3D Printing Technology for Clinical Applications

James J. Yoo

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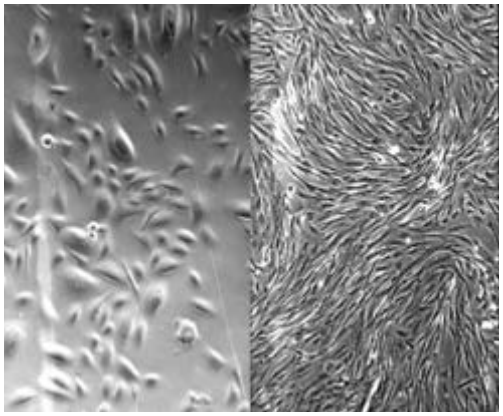
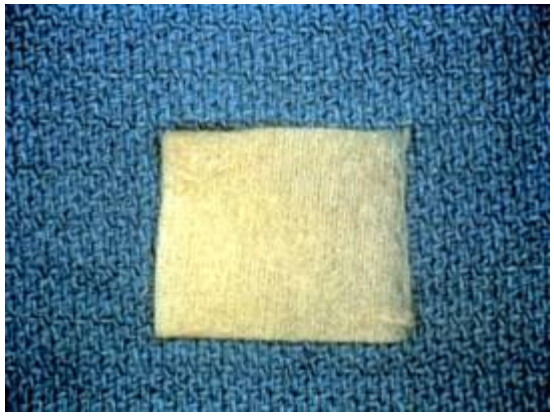
Strategy for Clinical Translation

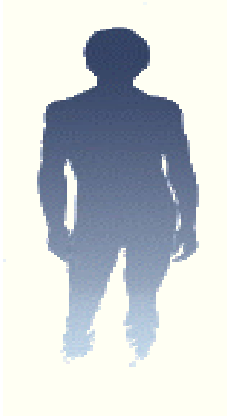
Cell



Scaffold



Patient



- Cells alone – Utilize or Enhance Cellular Function
  - Somatic Cells, Stem Cells
- Scaffolds alone – Bridging tissue defects, Regeneration
  - Natural, Synthetic, Hybrid Biomaterials
- Cells + Scaffolds – Engineered Tissues for Regeneration

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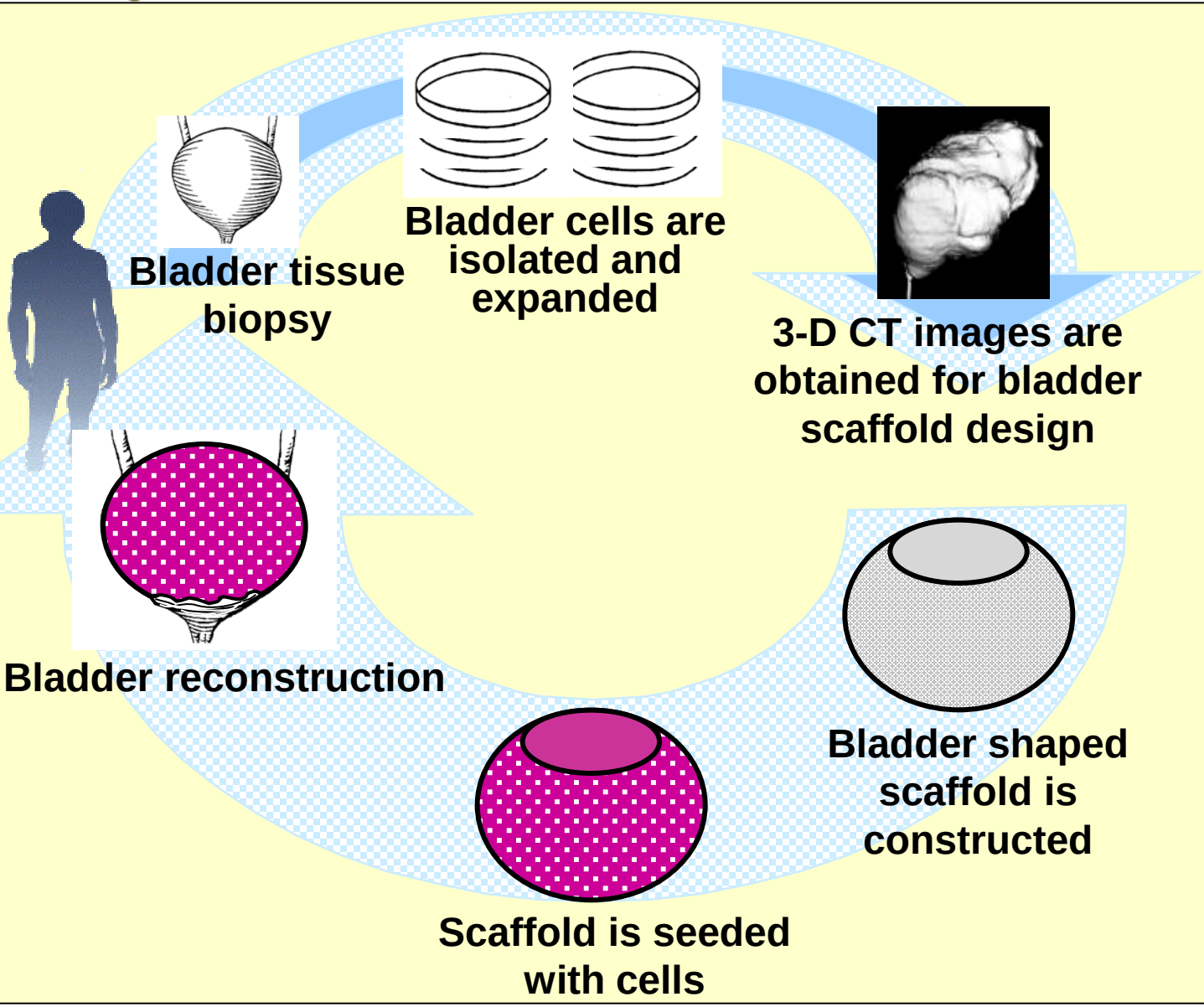
Tissue Engineering of the Bladder



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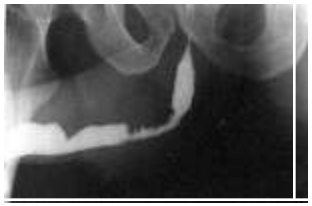
TRANSLATION

COMPLETED

Urethra

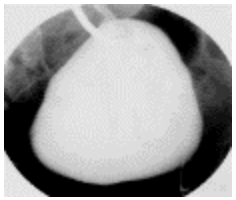


Vagina

Bl Neck

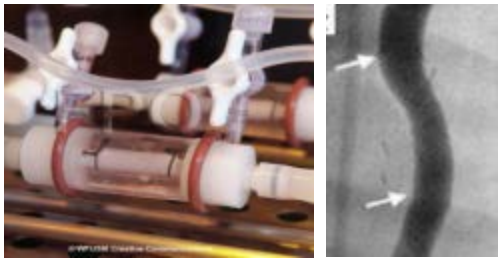
Ureter

Bladder

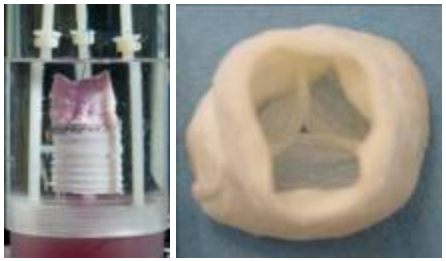


CURRENT DEVELOPMENT

Blood Vessel

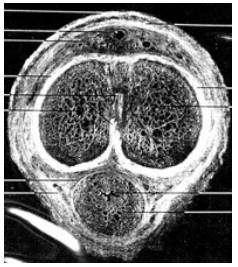


Heart Valve



Liver

Corpora

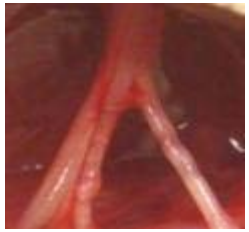


Ear

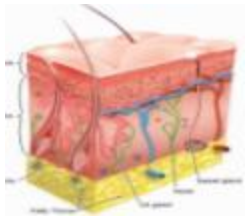
Finger

Kidney

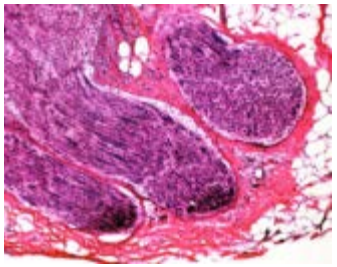
Nerve



Skin



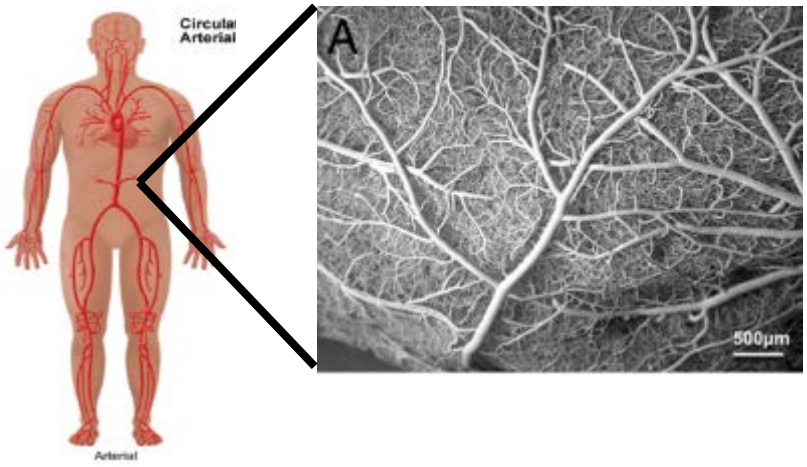
Muscle



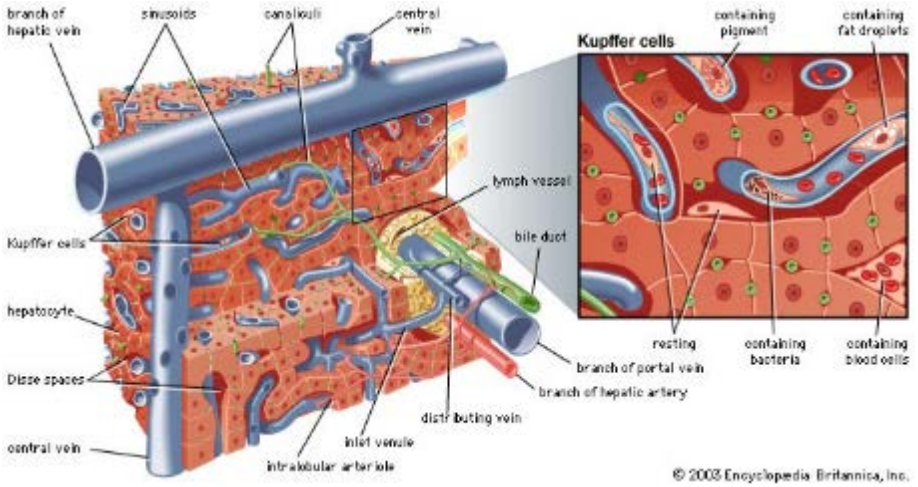
- **Tissue engineering has had initial successes with building a number of tissues clinically**

- **Challenges still exist in developing and translating complex tissue systems that require coordinated and systemic function**

Engineering Challenge



Vascular Network

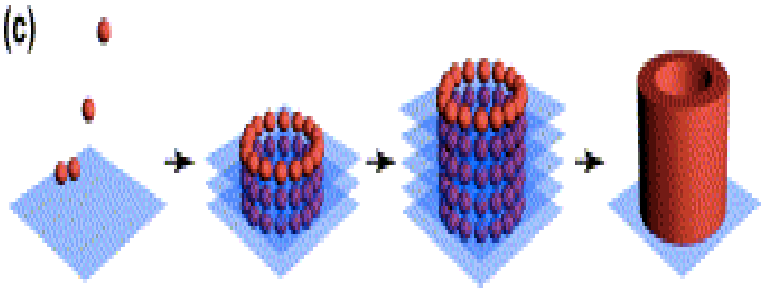


Organ and tissue complexity

Bioprinting Technology

- **Bioprinting technology is evolving into a viable method to fabricate tissue constructs for reconstruction**
- **Bioprinting technology can deliver 1)multiple cell types, 2) gel biomaterials, and 3) genes, proteins and macromolecules to target sites**
- **Multi-cellular tissue/organ constructs can be generated simultaneously with the maintenance of their structural and spatial orientation *in vivo***

Layer-by-layer assembly of 3-D constructs



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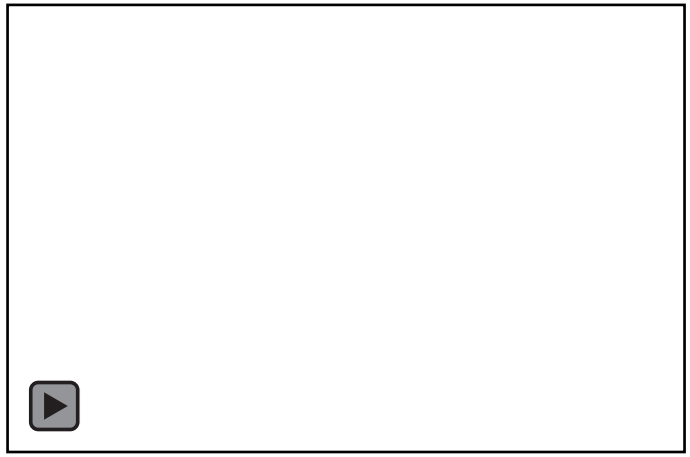
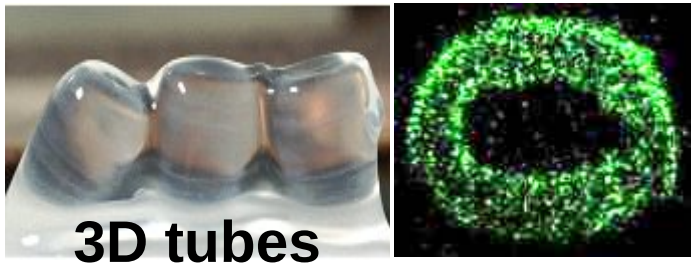
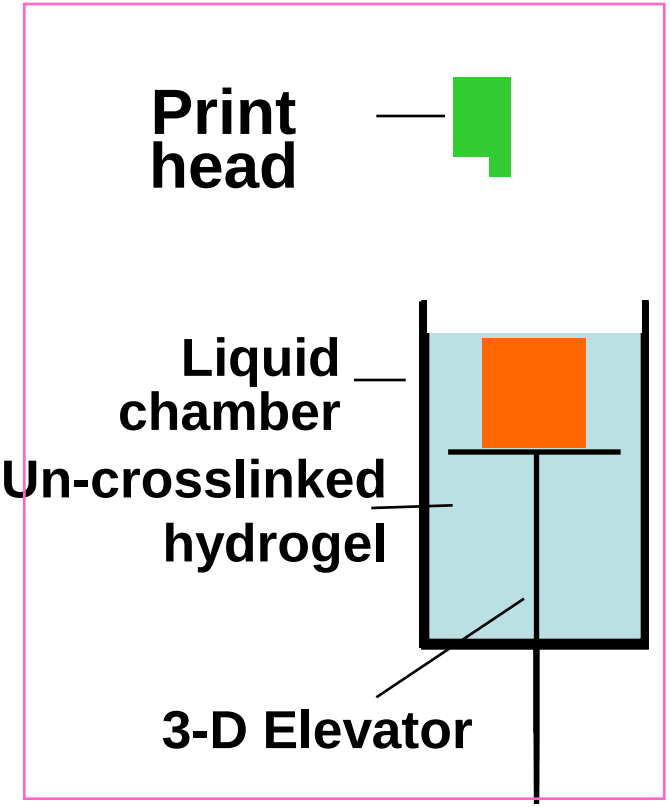
Bioprinting Technology

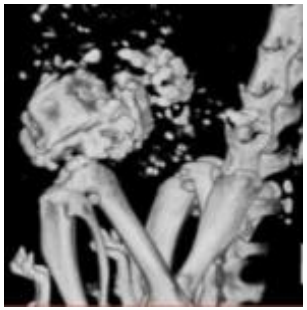




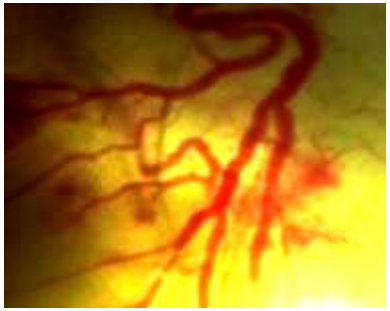


Heart

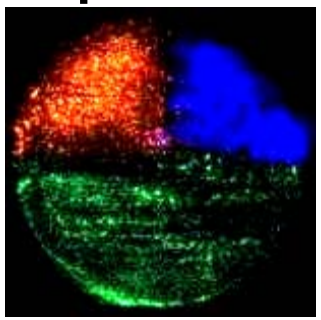
Bone



Blood Vessels



Composite Tissue



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Skin Printer for Burn

- **Burns comprise 5-20% of injuries in combat zones**
- **Large area burns are difficult to stabilize in the combat theater**

<u>Proposed Solution:</u>

- **Develop a novel delivery system that would repair battlefield burn injuries *in situ* using a portable skin printing system**

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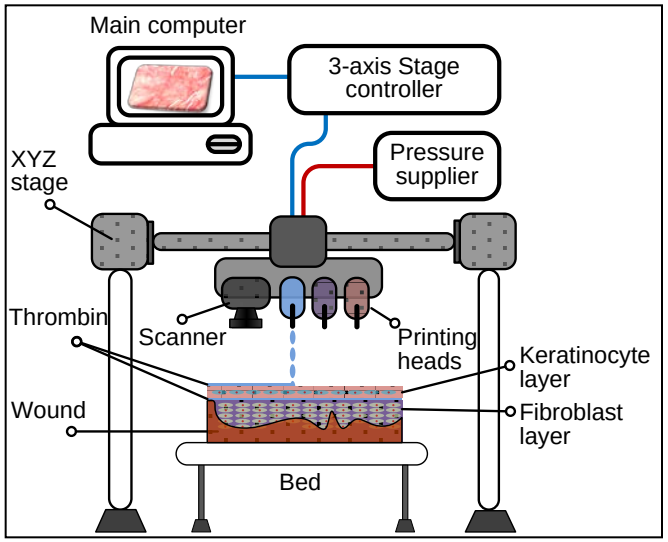
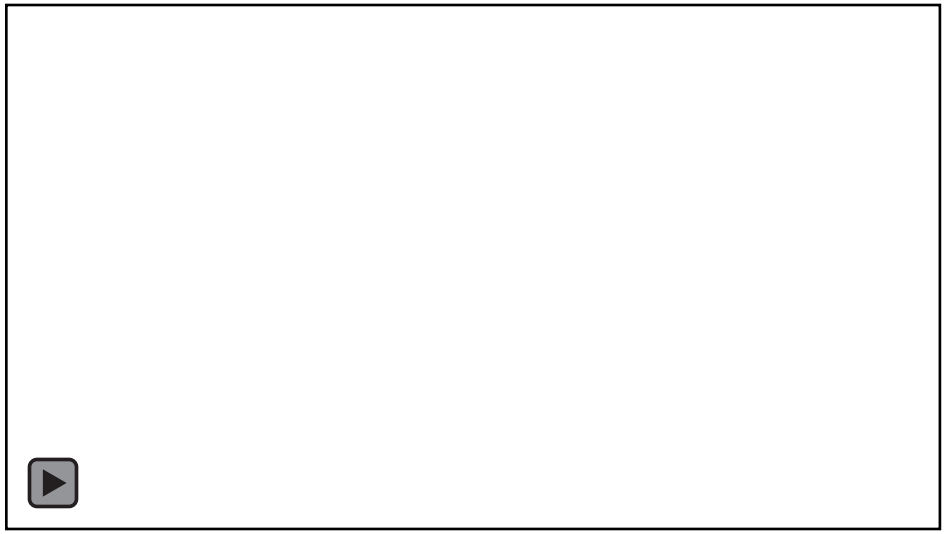
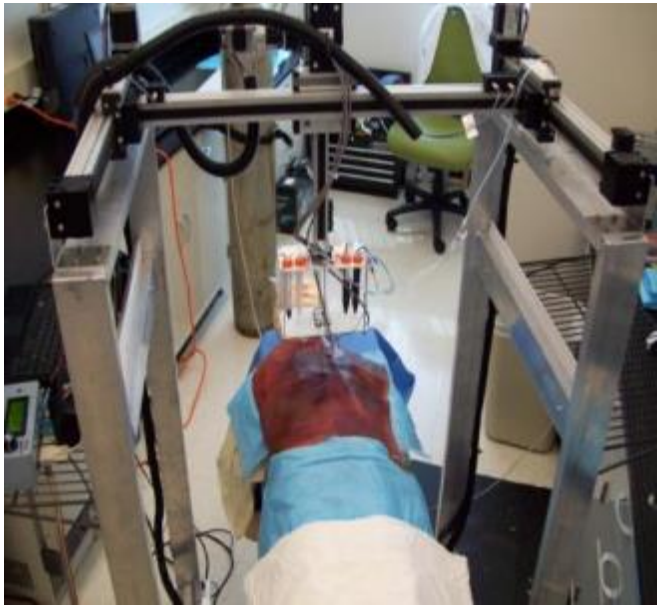
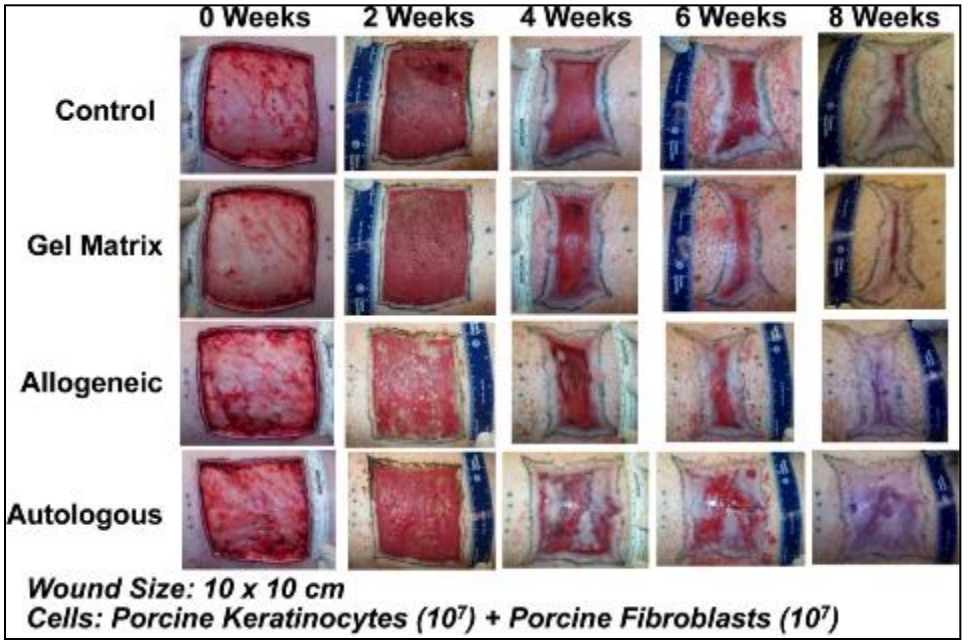
Mobile Skin Bioprinter System









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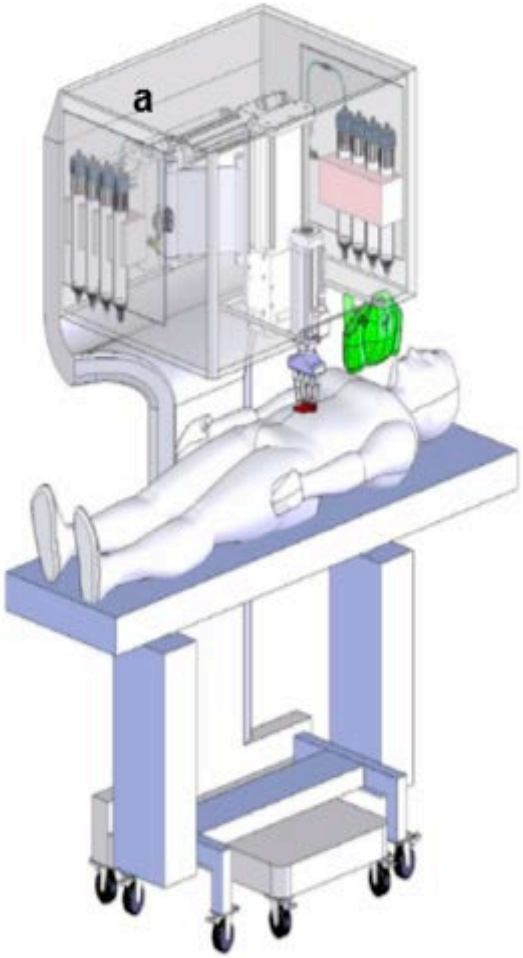
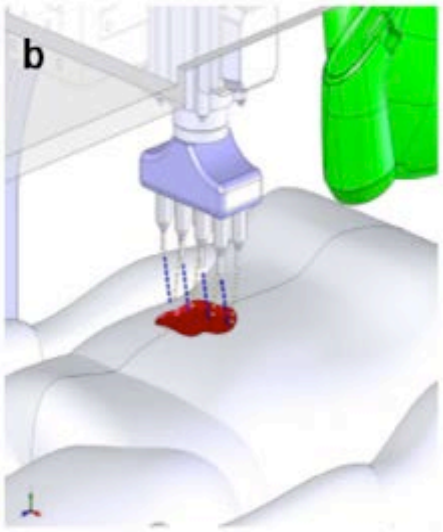
Design and Construction of Clinical Skin Bioprinter





Supported, in part, by TATRC

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Validation Study Design

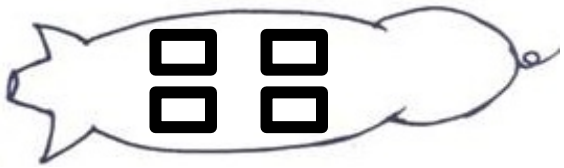
Skin Biopsy

Cell Isolation and Expansion

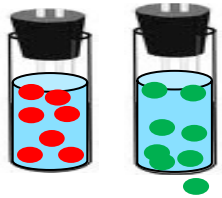
Keratinocytes

Fibroblasts

100 cm² Full-Thickness Wound (n=6)

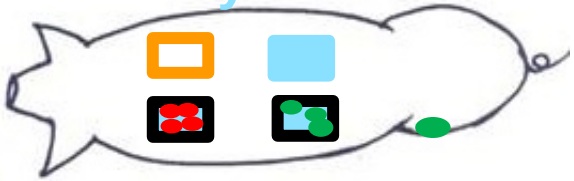


Cell Delivery



Suspension of 10^6 cells in solution

MAT Only Cell Printing



Untreated Cell Spraying

Data Collection & Analyses

- ❑ Gross observation
  - ❑ Wound closure
  - ❑ Contracture
  - ❑ Re-epithelialization
- ❑ Histology
  - ❑ H & E
  - ❑ Masson Trichrome
  - ❑ Immunohistochemistry

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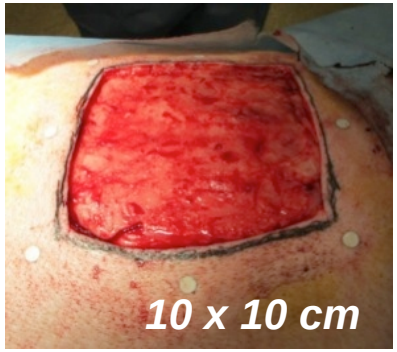
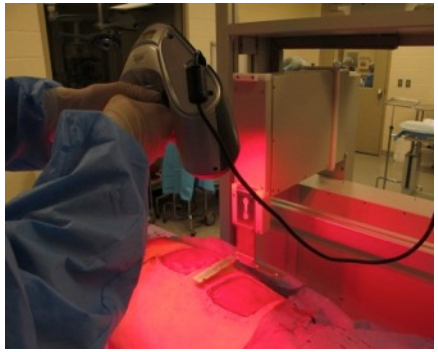
Printing Process

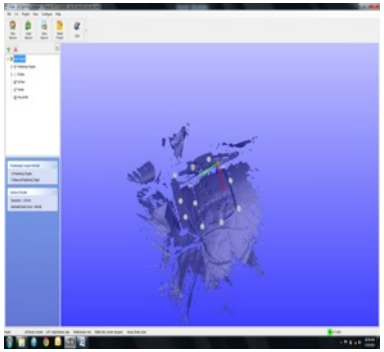
Skin Wound



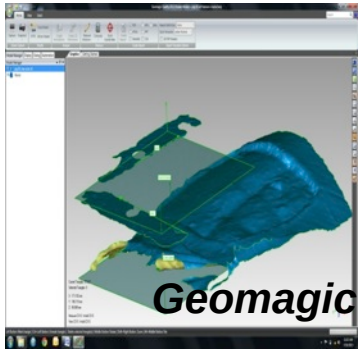
Scanning Wound



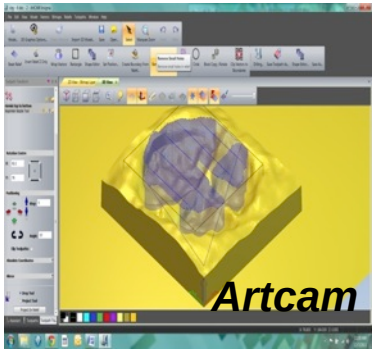
Raw Image



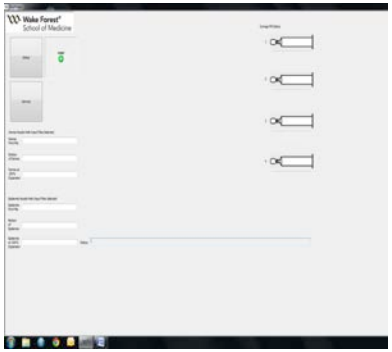
Standard Coordinates



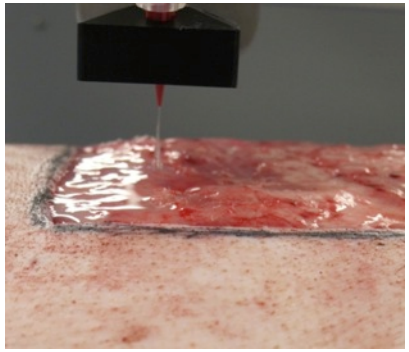
Generation of Nozzle Path



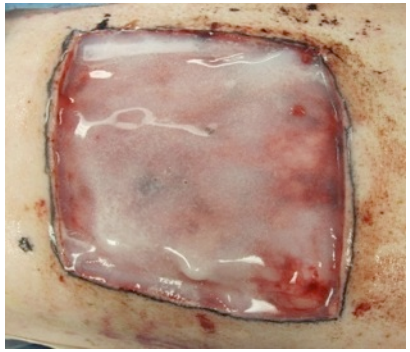
Operating Software



Bioprinting



Printed Wound



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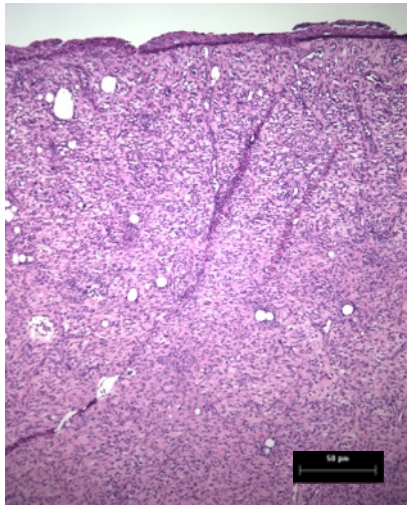
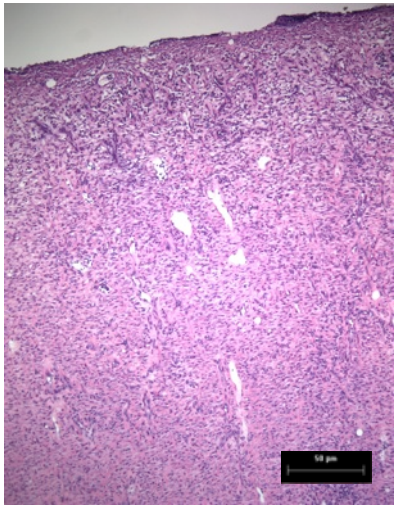
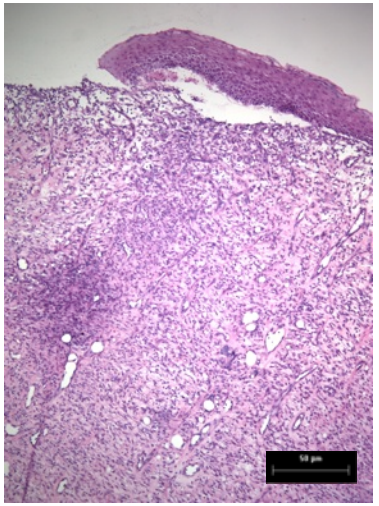
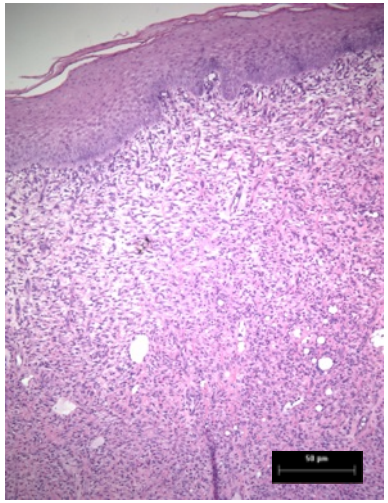
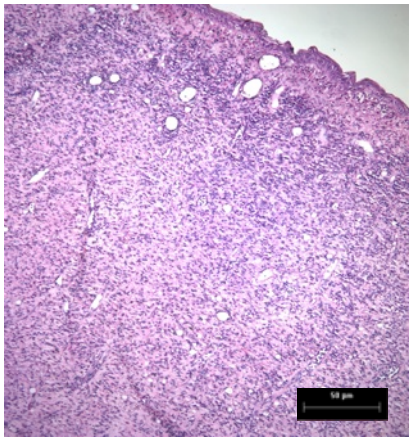
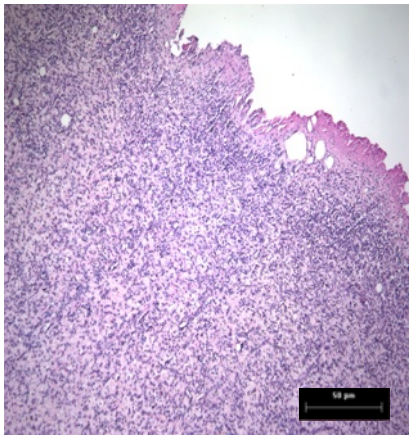
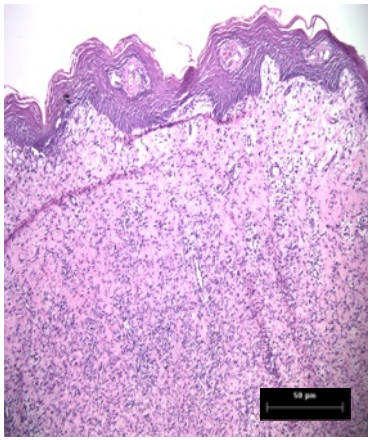
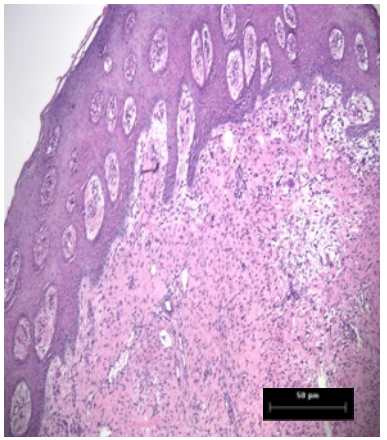
Morphological Analysis

	Untreated	Matrix Only	Cell Spray	Cell Printed
2 wks				
4 wks				

Wounds bioprinted with cells showed well organized re-epithelialization by 2 weeks

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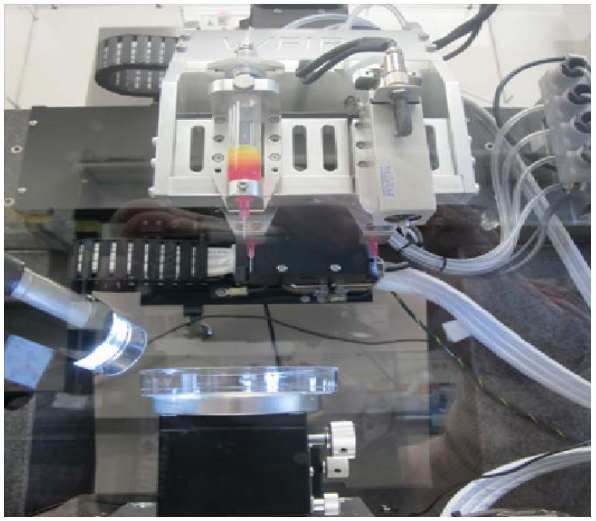
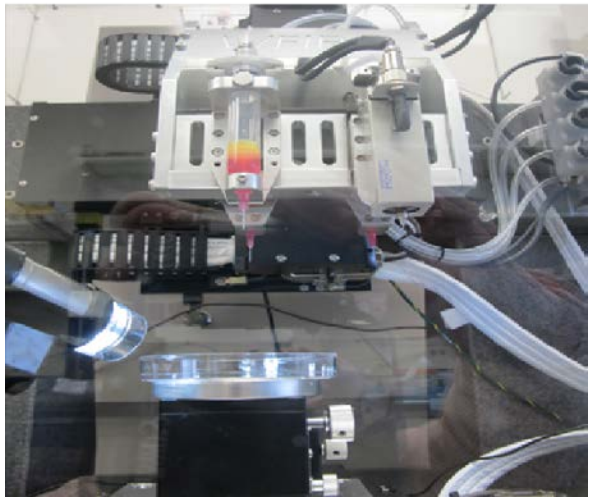
Integrated Organ Printing System for Building Complex Tissues

- Generation of 3D freeform shaped constructs with precision
  - Multiple cell types, biomaterials, drugs
- High strength constructs:
  - Gel and polymeric materials (~12)
- Printing resolution:
  - Cell printing: ≥ 50 µm
  - Structural material printing: ≥ 2 µm





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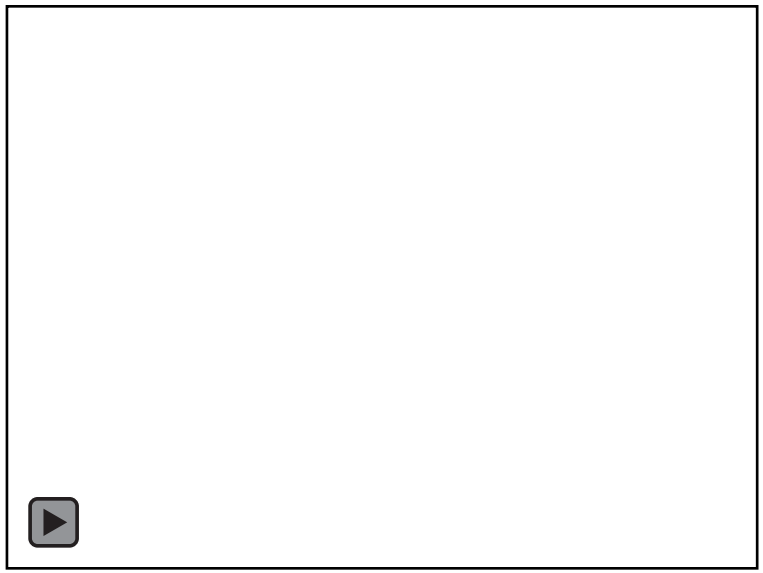
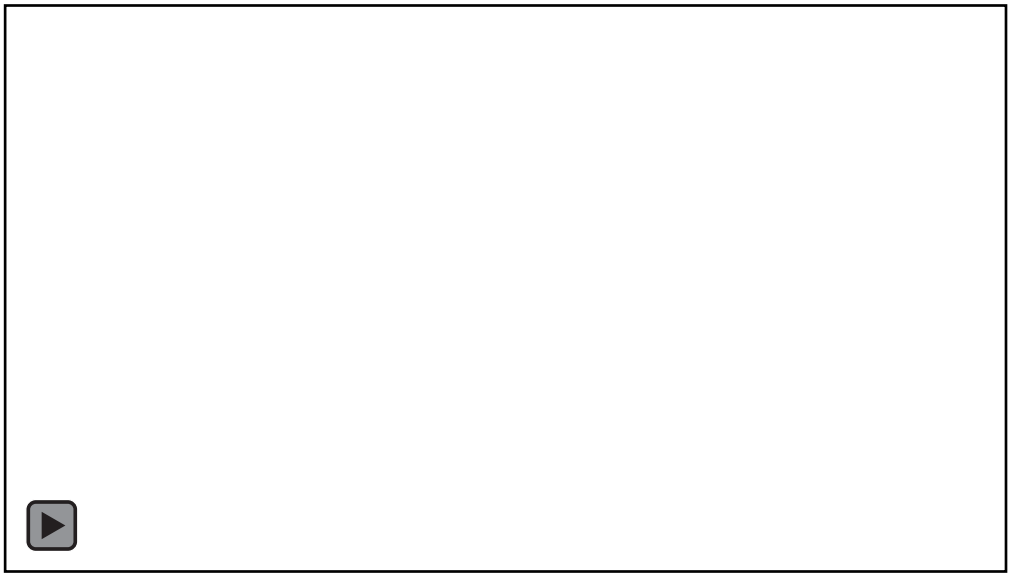
Integrated Organ Printing System



Working Principle



Fabrication Process

- ***White: Polycaprolactone***
- ***Pink: Cell mixed Hydrogel***

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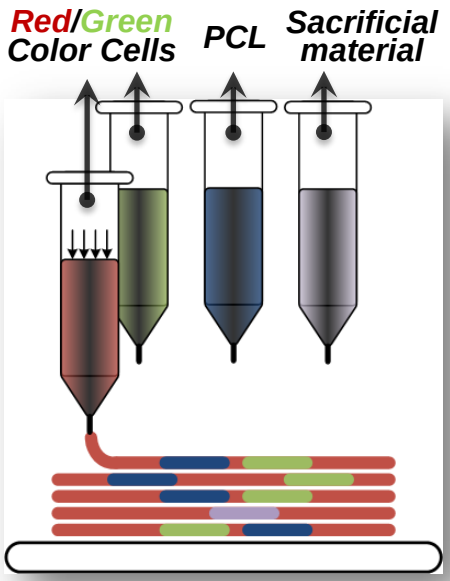
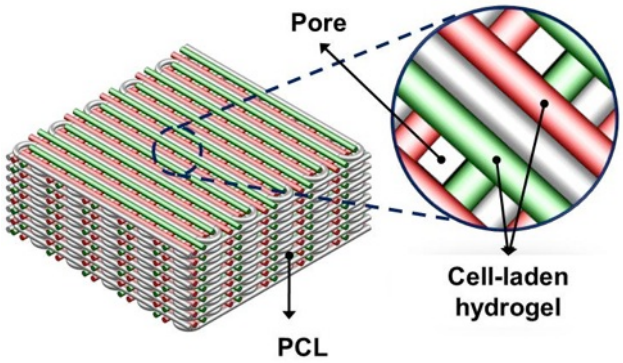
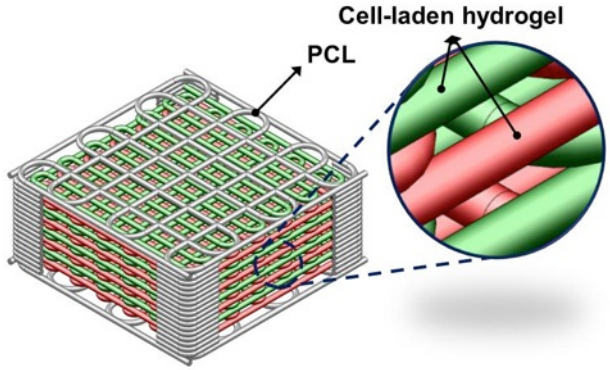
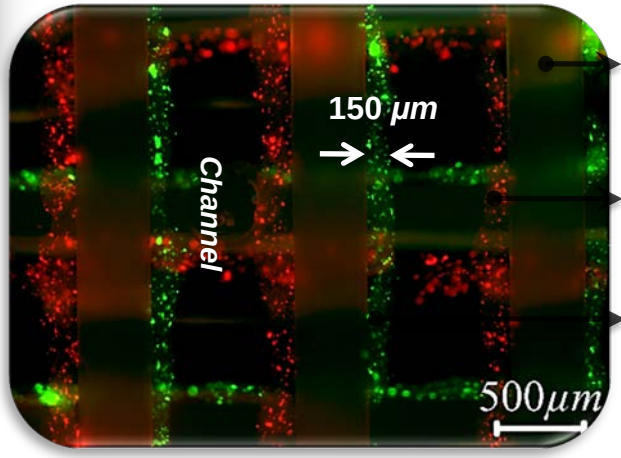
3-D Cell Patterning



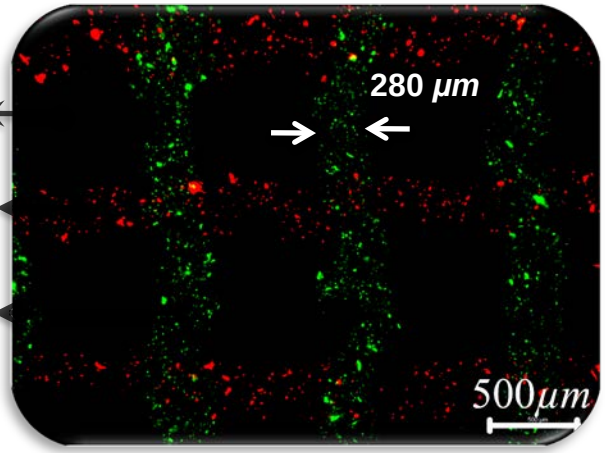
Printing w/ Synthetic polymer & fluorescent labeled cells







PCL
Channel
Red color cell
Green color cell



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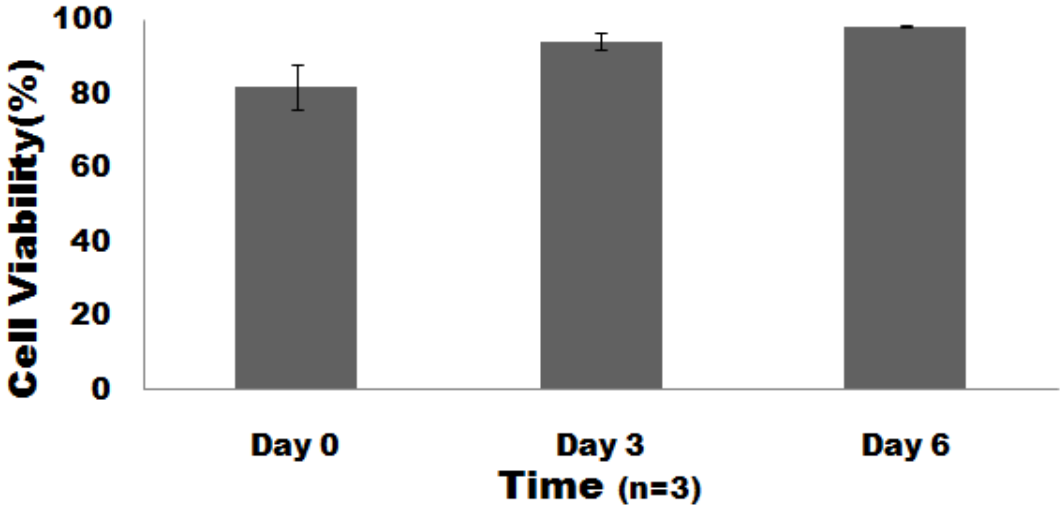
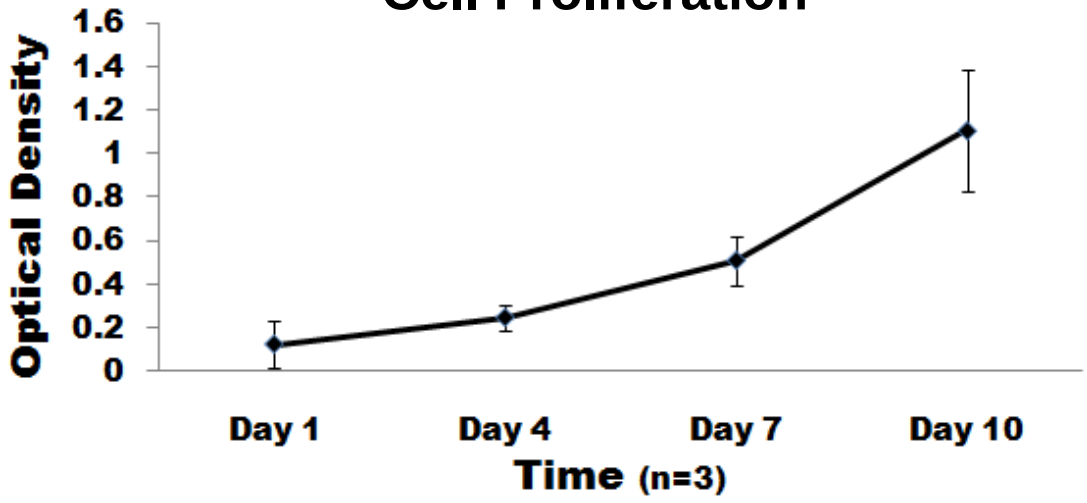
Cell Viability

Cell Viability

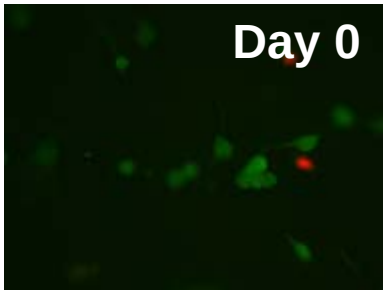


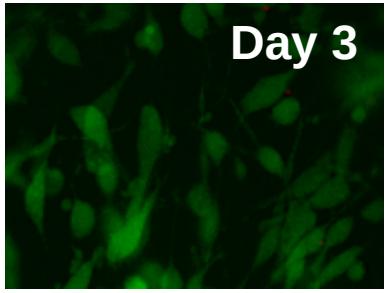
Cell Proliferation



Fluorescence Images

NIH3T3 Cells
Live Cell (Green) /Dead Cell (Red)





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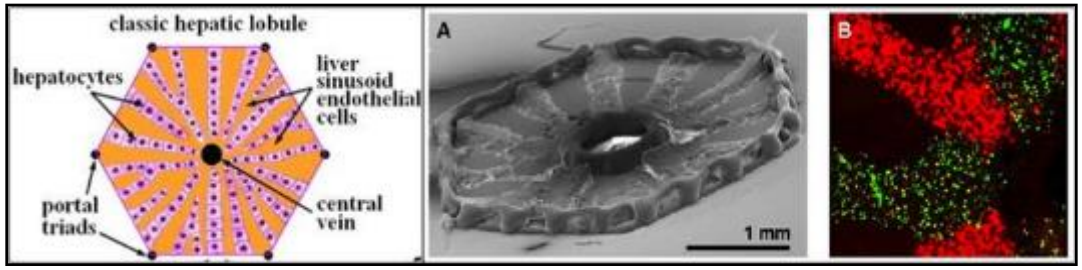
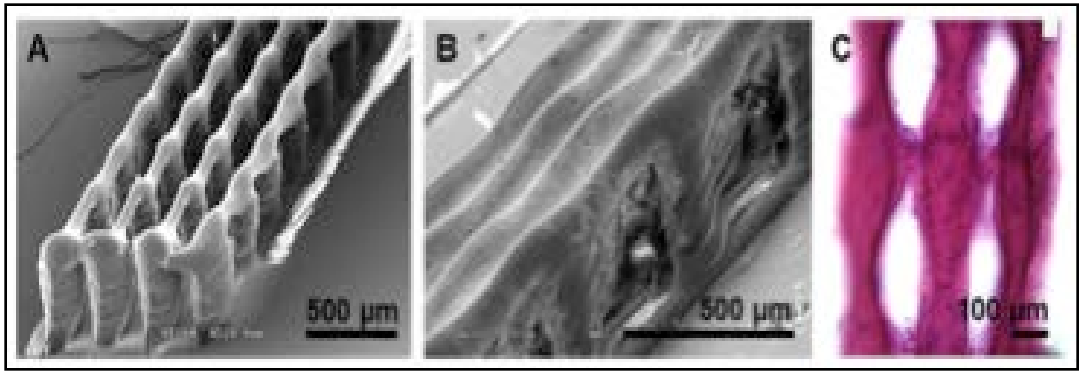
Micro-Organoids

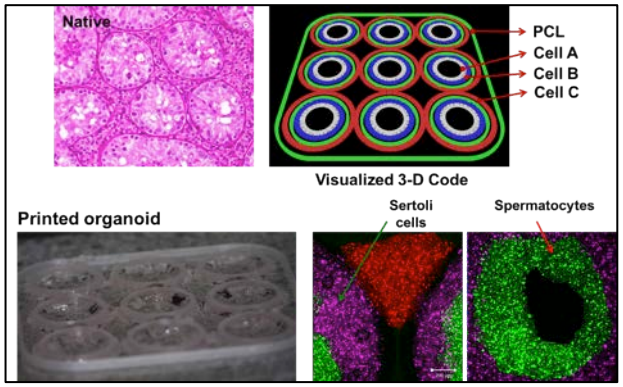
Liver Structure



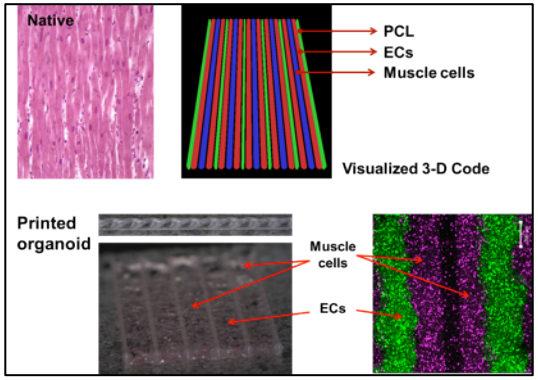
Bladder Tissue Structure



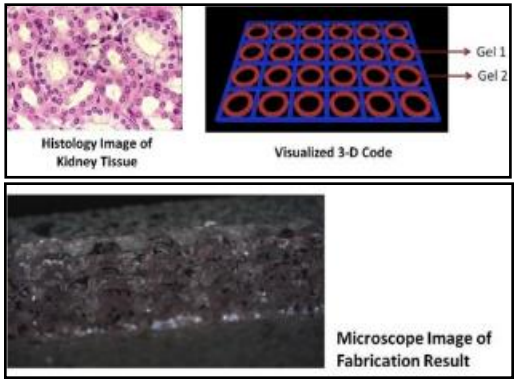
Testis



Cardiac Muscle



Kidney Structure



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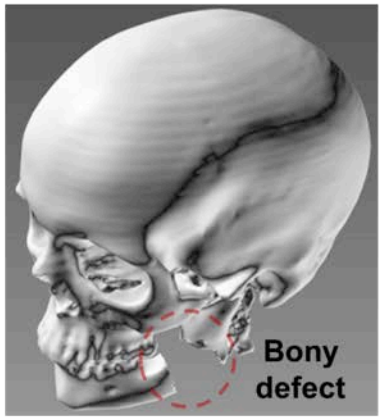
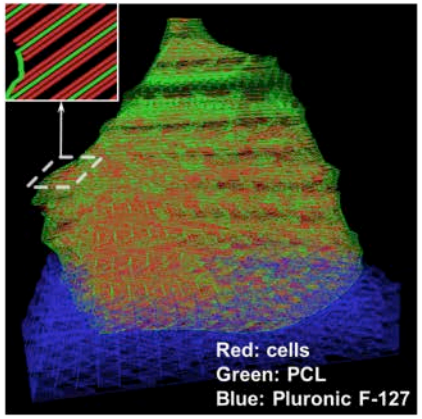
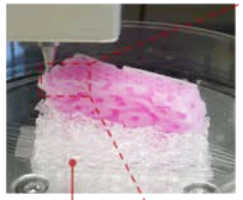
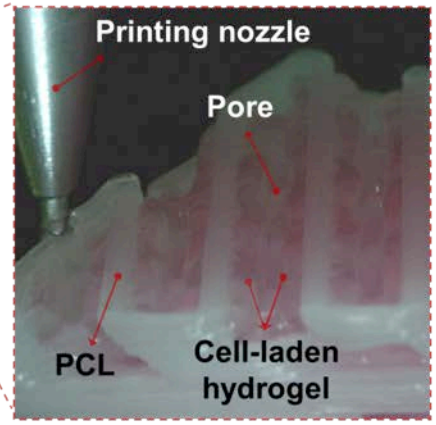
Fabrication of Engineered Constructs: Bone

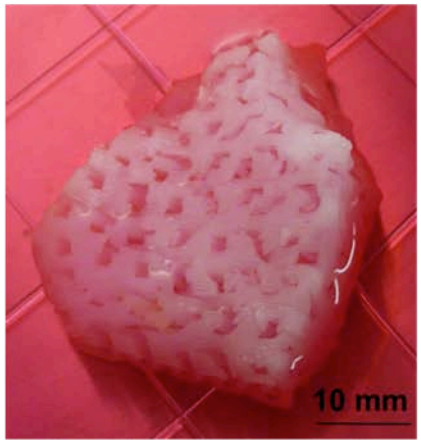


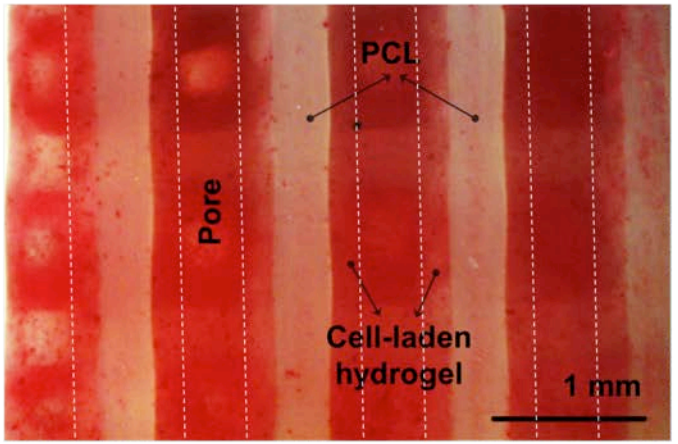




Pluronic F-127 (sacrificial material)







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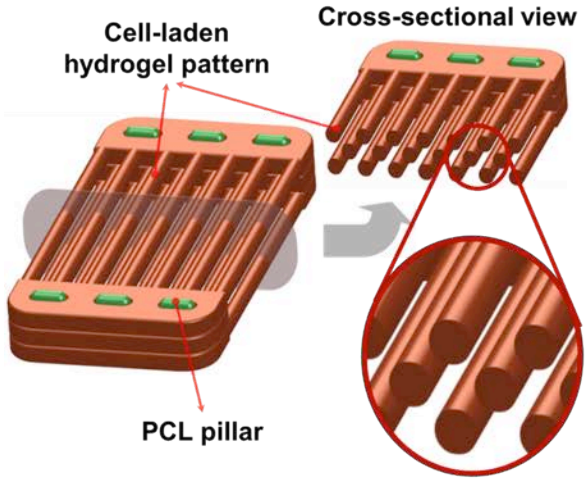
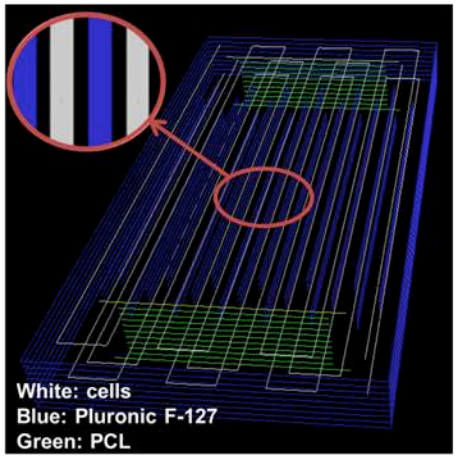
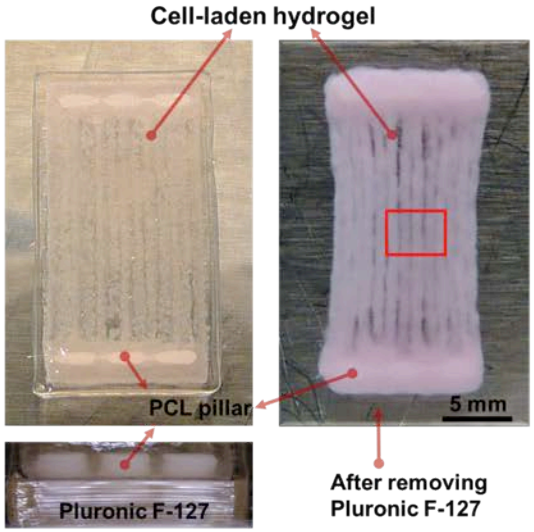
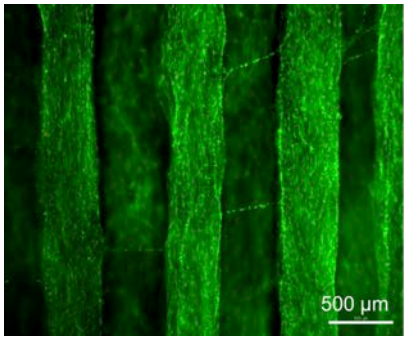
Fabrication of Muscle Constructs

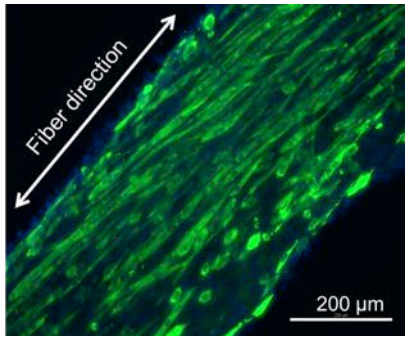




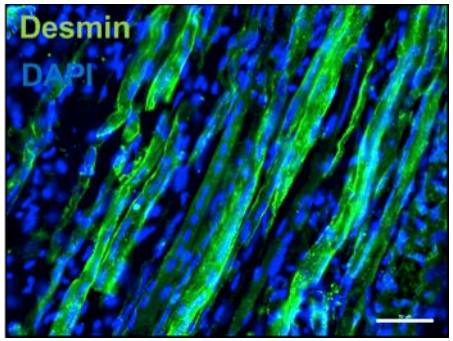


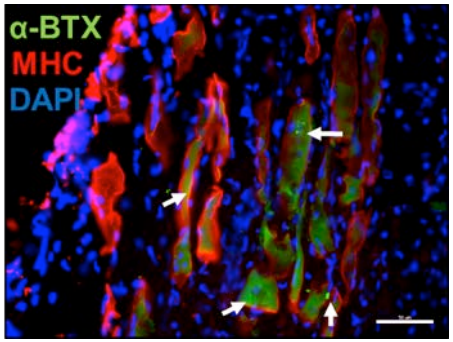
In vitro





In vivo





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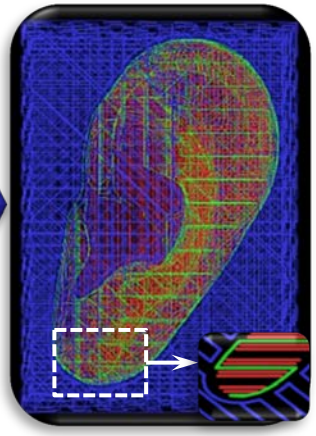
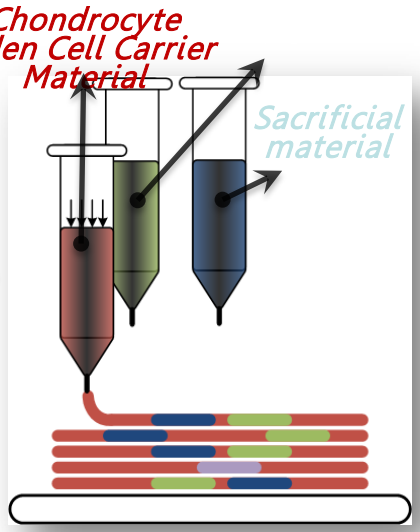
Engineered Ear Cartilage

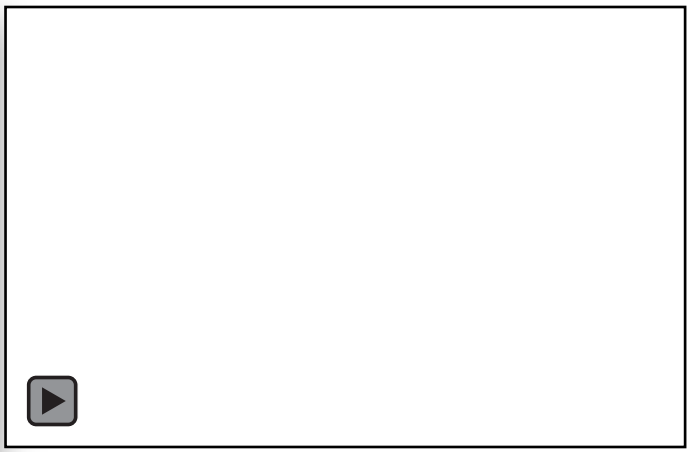
Ear CAD model



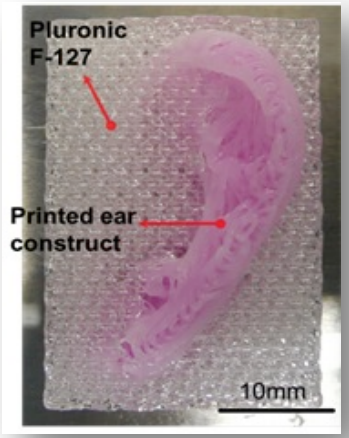
Scanning Path



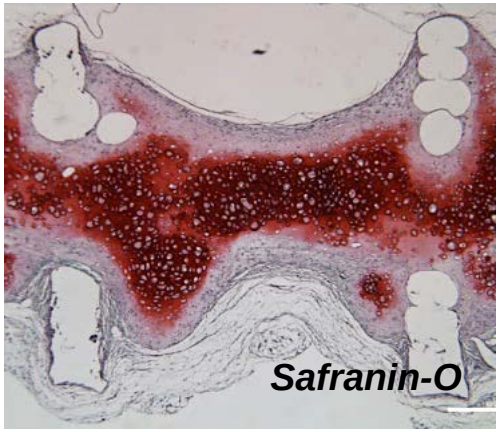
Printing Condition



In Vitro



In Vivo



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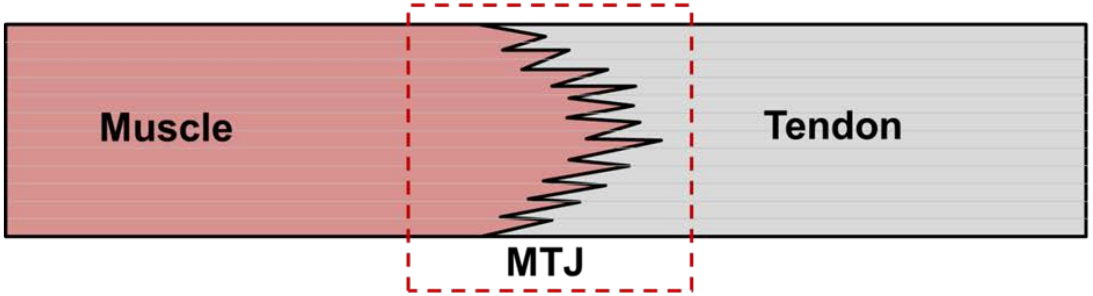
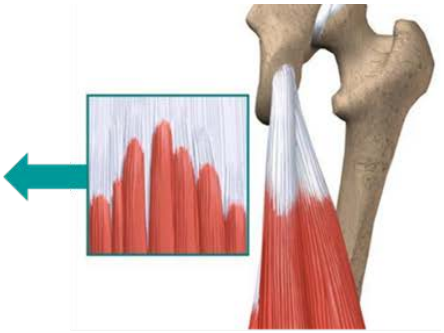
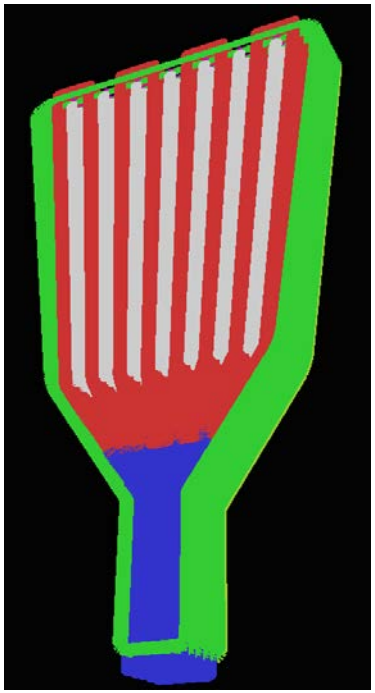
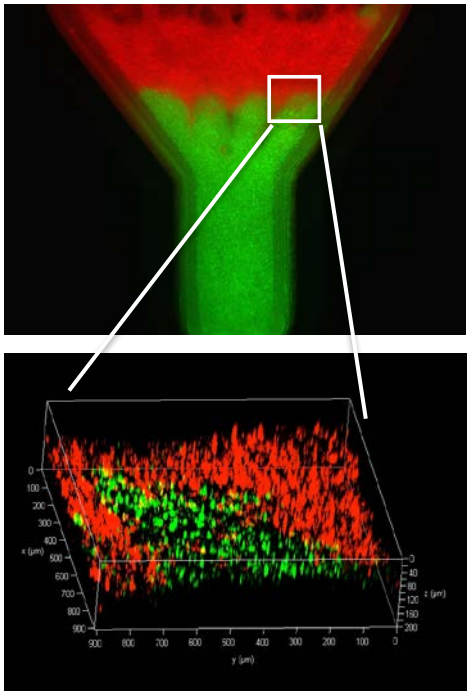
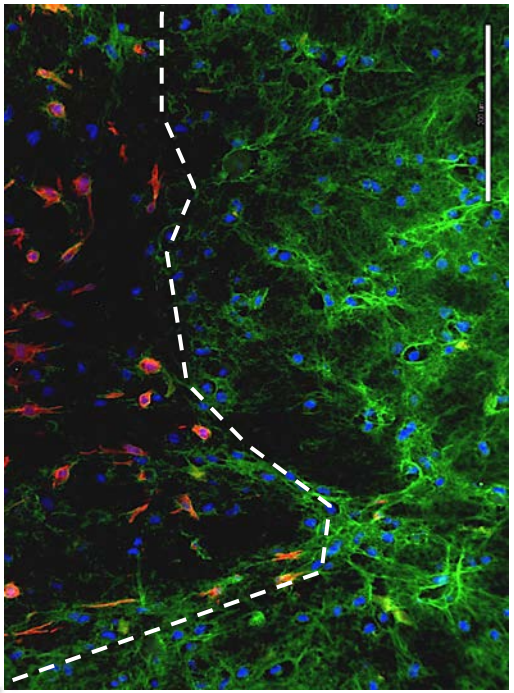
Muscle-Tendon Junction (MTJ)











Red: Desmin; Green: Collagen type I

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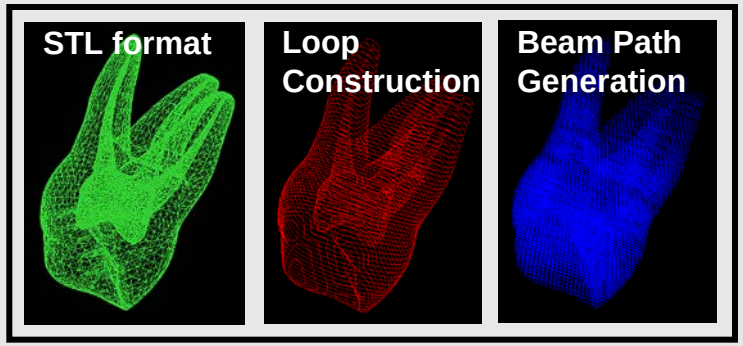
Integrated Organ Printing System

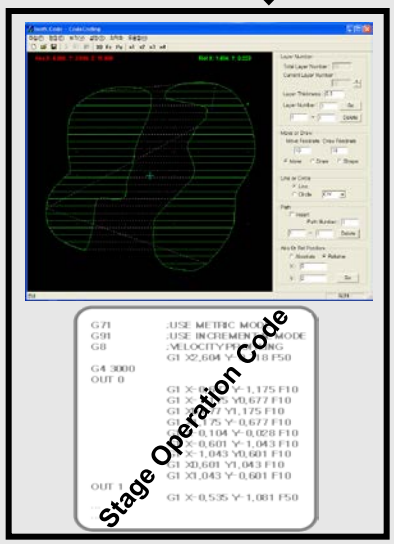
Reverse Engineering

Medical Data

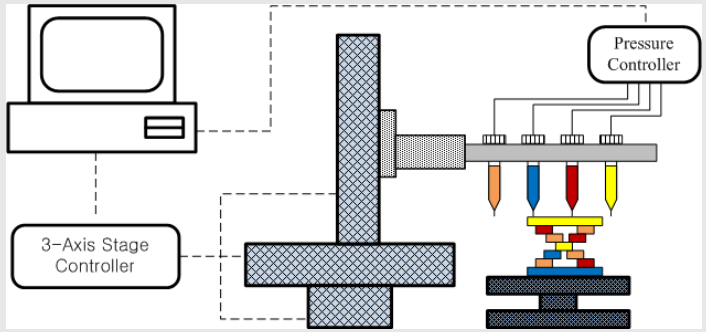
3D CAD Model



CAD/CAM System



Code Generation



Fabrication System

3D Structure

Courtesy of Prof. DW Cho, Postech

Conclusion

- **Bioprinting technology is able to generate 3-D tissue constructs with precision**
- **Delivered cells are uniformly distributed within the construct and remain viable over time**
- **This system can be used as a cell delivery tool for building complex and composite tissue and organ systems**

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Future Direction

- **Technology Development**
  Hardware, Software, Bioink (cells, materials)
- **Regulatory Pathway**
- **Commercialization Strategy**

Wake Forest Institute for Regenerative Medicine

- **WFIRM: >300 Members: (Dr. Anthony Atala-Director)**
- **Working on over 30 different tissues and organs**

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NIH: HLI
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National Kidney Foundation
Muscular Dystrophy Association
The Crown Foundation
The Frase Foundation
The Nakos Foundation
The Chapman Foundation
JDRF
Musculoskeletal Transplant Foundation
Tengion, Inc
Plureon
Errett Fisher Foundation