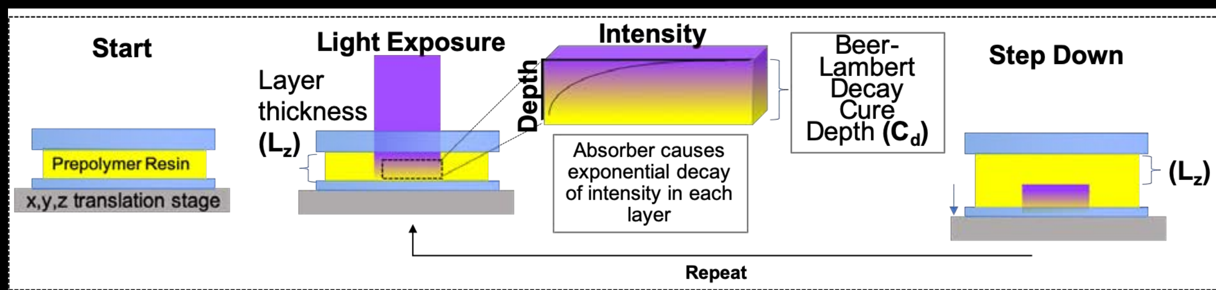
Gradients in stiffness found within individual zones of physeal cartilage.

- Sharp decline in stiffness in hypertrophic region.†

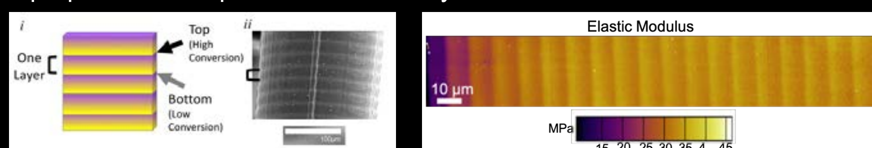
Camila Uzcategui

3D Printing Technology

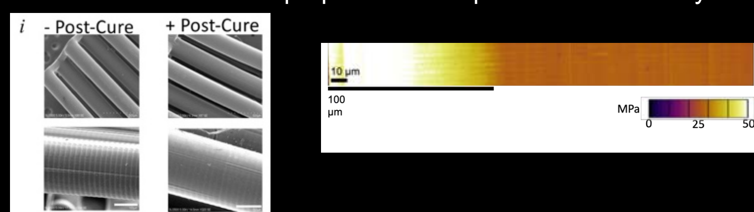
- Layer-by-layer 3D printing by stereolithography (SLA)



Variable properties in 3D printed structures by SLA



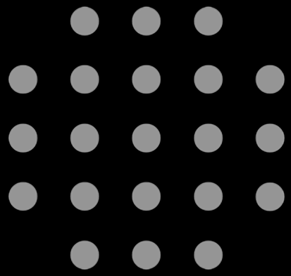
Developed methods to achieve uniform properties in 3D printed structures by SLA

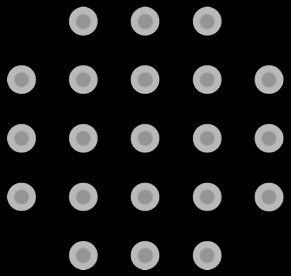


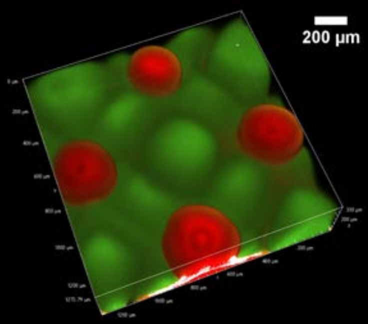
Uzcategui AC *et al.* Adv Eng Mater. 2018; 20(12)

Integration of Cartilage Mimetic Hydrogel with Stiff Structure

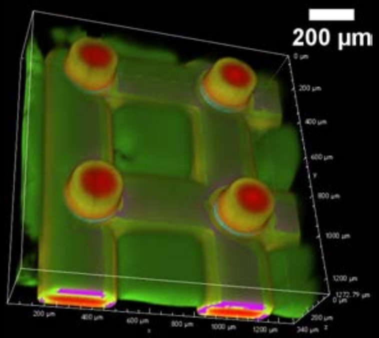
Archish Muralidharan







High conversion in pillars
- No integration



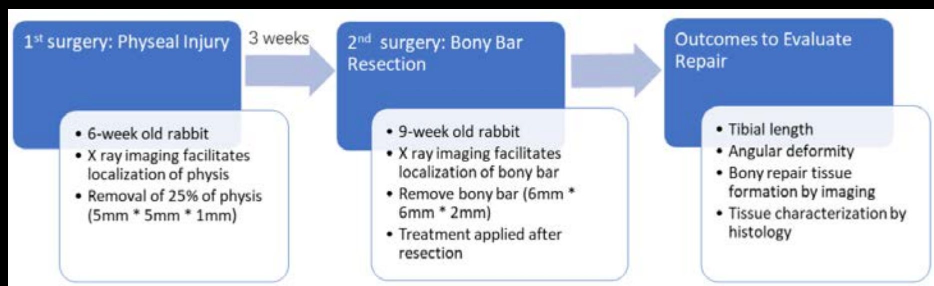
Shell of low conversion
around pillars
- Integration

Red = stiff pillars
Green = cartilage mimetic hydrogel

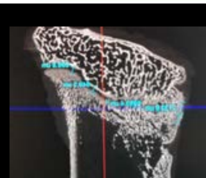
Testing the 3D printed construct *in vivo*

Yangyi Yu, MD

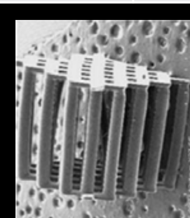
Rabbit model of proximal tibia physeal injury



Right tibia: injured
Left tibia: intact

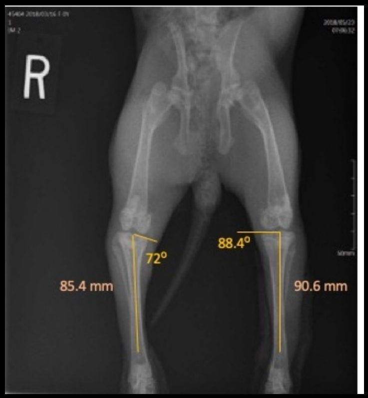


Groups	Total number of rabbits
Untreated	10
Fat Graft	9
Cartilage mimetic hydrogel	10
3D structure infilled with cartilage mimetic hydrogel	10

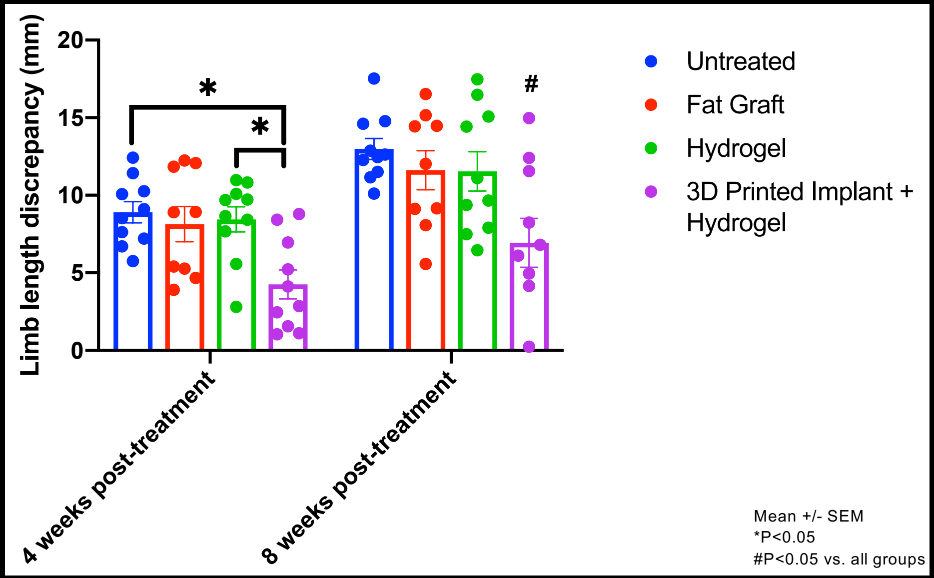


6 mm x 6 mm x 2 mm

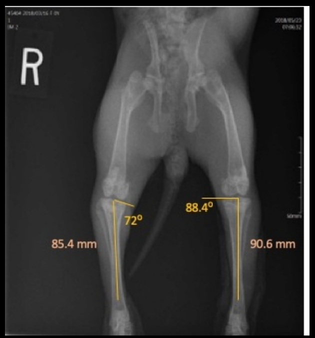
3D Printed Implant Led to Decreased Limb Length Discrepancy



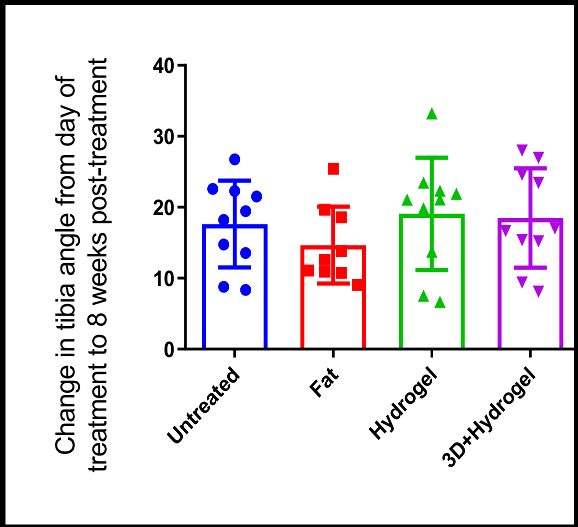
Limb length discrepancy =
Left tibia (Intact) – right tibia (Injured)



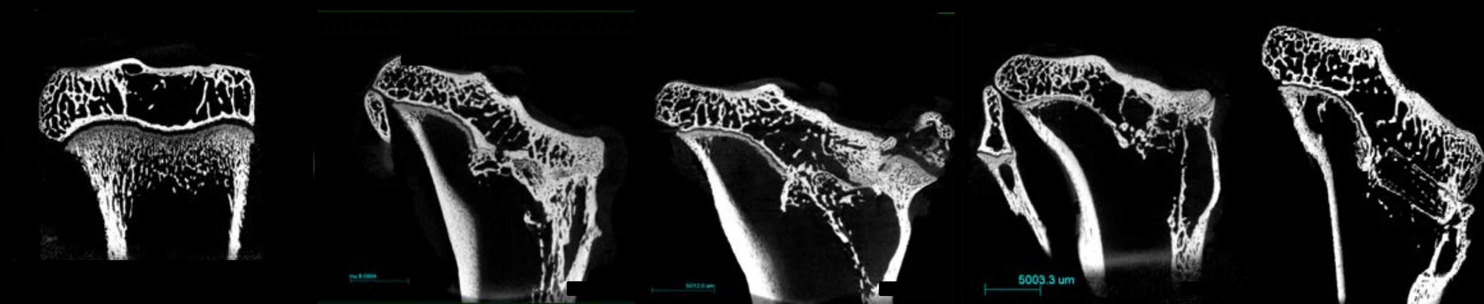
No Treatment Led to an Improvement in Tibial Angle

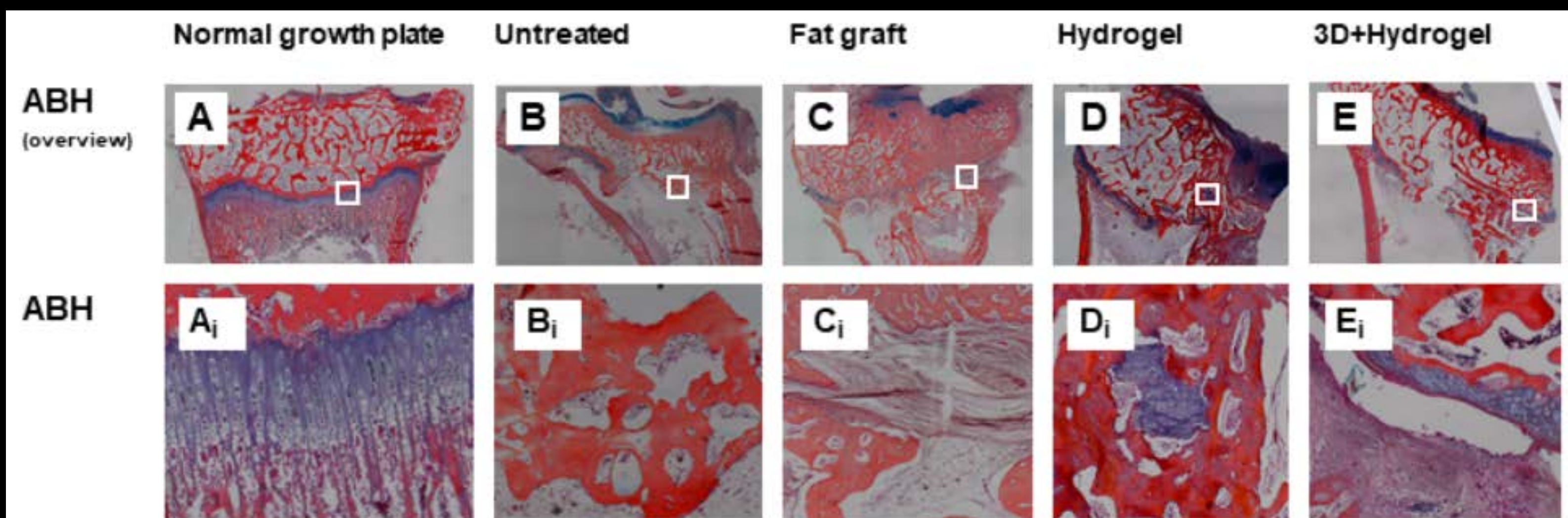


Change in tibial angle =
Tibia angle (day 0)
– Tibia angle (8 weeks post-treatment)



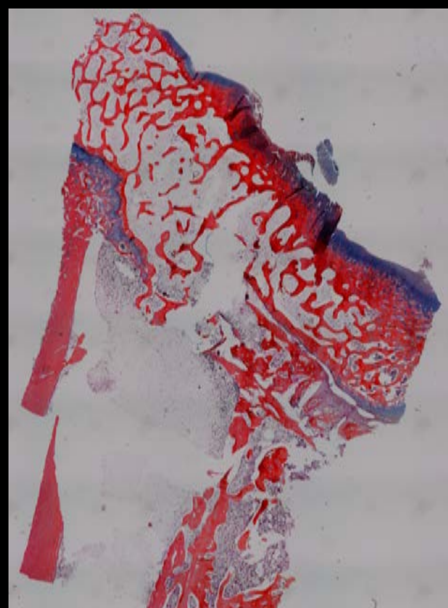
MicroCT and Histology 8 weeks post-implantation

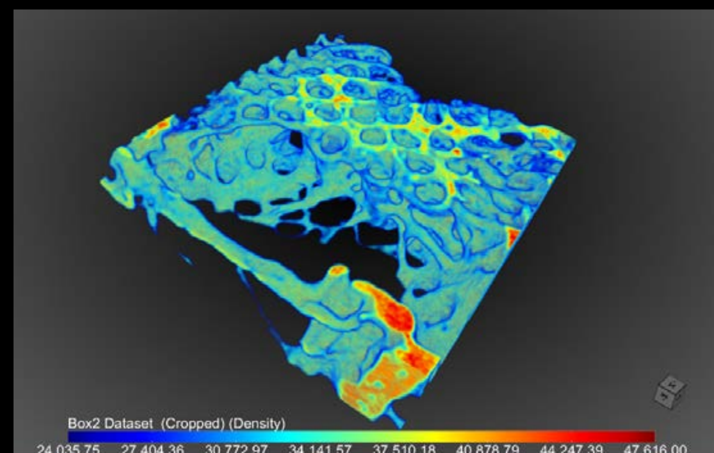




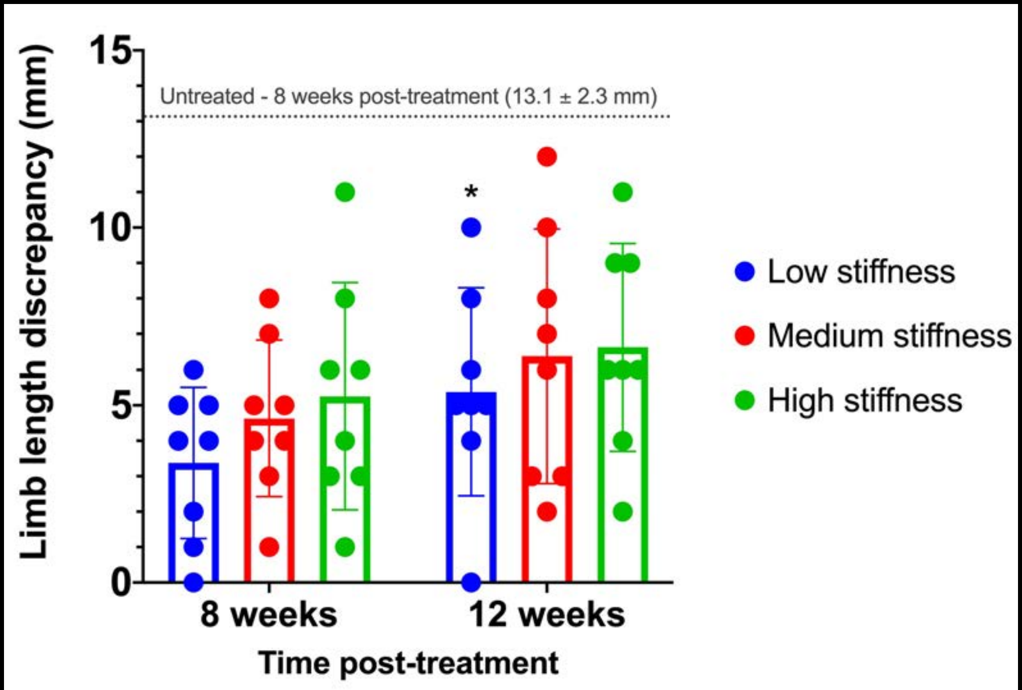
Mineralization within 3D Printed Implant

Kristine Fischenich, PhD





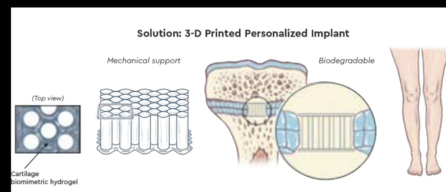
Effect of mechanical stiffness of the implant



100 kPa
500 kPa
1 MPa

Mean +/- SD
N=8/group
* 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

→ Provides input for 3D printing/scale-up

- 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

Conclusion/Clinical Translation

Prevent Bony Bar Formation
Block angiogenesis

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

Restore Normal Bone Elongation

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- Kevin Eckstein (PhD candidate)
- Kristine Fischenich, PhD

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R21 AR071585

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MTF Musculoskeletal Transplant Foundation

POSNA — PEDIATRIC ORTHOPAEDIC SOCIETY OF NORTH AMERICA

SPARK

Thank You!


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