KU LEUVEN



Bioprinting for skeletal tissue regeneration

Current strategies and future perspectives

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Biofabrication Lab
Faculty of Engineering Technology
Prometheus - Division of Skeletal Tissue Engineering

Will we soon be printing organs?

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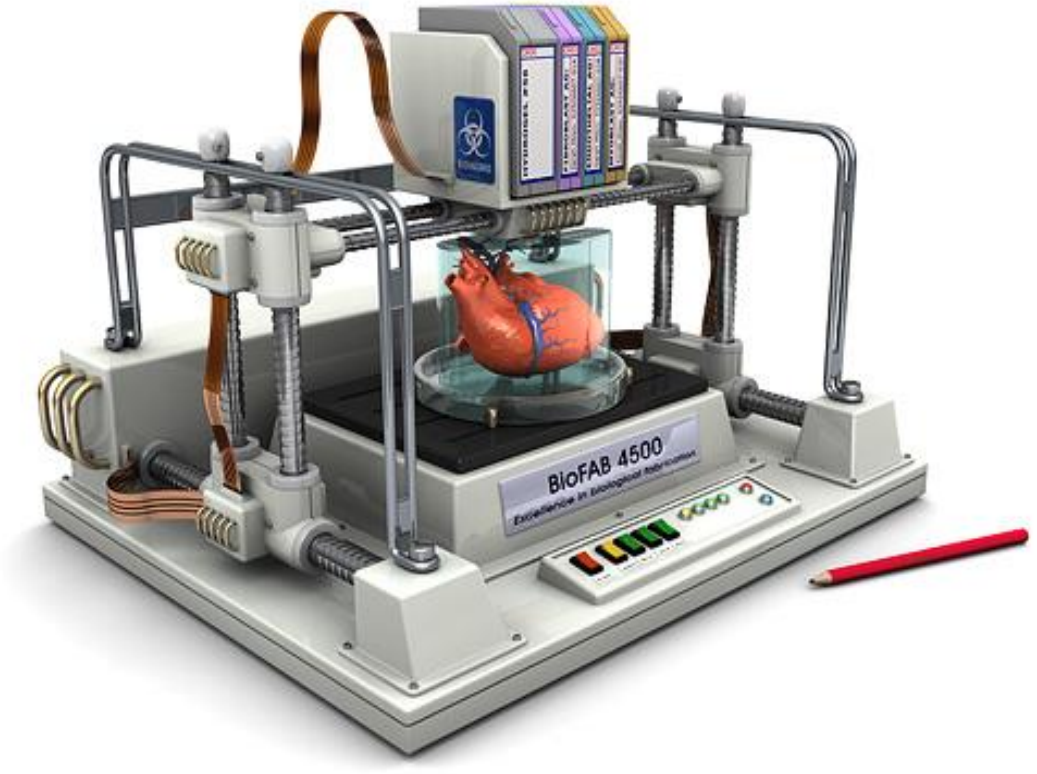



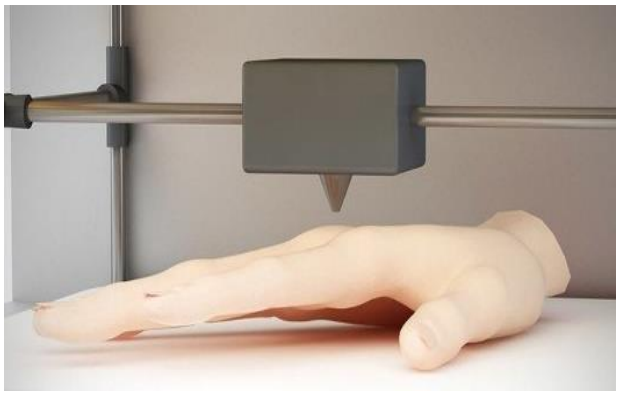

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YES?



Incredible breakthrough in medicine: 3D printed organs are a real thing now!

Nexter.org • 7.9K views • 1 year ago

Follow Nexter for more: https://nexter.org/ Many people need to wait a long time before receiving some body parts to improve their ...



Scientists Can Now 3D Print Functional Organs

Futurism 1.0 • 99K views • 2 years ago

They mimic real **organs** and even bleed when cut. WEBSITE: http://futurism.com FACEBOOK: https://www.facebook.com/futurism ...



Printing a human kidney - Anthony Atala

TED-Ed ✓ 81K views • 6 years ago

View full lesson: http://ed.ted.com/lessons/printing-a-human-kidney-anthony-atala Surgeon Anthony Atala demonstrates an ...

CC

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A few important remarks

- A model of an organ is not a functional organ
- Bioprinting involves biological material such as living cells or proteins
- An organ has a complex architecture related to its function

YES? -> NO

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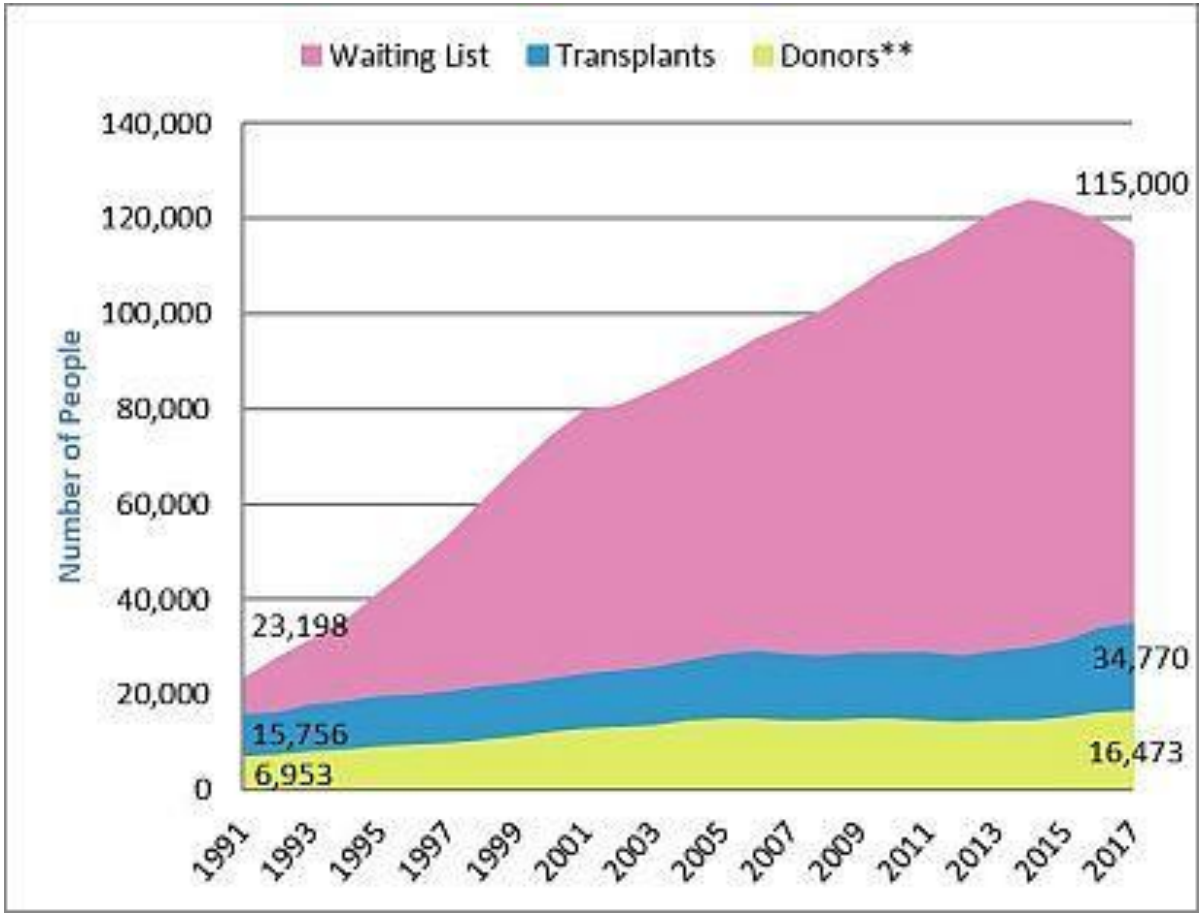
There is a need



Data from optn.transplant.hrsa.gov and OPTN/SRTR Annual Report.

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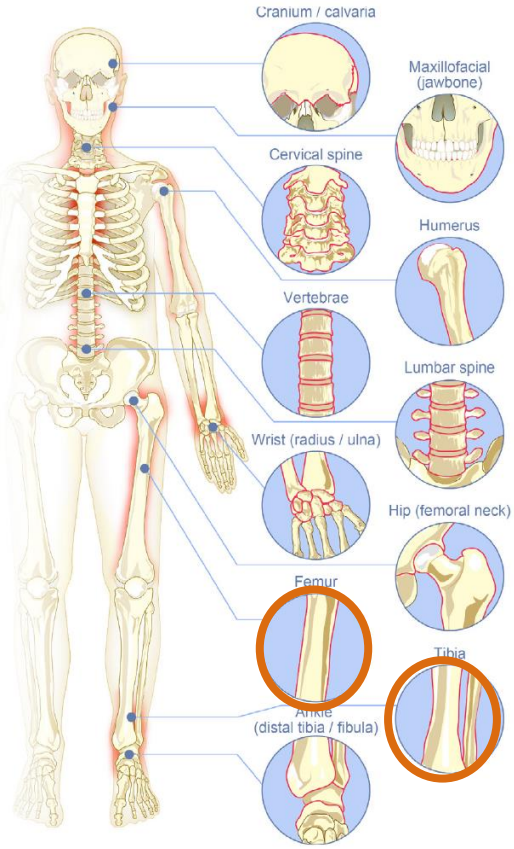
Critical skeletal defects

Critical bone defects





Osteochondral defects



Ho-Shui-Ling *et al*., 2018

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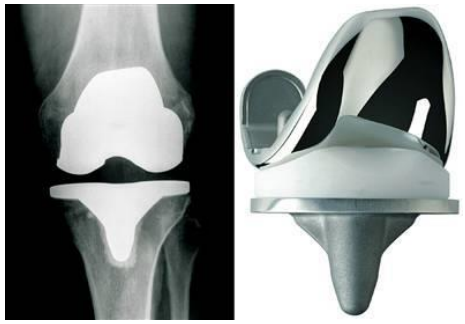
Current treatments

(replacement strategies)

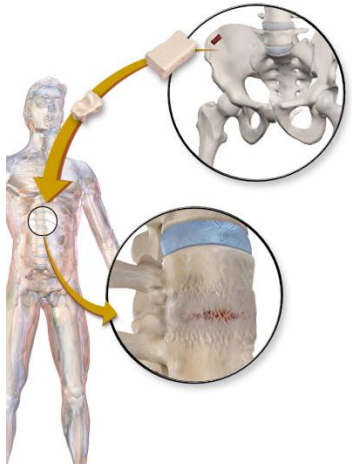
Material-based implants

- Metal implants
- Ceramic implants



Tissue transplants

- Autografts
- Allografts
- Xenografts



Several limitations:

- Minimal tissue availability
- Donor side morbidity
- Wear -> 2nd surgery
- Limited implant integration
- …

From replacement towards regeneration

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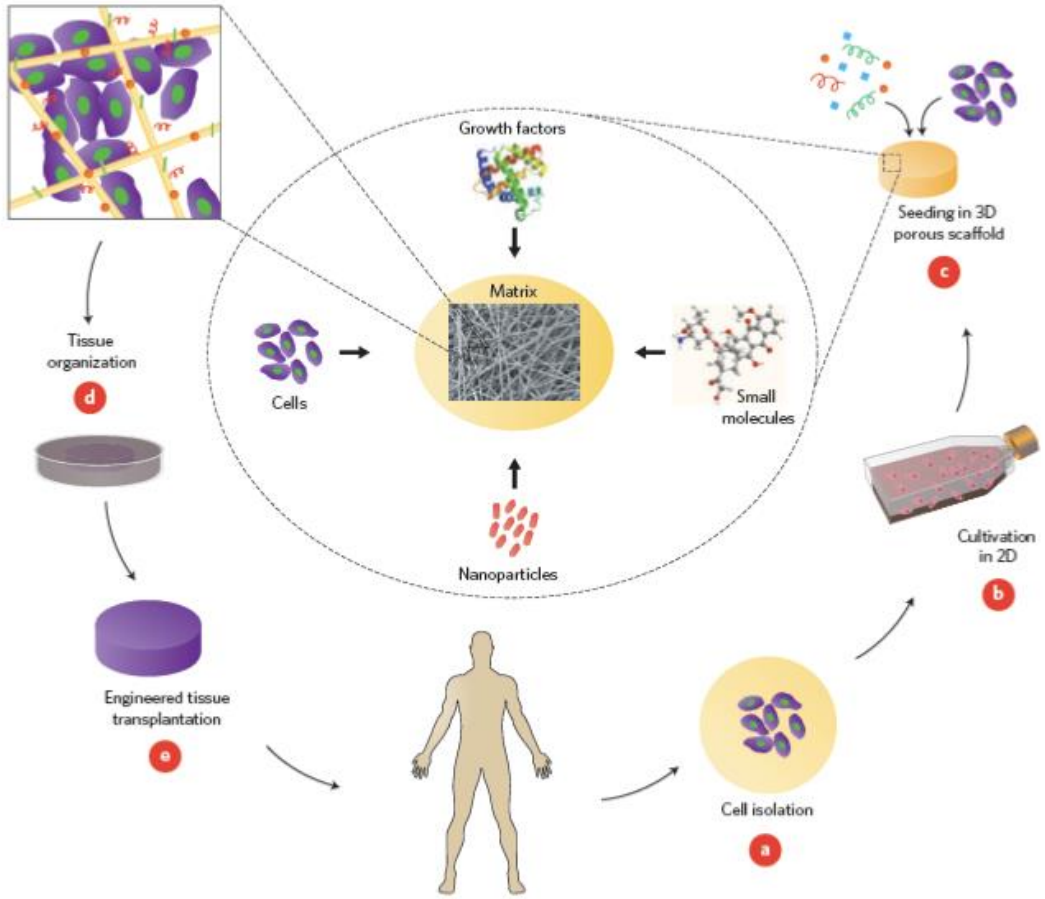
The traditional concept of Tissue Engineering



The development of cell-based implants for tissue regeneration

prometheus

Skeletal tissue engineering

Dvir *et.al.*, 2011

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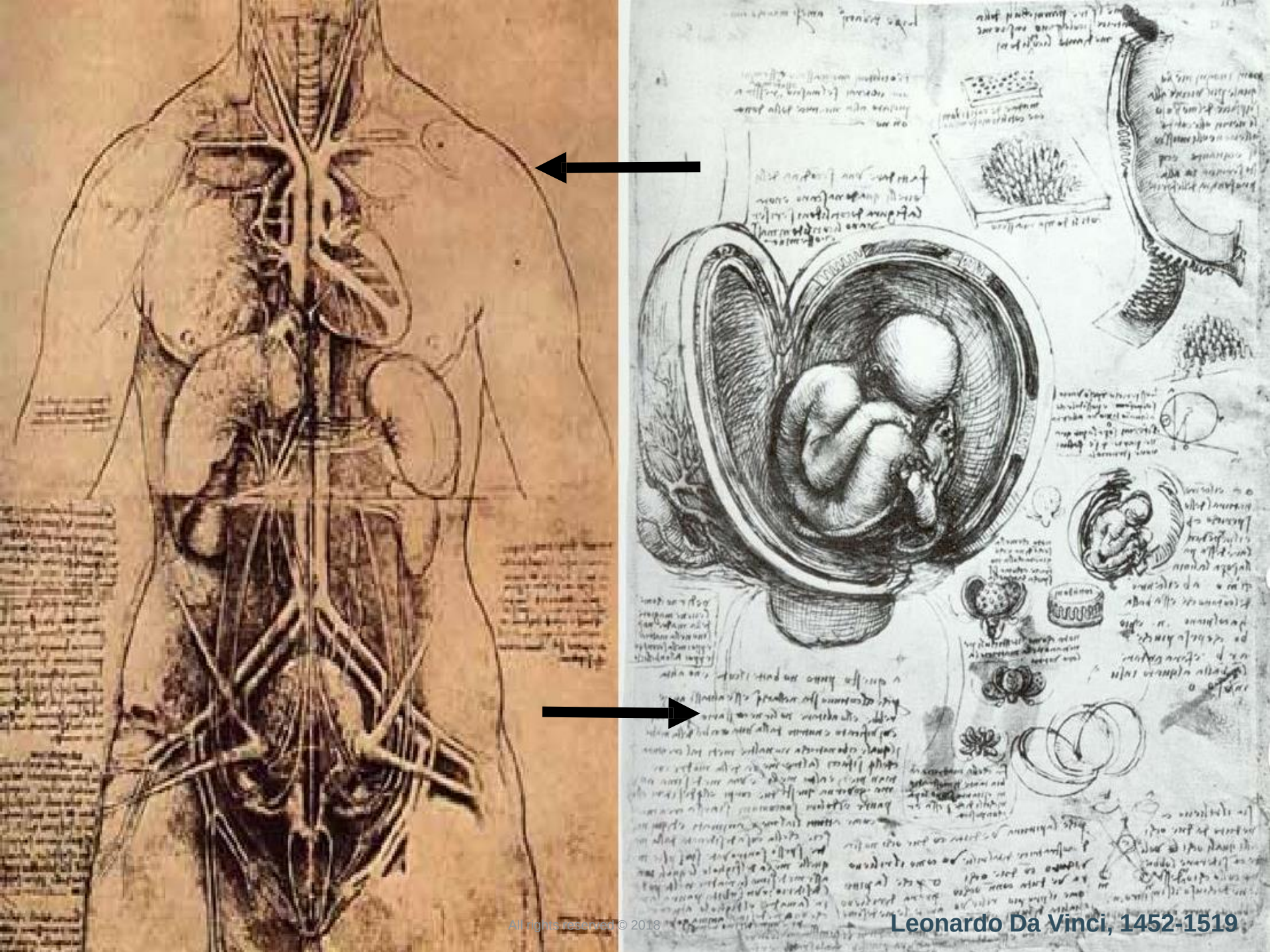

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Leonardo Da Vinci, 1452-1519

Increase robustness

TISSUE ENGINEERING: Part B
Volume 15, Number 4, 2009
© Mary Ann Liebert, Inc.
DOI: 10.1089/ten.teb.2008.0575

Developmental Engineering: A New Paradigm for the Design and Manufacturing of Cell-Based Products. Part I: From Three-Dimensional Cell Growth to Biomimetics of *In Vivo* Development

Petros Lenas, Ph.D.,[1,2] Malcolm Moos, Jr., M.D., Ph.D.,[3] and Frank P. Luyten, M.D., Ph.D.[4]

Recent advances in developmental biology, systems biology, and network science are converging to poise the heretofore largely empirical field of tissue engineering on the brink of a metamorphosis into a rigorous discipline based on universally accepted engineering principles of quality by design. Failure of more simplistic approaches to the manufacture of cell-based therapies has led to increasing appreciation of the need to imitate, at least to some degree, natural mechanisms that control cell fate and differentiation. The identification of many of these mechanisms, which in general are based on cell signaling pathways, is an important step in this direction. Some well-accepted empirical concepts of developmental biology, such as path-dependence, robustness, modularity, and semiautonomy of intermediate tissue forms, that appear sequentially during tissue development are starting to be incorporated in process design.

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Insights from traditional TE-results

- Implanted cells significantly contribute to tissue regeneration
- A biomimetic approach improves tissue formation: using engineering strategies based on principles in developmental biology
- Large variability in the *in vivo* outcome
- One size does not fit all

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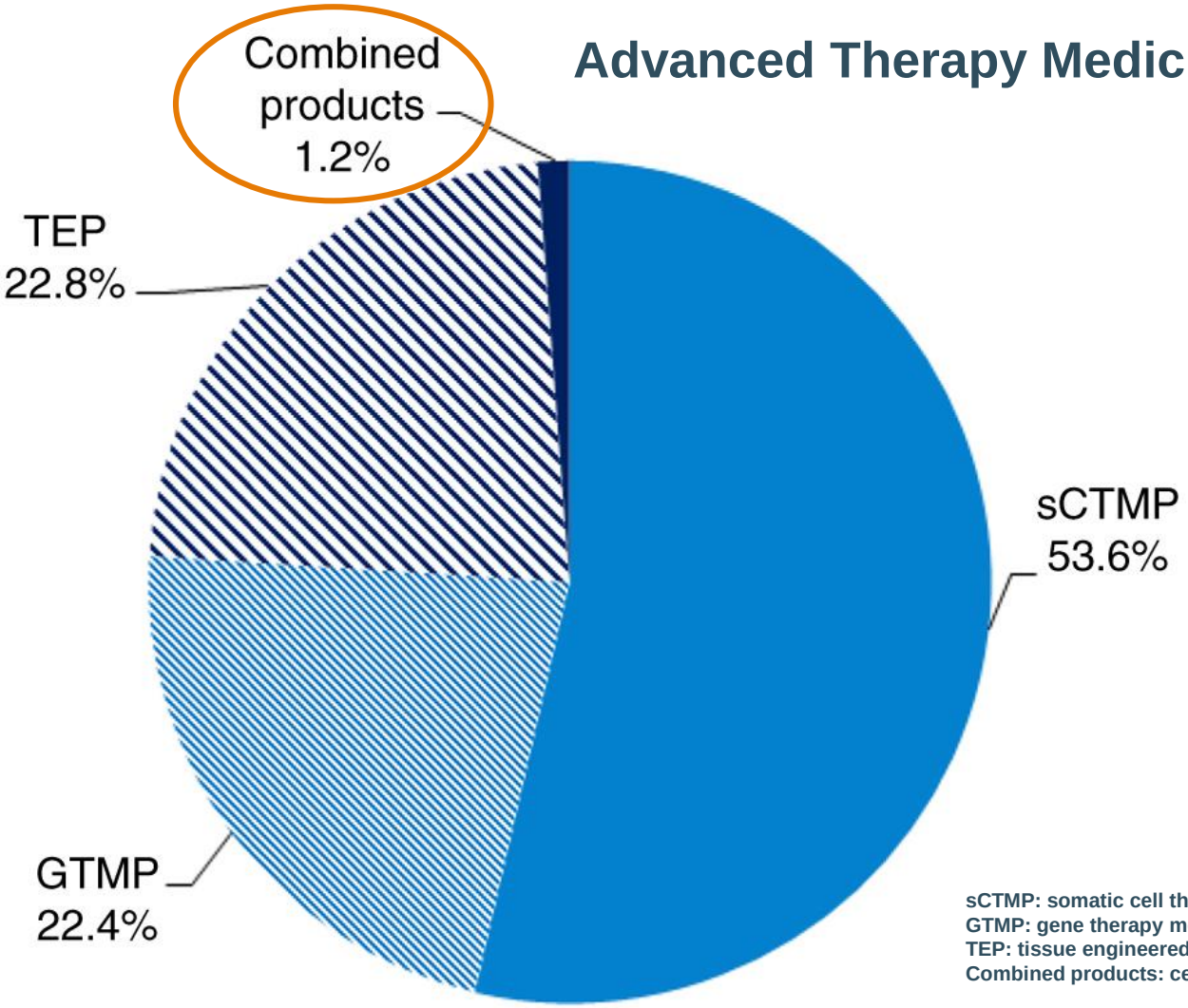
Advanced Therapy Medicinal Products



sCTMP: somatic cell therapy medicinal product
GTMP: gene therapy medicinal product
TEP: tissue engineered product
Combined products: cellular/tissue part + medical device

Tissue Engineering approaches are challenged in terms of reproducibility and clinical relevance of the cell-based product

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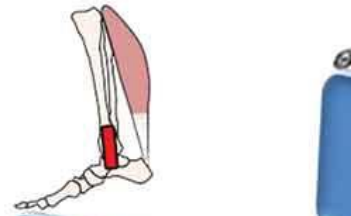
Customization and Fabrication of Personalized Tissue-like Substitutes



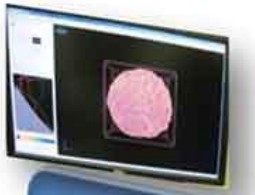
Lesion or Defect

- Lesions with particular dimensions and irregular shapes that can occur in tissues with different biomechanical and physiological properties



Lesion Scan

- Routine imaging techniques as CT and MRI to provide diagnosis will collect 3D data on the specificities of the patient defect



Computer Modeling

- CAD like software will provide the digital cross-sections of the defect scans from imaging techniques to be used as guidelines for printing a 3D model



BioFabrication Technologies

- The virtual data is translated into successive layers of a biomaterial with or without cell or GFs enrichment

Personalized tissue substitute

- The 3D template will be :
i) implanted to replace lesion or defect
ii) storage & preserved until implant surgery
iii) *in vitro* cultured until maturation, e.g. in a bioreactor

At this stage, it is relevant to have the biomaterial (often natural or synthetic polymers) selected to fabricate the 3D template as well as the type and/or the state of cells (iPSCs, stem cells or differentiated cells) to be incorporated within the biomaterial.

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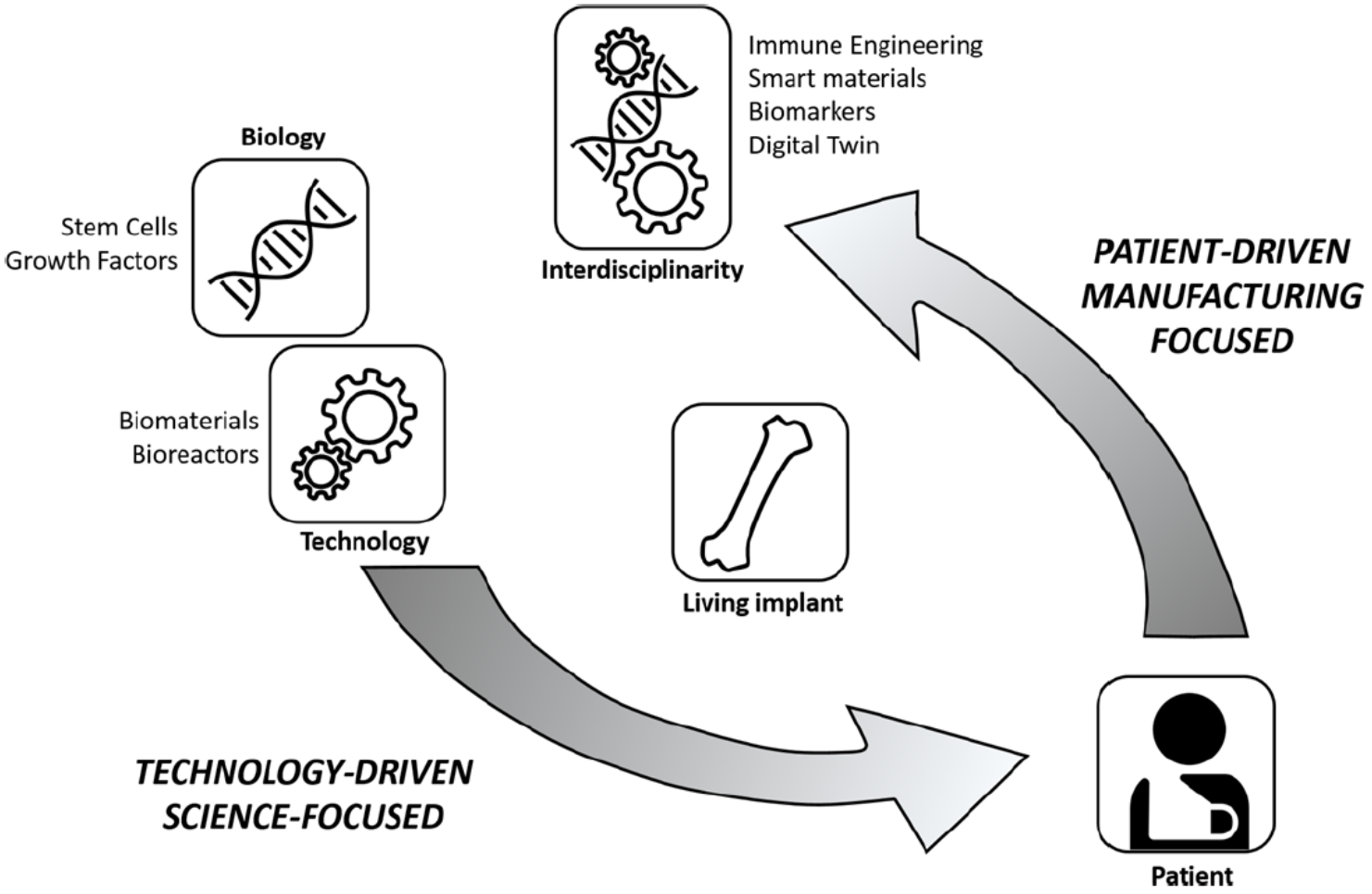

Papantoniou *et al* , 2019

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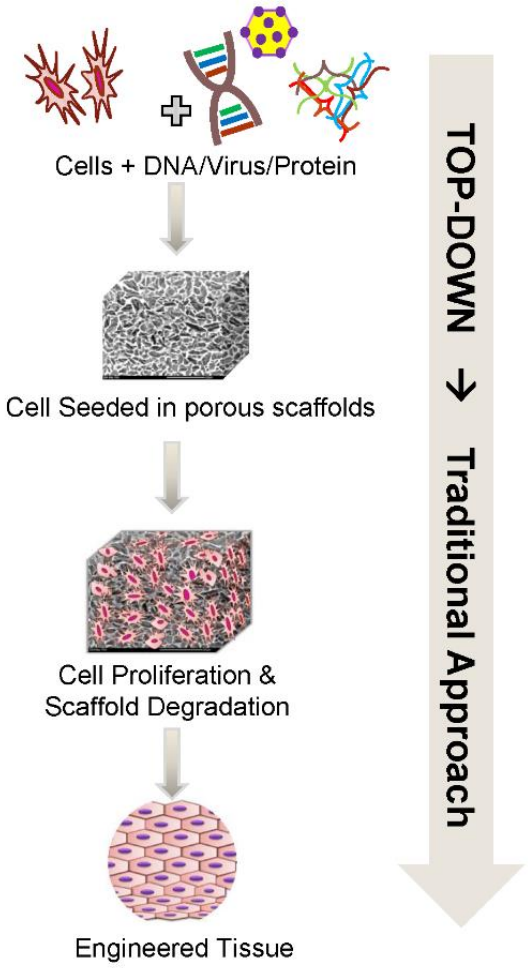
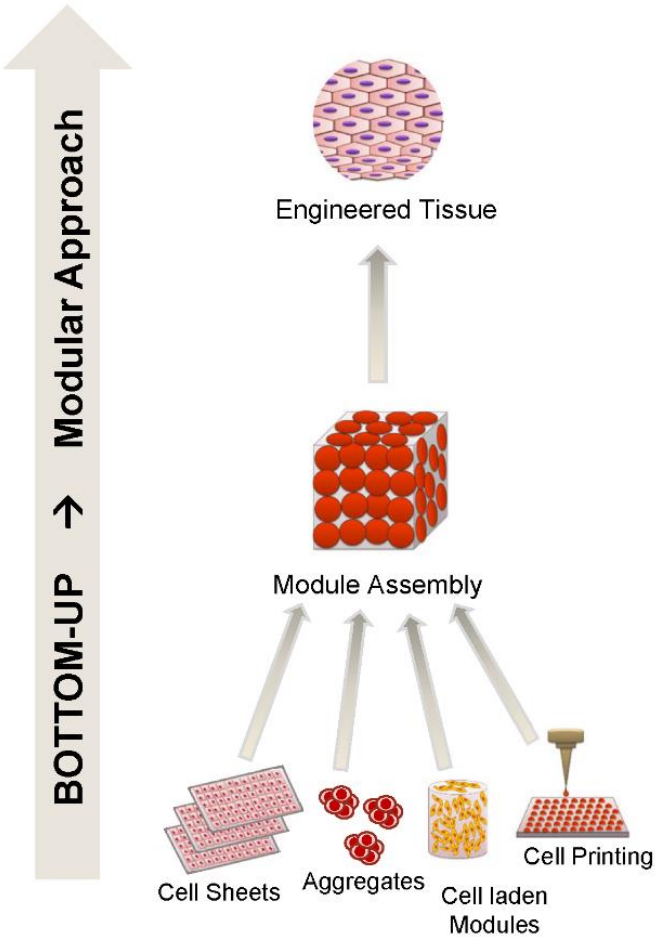
Top-down versus bottom-up Tissue Engineering





Tiruvannamalai-Annamalai *et al*, 2014
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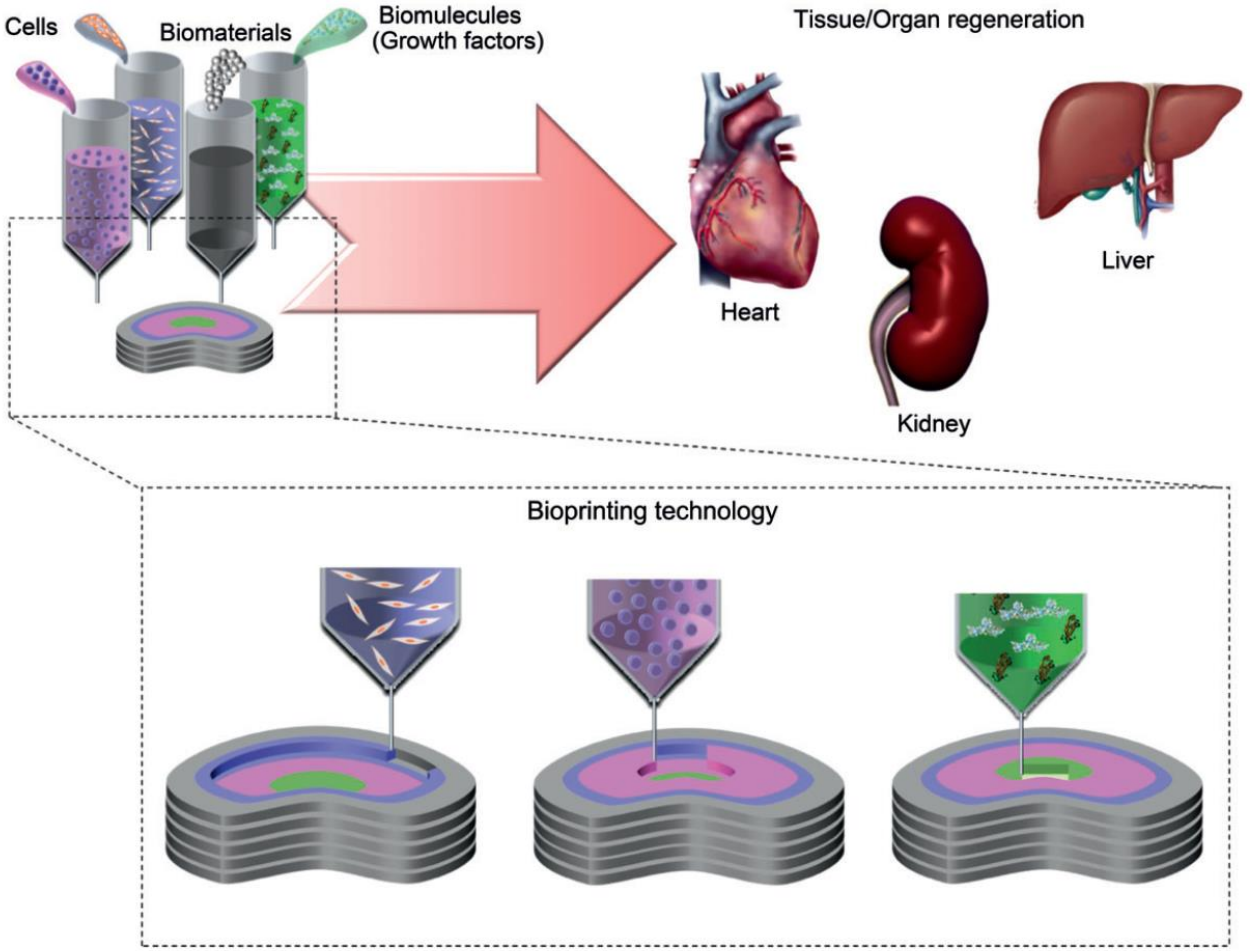
Bioprinting as a tool for the precise fabrication of complex architectures



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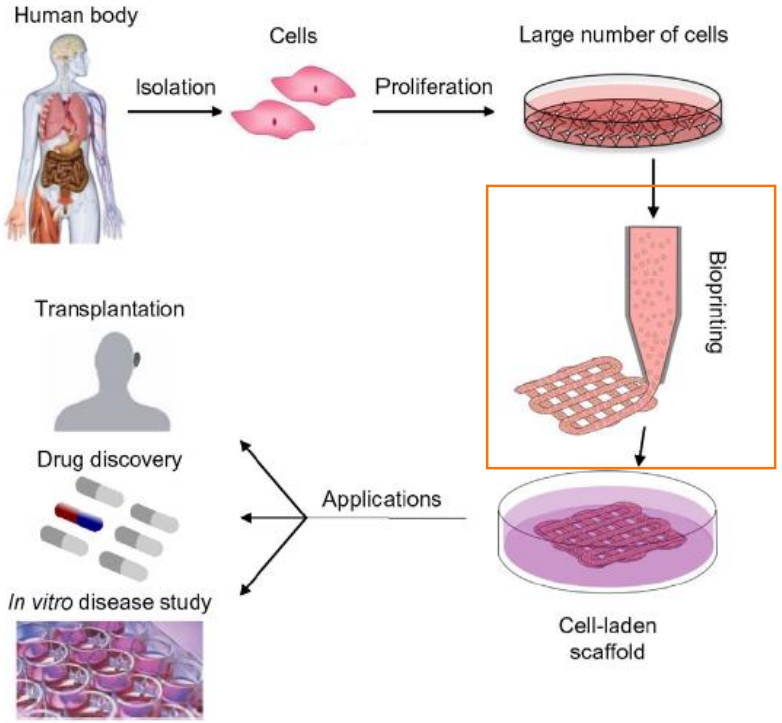

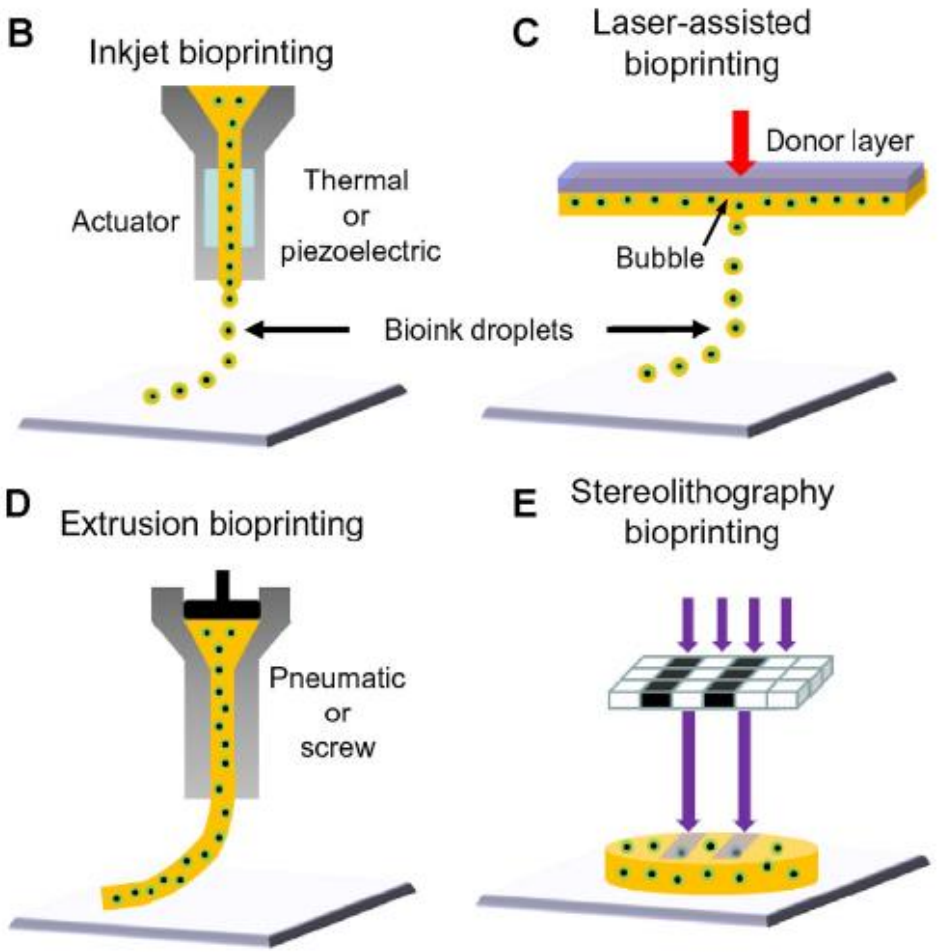

Mandrycky *et al* , 2015
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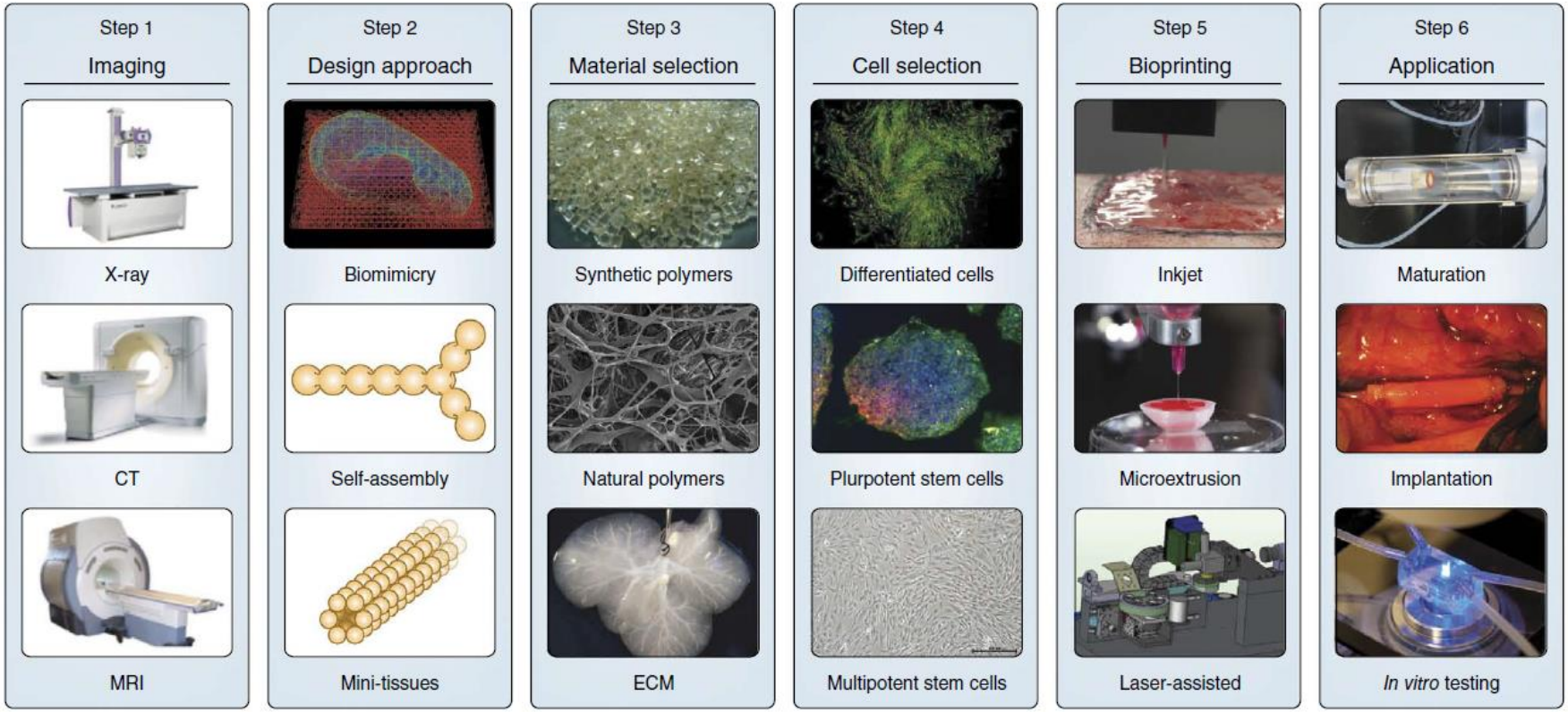
The process of bioprinting



Murphy *et al* , 2015

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Bioprinting for osteochondral regeneration

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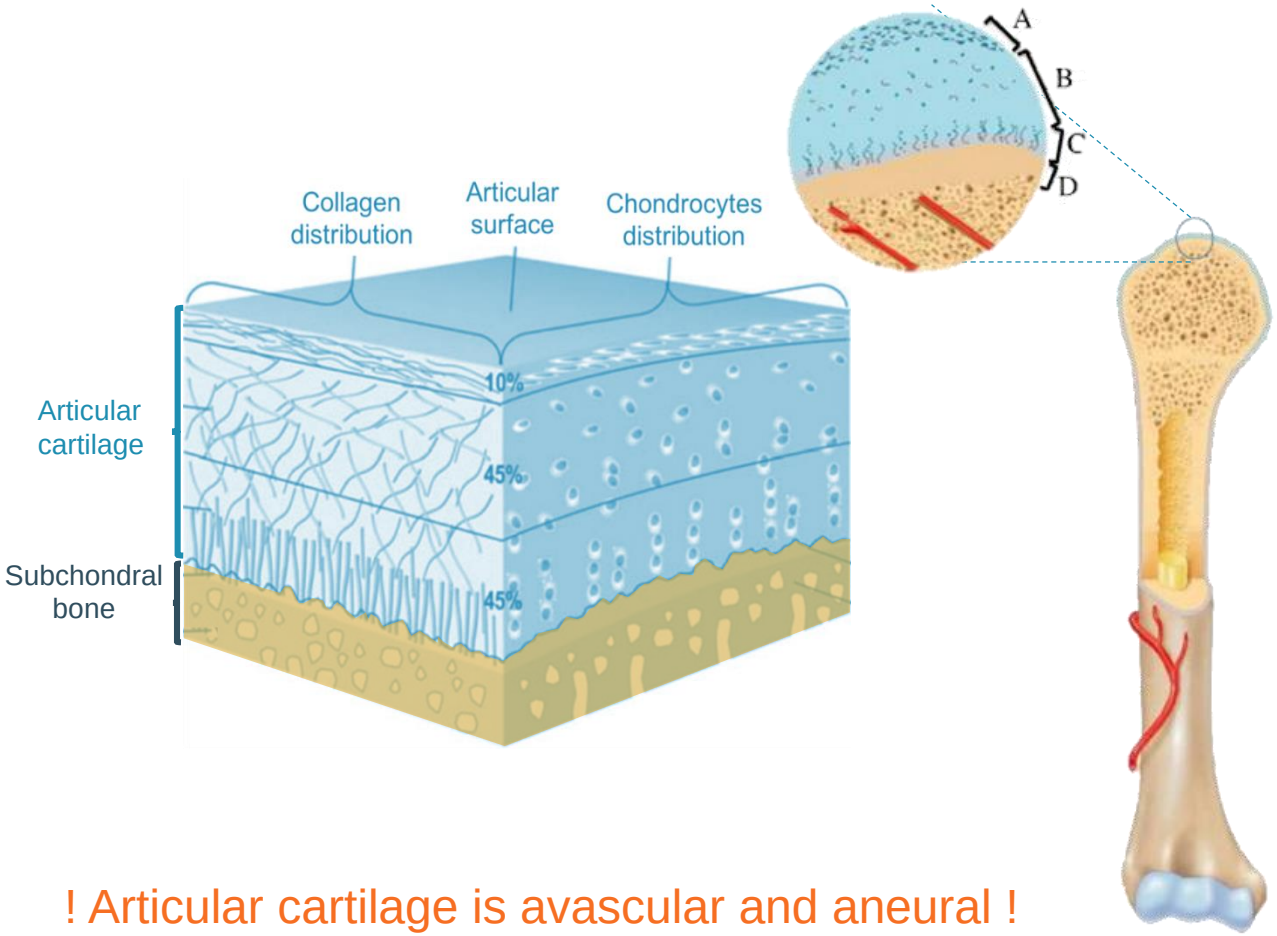
Osteochondral tissue



- Articular cartilage
  - Superficial zone (A)
  - Middle zone (B)
  - Deep zone (C)
    Calcified cartilage
- Subchondral bone (D)

! Articular cartilage is avascular and aneural !

Nukavarapu et al. 2013 *Biotechnol Adv*
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Osteochondral tissue

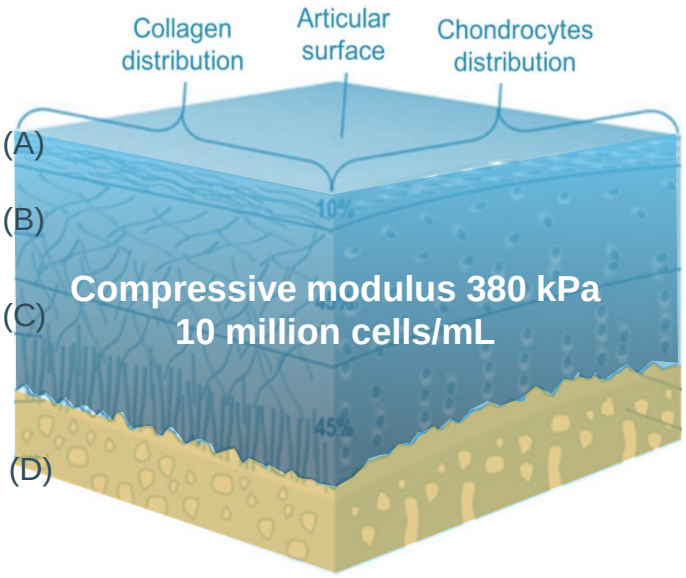
Superficial zone (A)

Middle Zone (B)

Deep zone (C)

Calcified cartilage

Subchondral bone (D)



PRG-4

CILP

COMP

Matrix stiffness

100 kPa

2000 kPa

Cell density

$24 \cdot 10^6$ cells/mL

$7 \cdot 10^6$ cells/mL

Schinagl et al. 1997 , Hunziker et al. 2002

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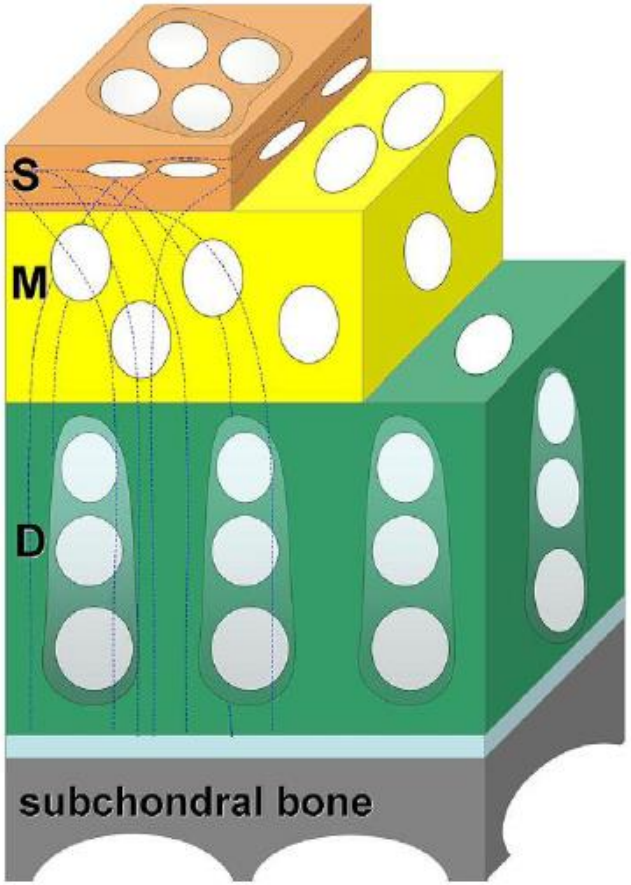
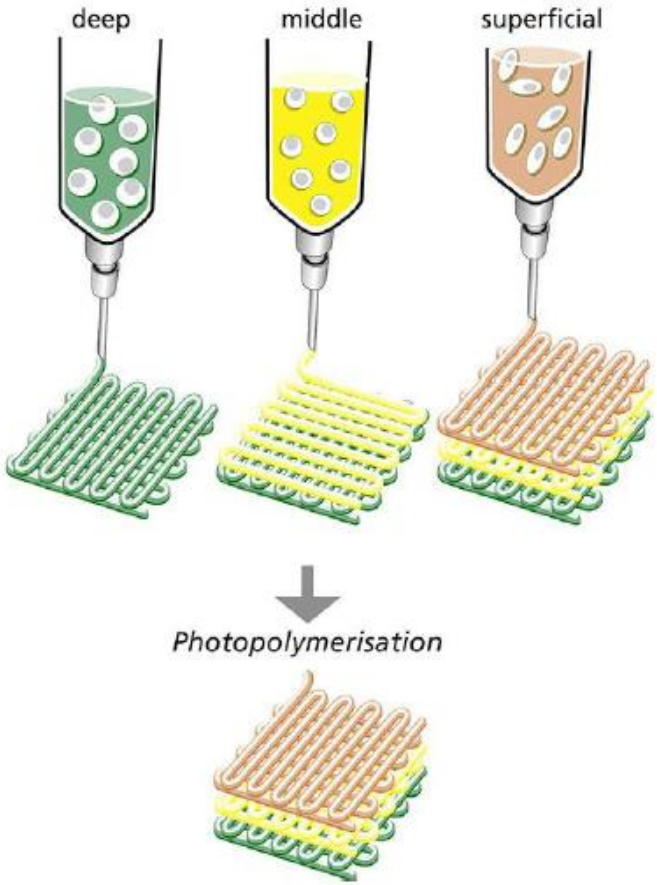
Bioprinting for osteochondral regeneration





Groen *et al*. 2017 ,

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Inkjet-based bioprinting of a cartilage construct

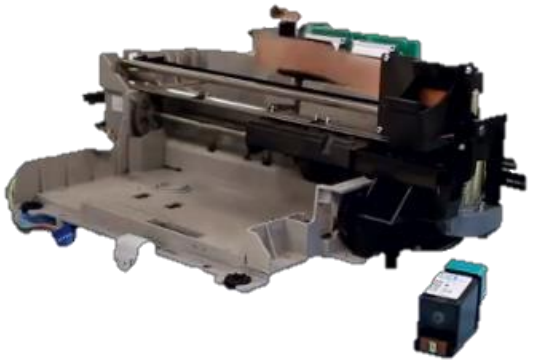
Modified HP Deskjet 500 thermal inkjet printer

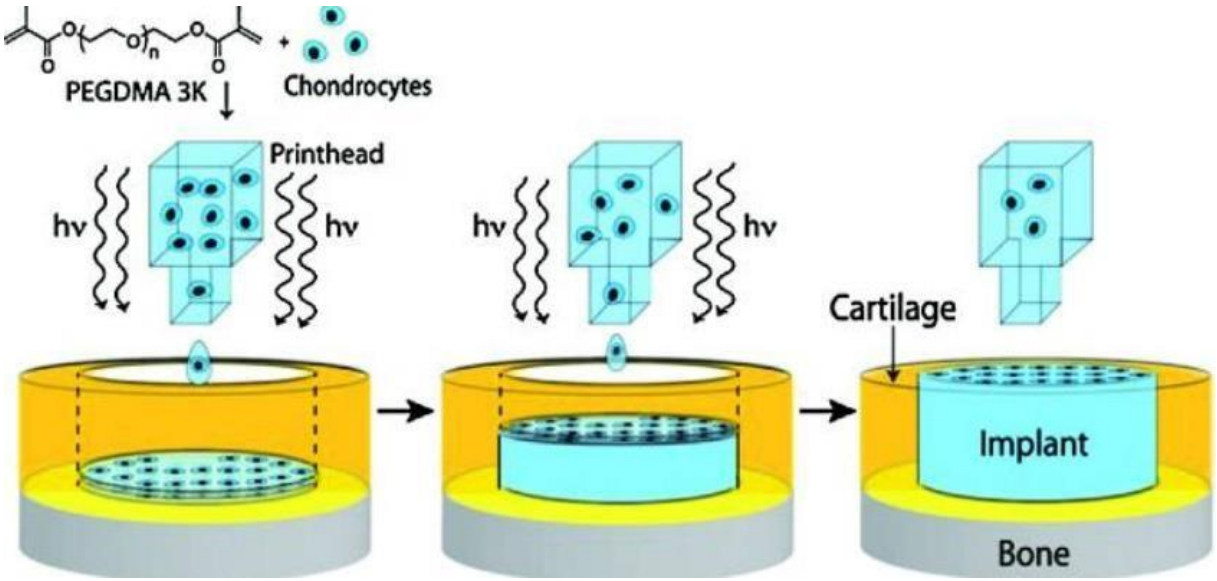
10 % PEGDMA in PBS

0,05 % I-2959

5 x 10^6 cells/mL human chondrocytes

⌀4 mm x 2 mm







Cui *et al* , 2012

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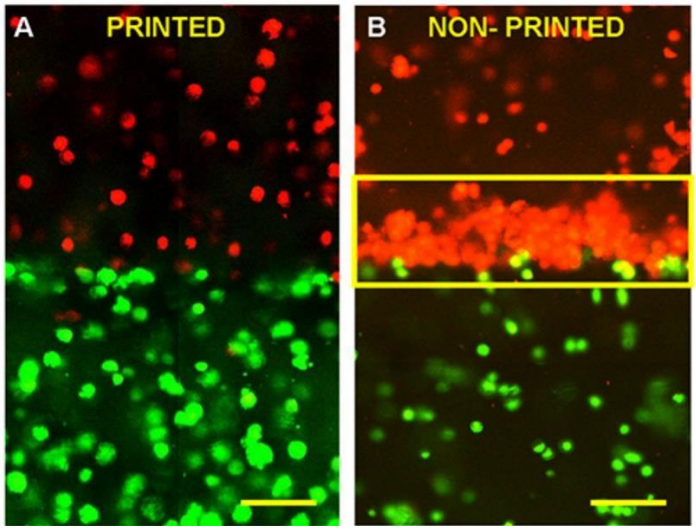
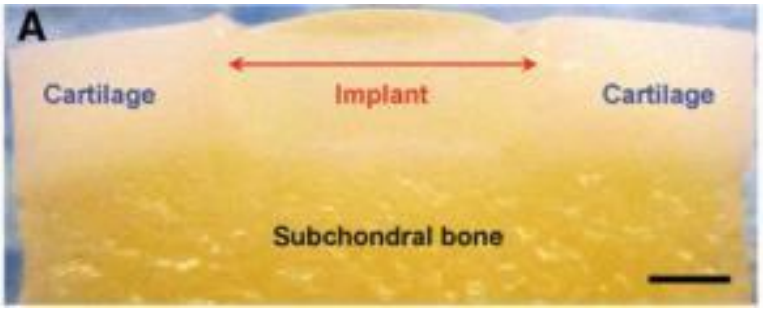
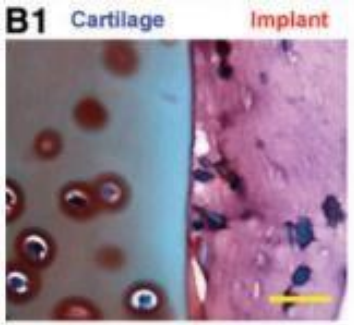
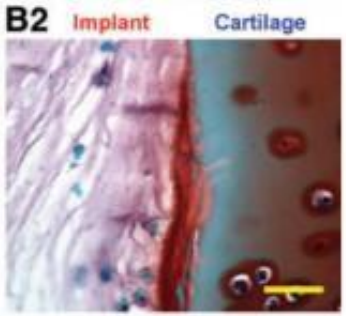


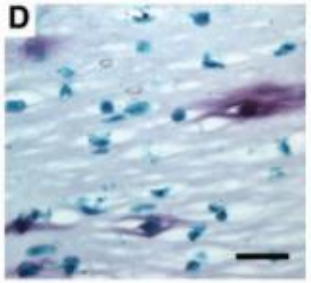
Distribution of fluorescently labeled chondrocytes in PEG
Scalebar = 100µm







Safranin-O staining after 6 weeks in chondrogenic medium.
Scale bar = 200µm



Construct printed in mold
Scalebar = 2 mm

- Simultaneous photopolymerisation → Lower cytotoxicity: cell viability 89% vs 63%
  → Maintained position and phenotype
- Printed in bovine osteochondral plugs more GAG/DNA than without plug

Cui *et al* , 2012
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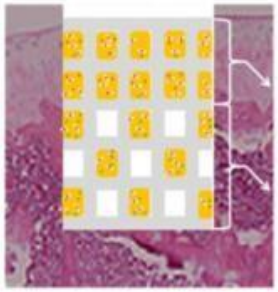
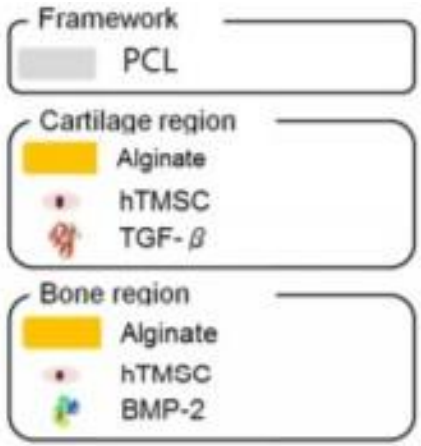
Multilayered constructs containing human MSCs for osteochondral tissue regeneration in rabbits

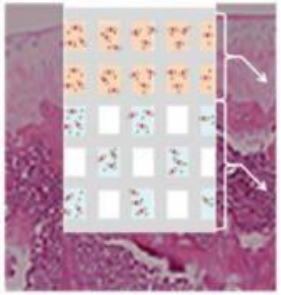
In-house built extrusion bioprinter

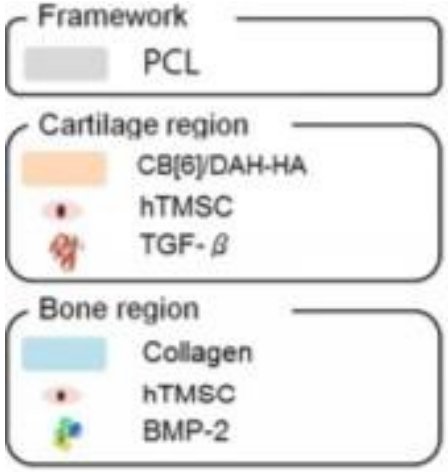
PCL
4 % alginate OR 5% CB[6]DAH-HA in DMEM
3% atelocollagen in DMEM
2 or 1 x10^6 cells/mL human MSCs
100 ng/mL TGF-β or 5 µg/mL rhBMP-2
⌀5 x 5 mm











Shim *et al* , 2016
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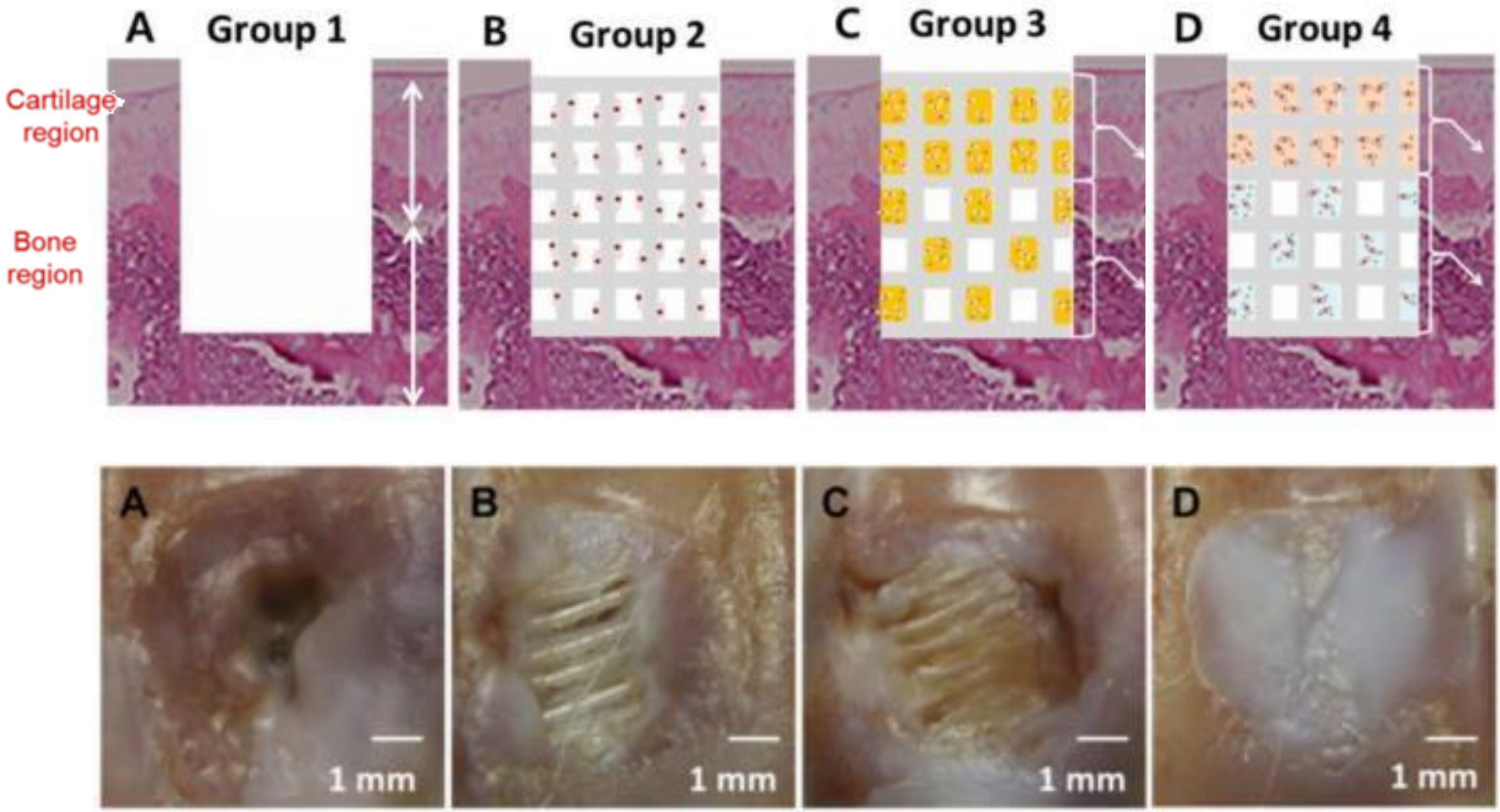

4 experimental groups, cultured in DMEM for 24h, then 8 weeks *in vivo*

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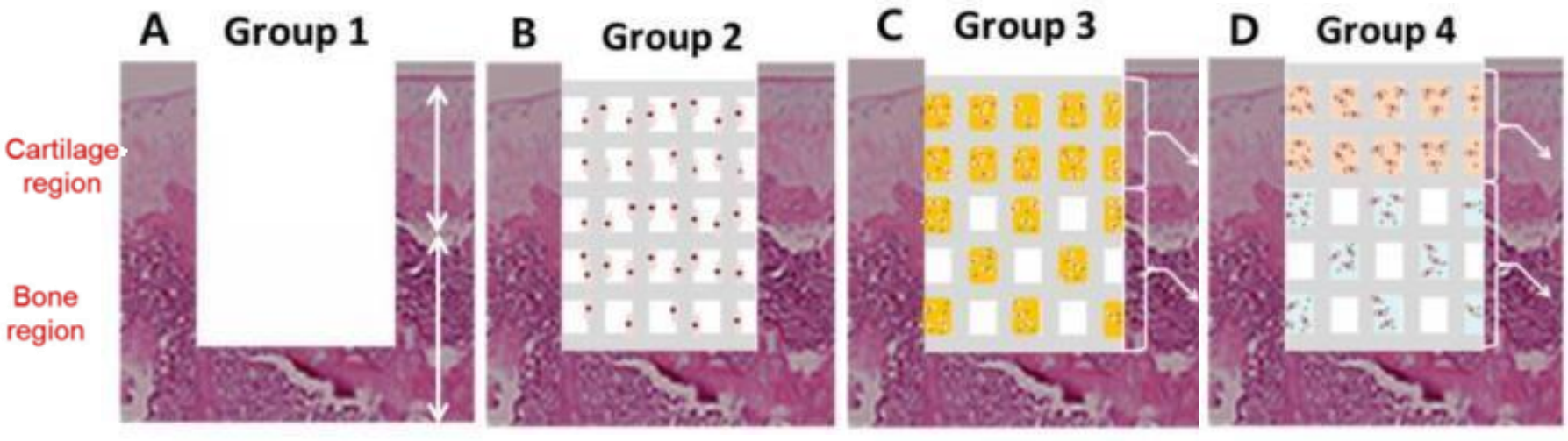

- Cell viability after printing atelocollagen 93%
  CB[6]/DAH-HA 86%
- Mechanically stabilized atelocollagen gel and new crosslinked HA hydrogel with MSCs and growth factors promote heterogeneous neotissue formation *in vivo*

Shim *et al* , 2016
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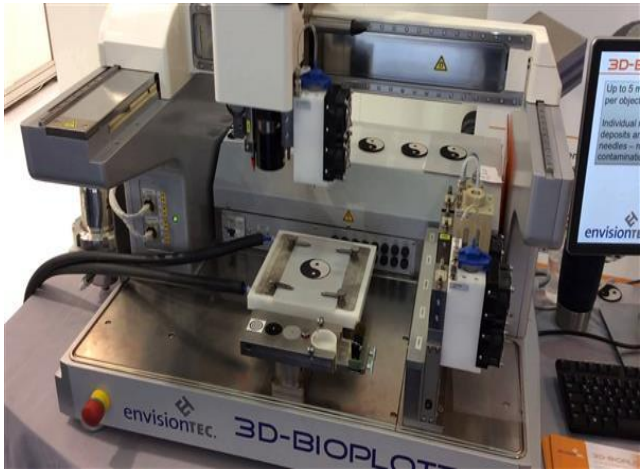
Commercial systems



Organovo – Novogen MMX



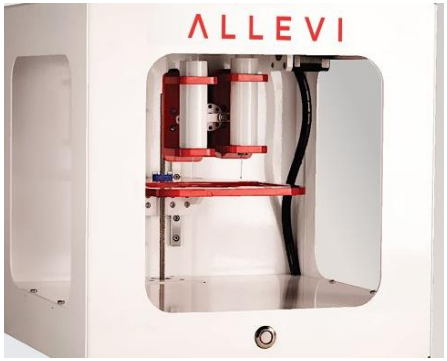
EnvisionTec – 3D-Bioplotter



RegenHu - Biofactory



Cellink Bio-X



Allevi (former biobots)

■ ■ ■

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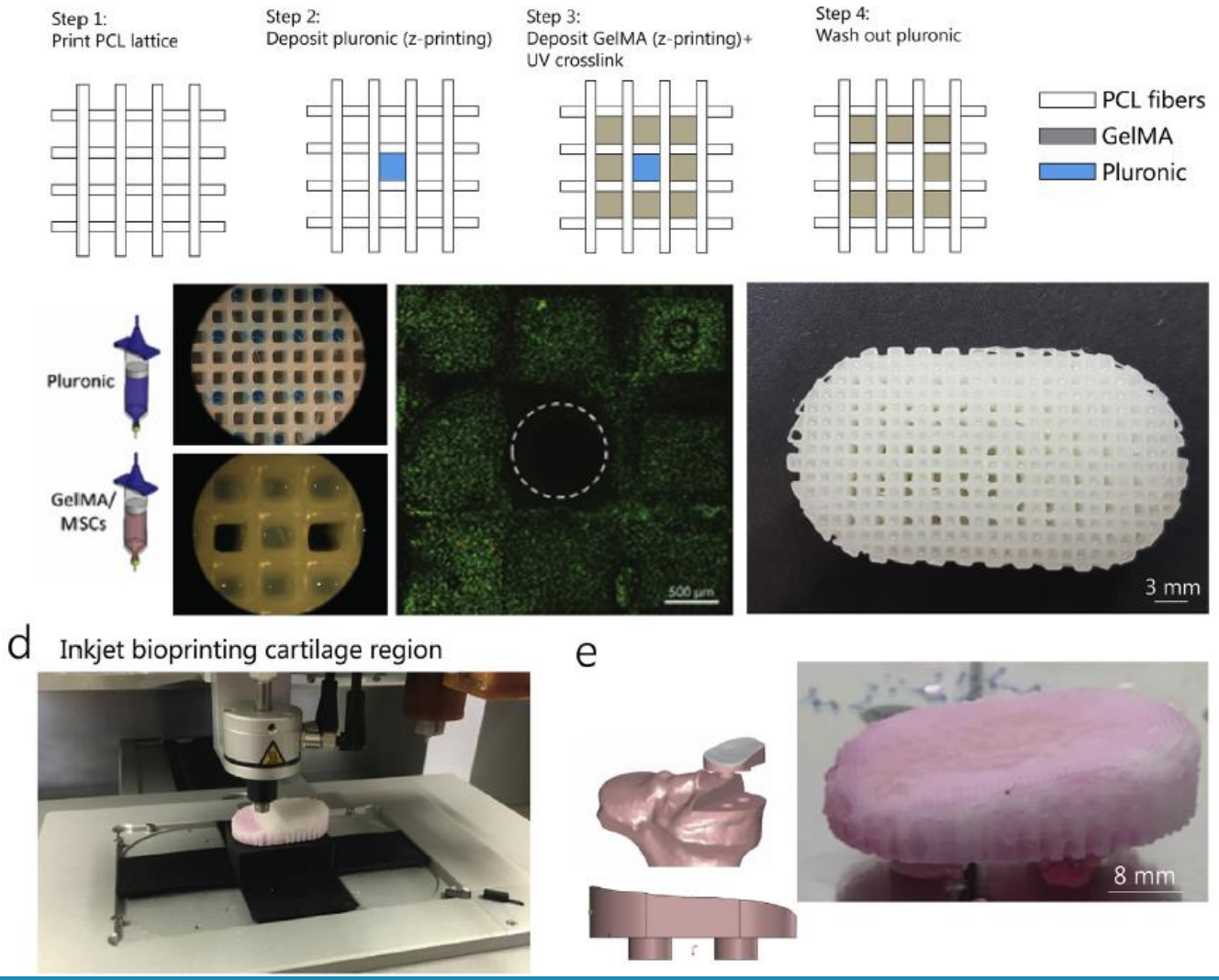
Biofabrication of spatially organised tissues



Daly *et al* , 2019

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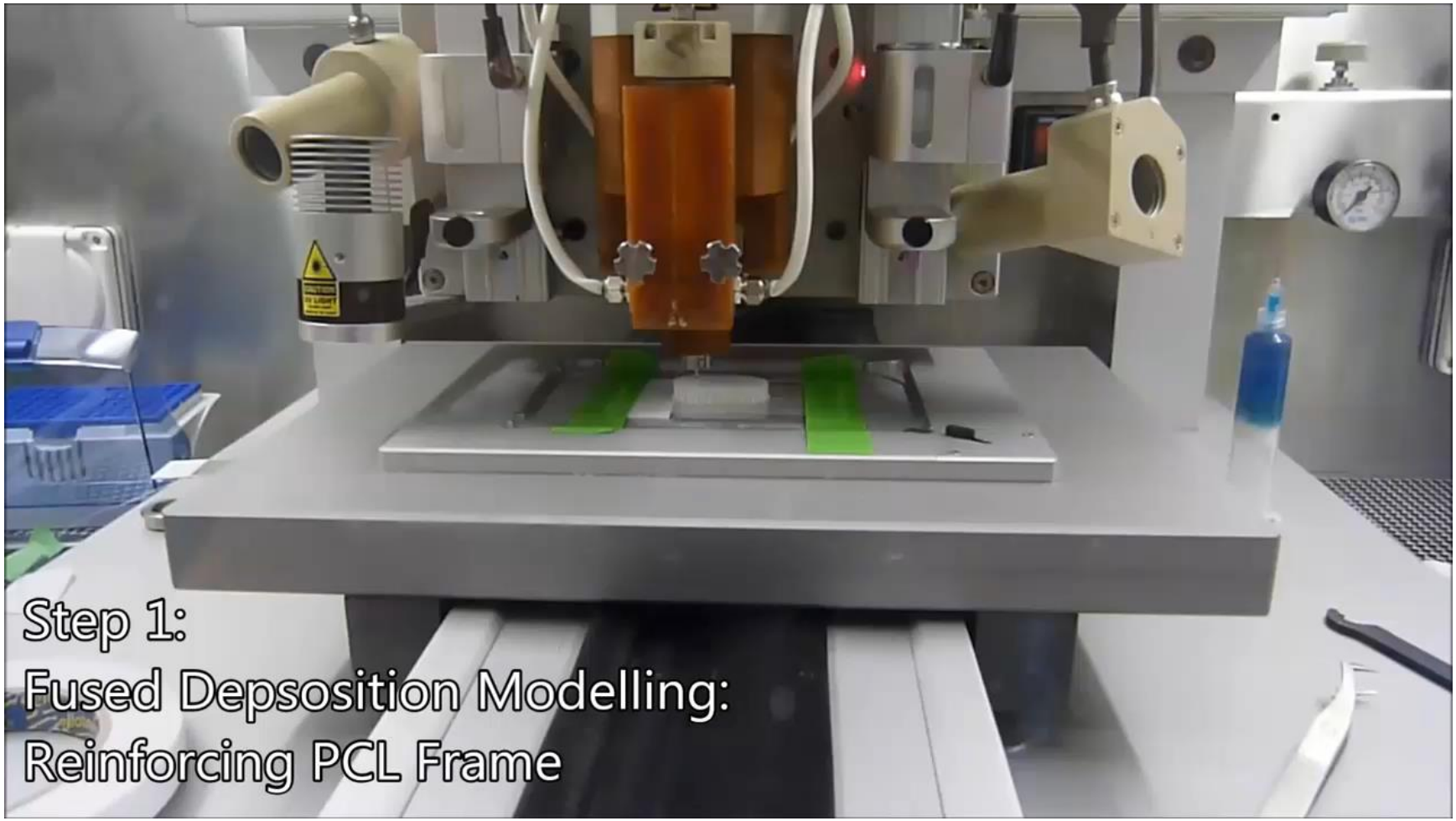

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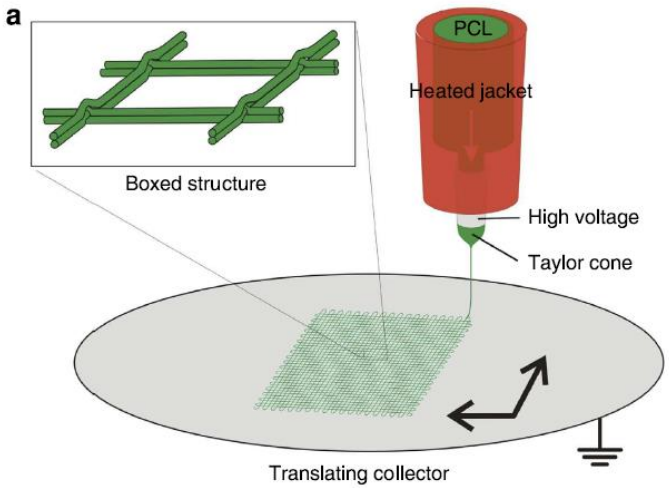
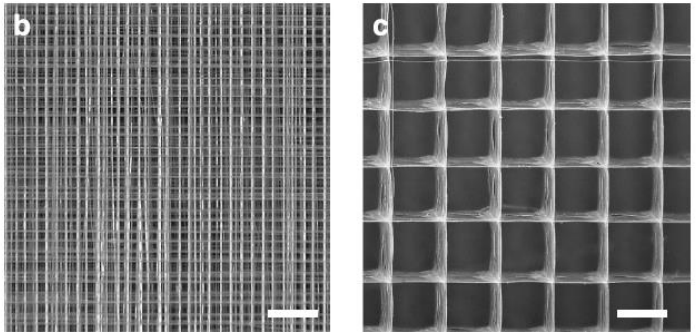
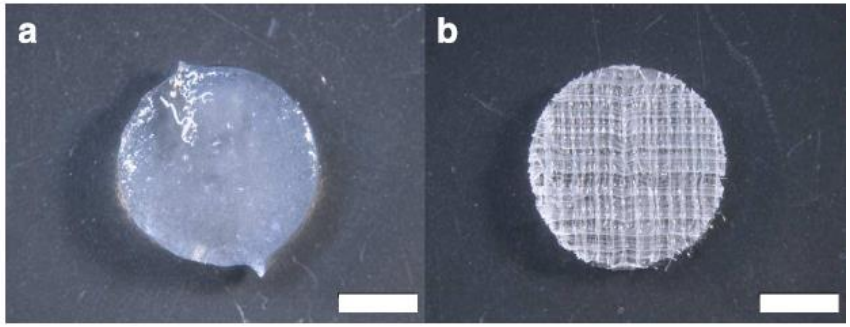
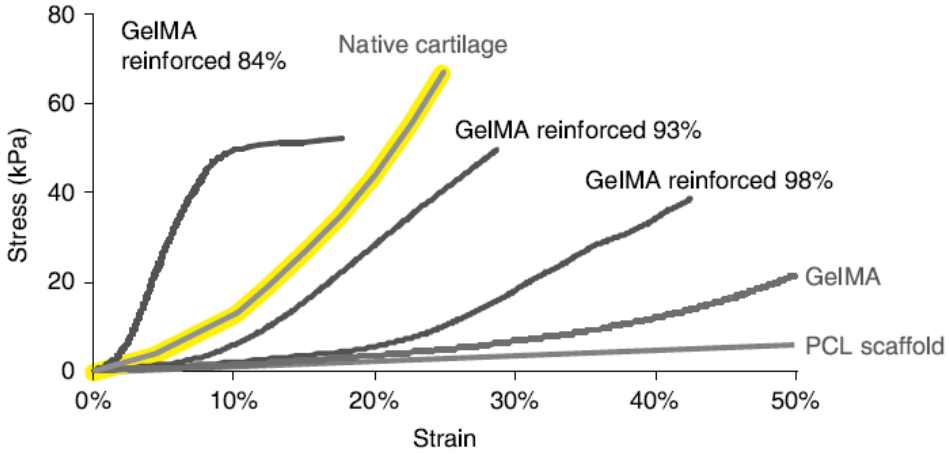
Melt electrowriting (MEW) for the reinforcement of hydrogels with 3D printed microfibers









Visser *et al* , 2015

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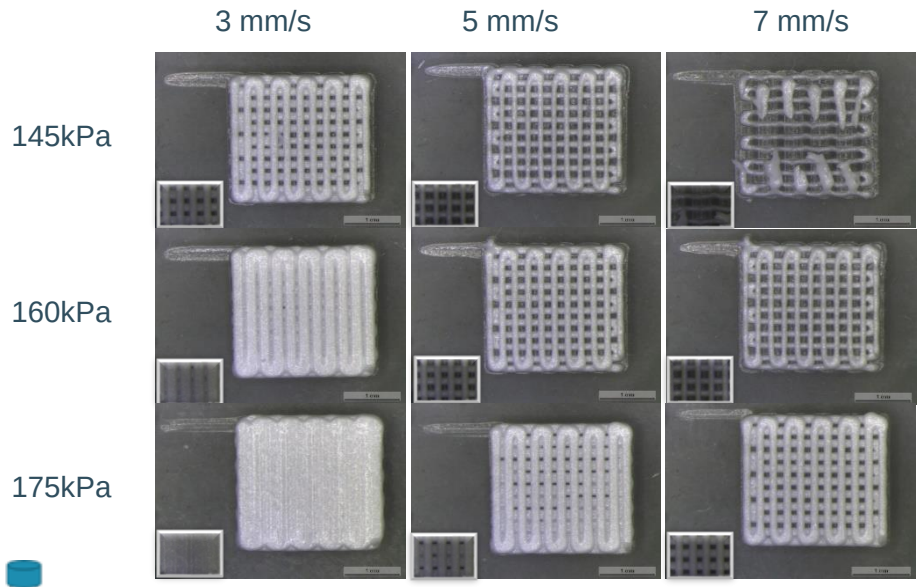
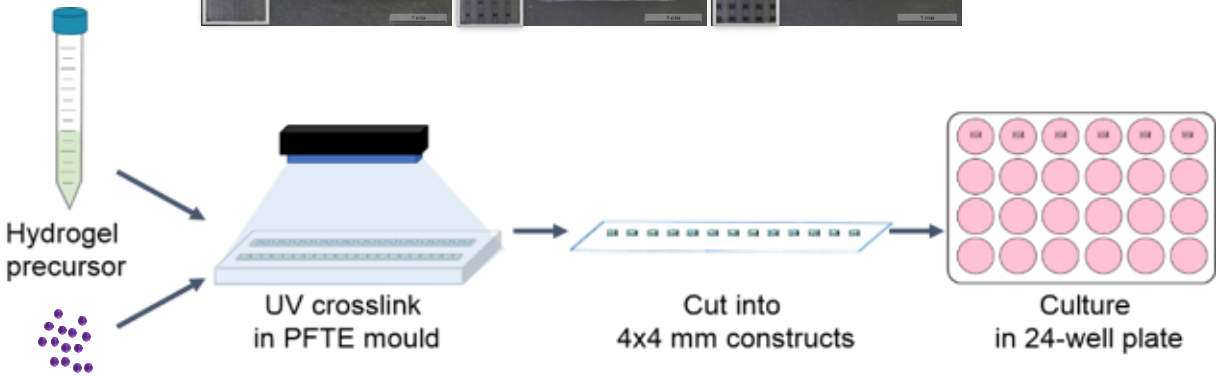
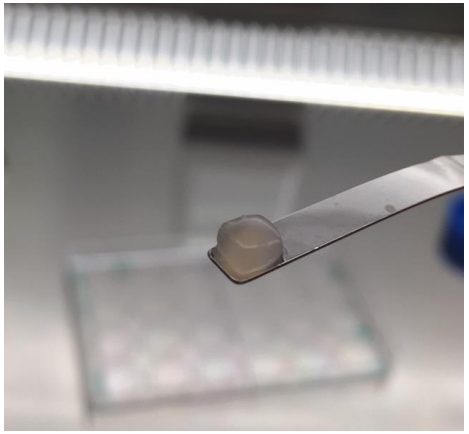
Bio-ink development for osteochondral regeneration: preliminary in-house data









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Bioprinting for long bone healing

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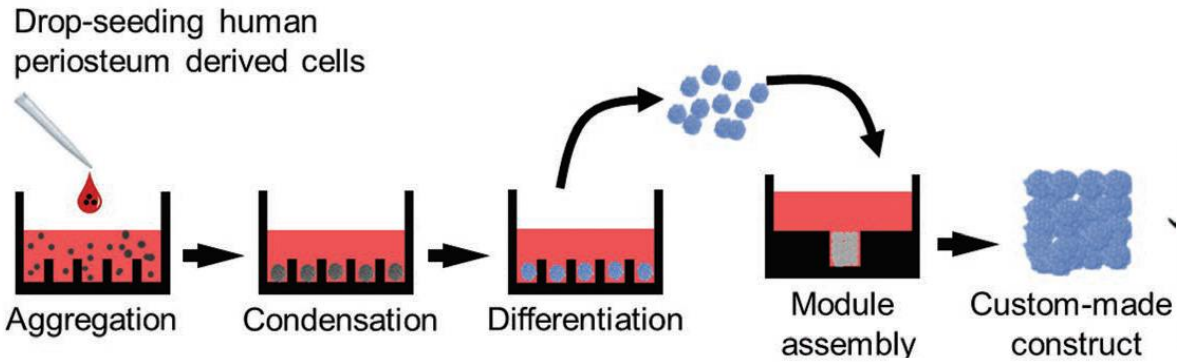
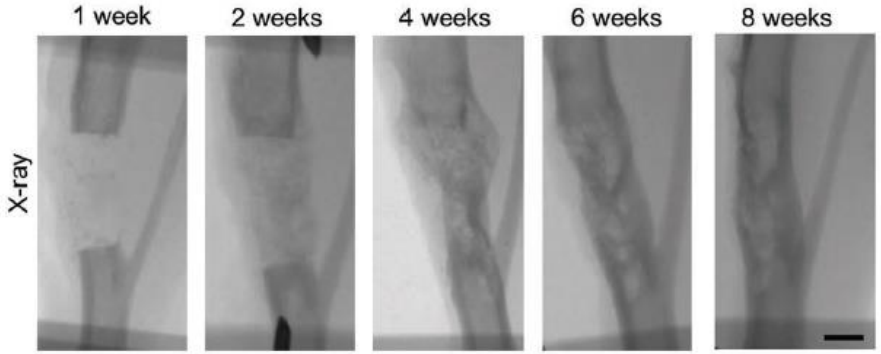
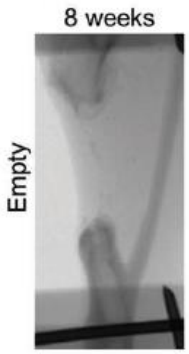
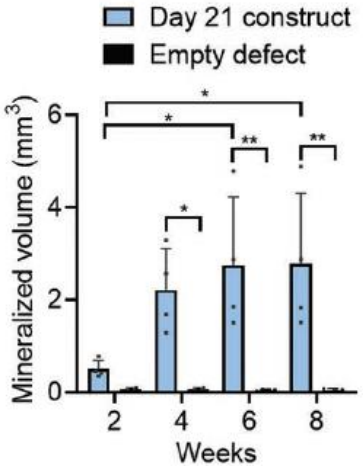
Developmentally engineered callus organoid bioassemblies for long bone healing











Nilsson-Hall *et al* , 2019
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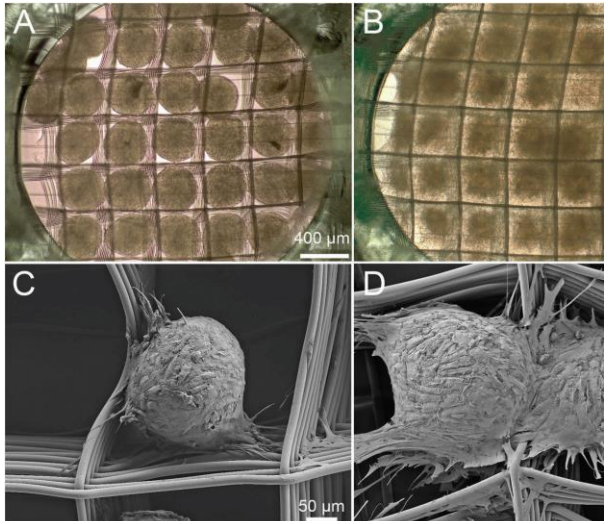
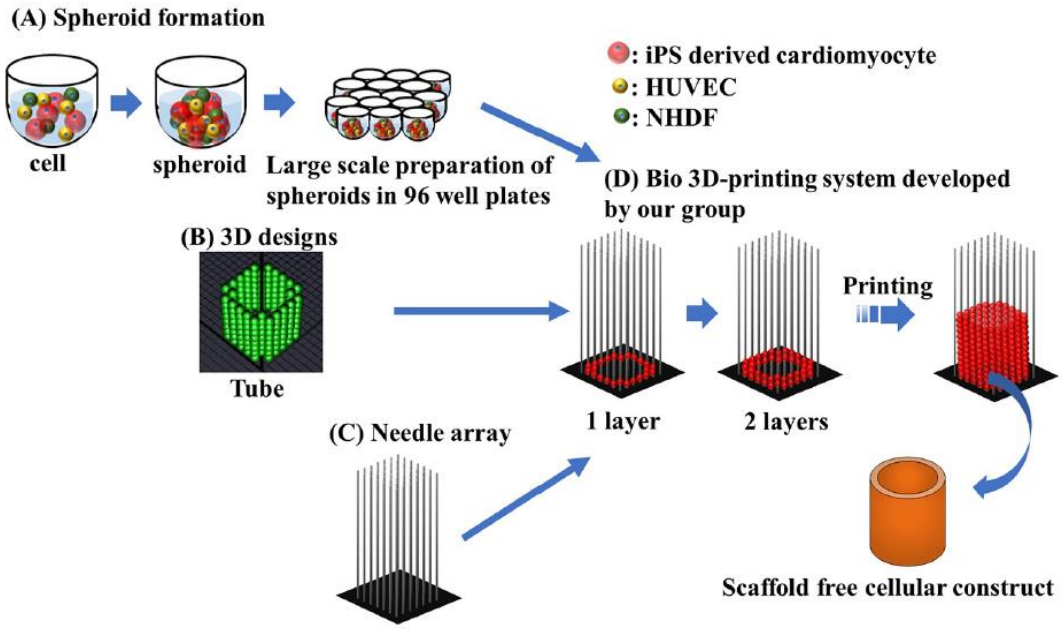
3D printing as a route for upscaling?



Sheet-like tissue constructs
from spheroids and MEW-scaffolds



The “Kenzan” method

Arai *et al* , 2018

McMaster *et al* , 2019

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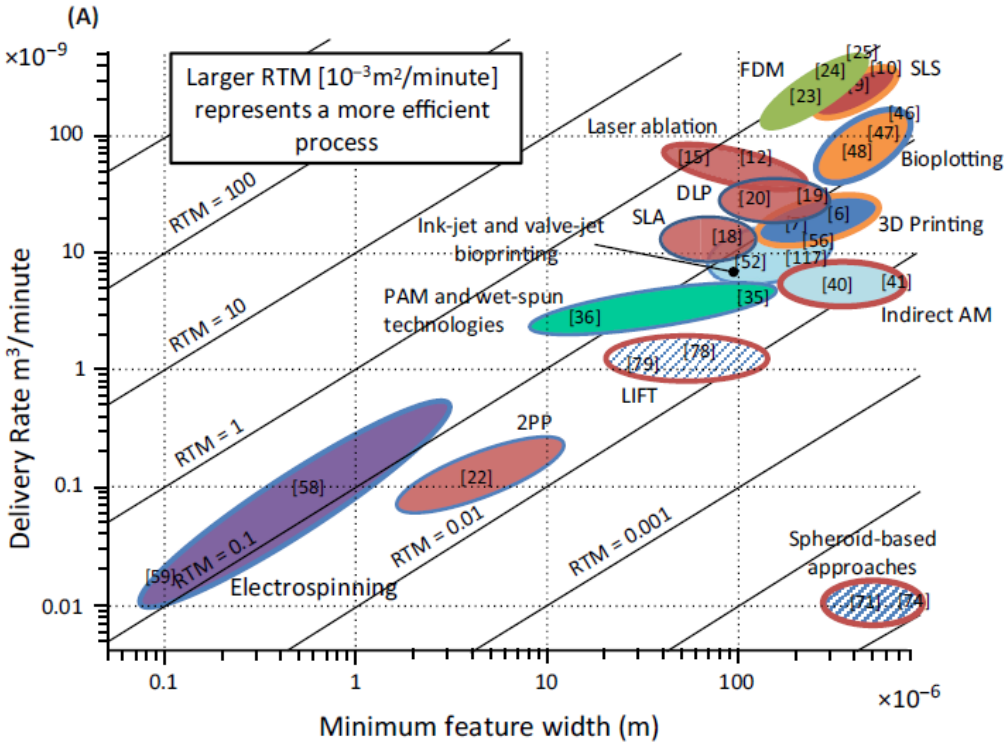
From bioprinting to biofabrication

‘the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as micro-tissues, or hybrid cell-material constructs, through bioprinting or bioassembly and subsequent tissue maturation processes’



Moroni *et al* , 2018

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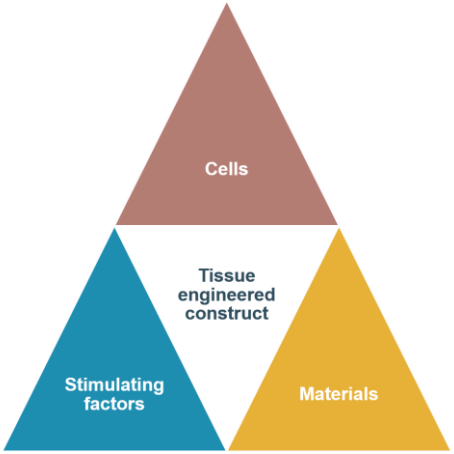
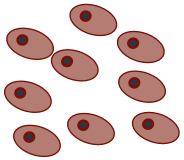
BIOFABRICATION

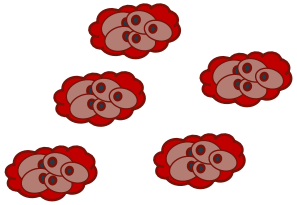
BIOASSEMBLY



BIOPRINTING

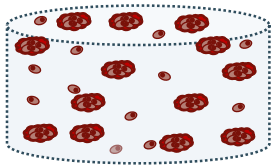


Single cells



Cell aggregates micro-tissues

(cell-driven self-organization)



Hybrid cell-material constructs

(living materials)

https://doi.org/10.1016/j.tibtech.2017.10.015

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In summary

Traditional TE needed…

- Scaling up
- Anatomically accurate and mechanically functional implants upon implantation
- A robust and controllable process that diminishes variability in the biological outcome
- Personalised approach

Biofabrication technologies offer…

- The gradient deposition of cells, proteins, materials…
- An automated, controlled process from design to fabrication allowing scale up
- Combined technologies that have shown potential to develop improved constructs

Challenges still remain…

- Vascularisation
- Material optimisation to increase shape fidelity
- Complex architectures
- Post-fabrication processing
- Regulatory and ethical challenges
- …

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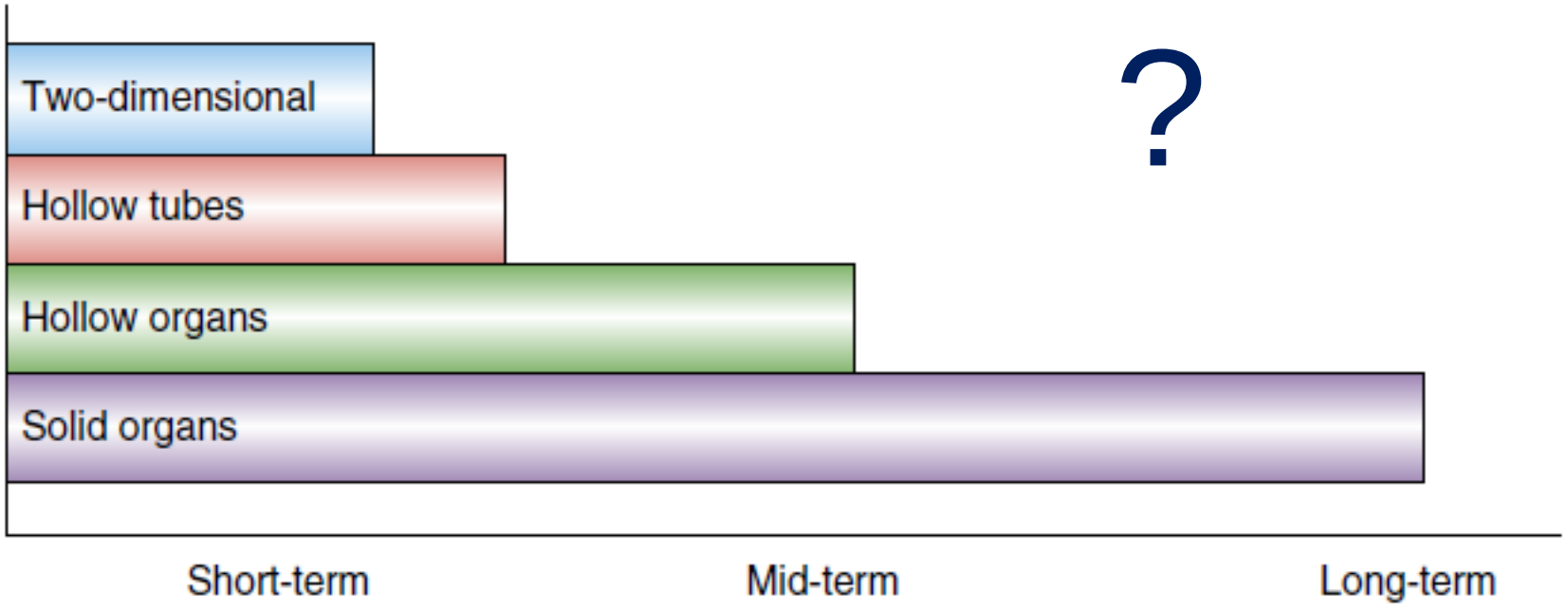
State-of-the-art and future perspectives



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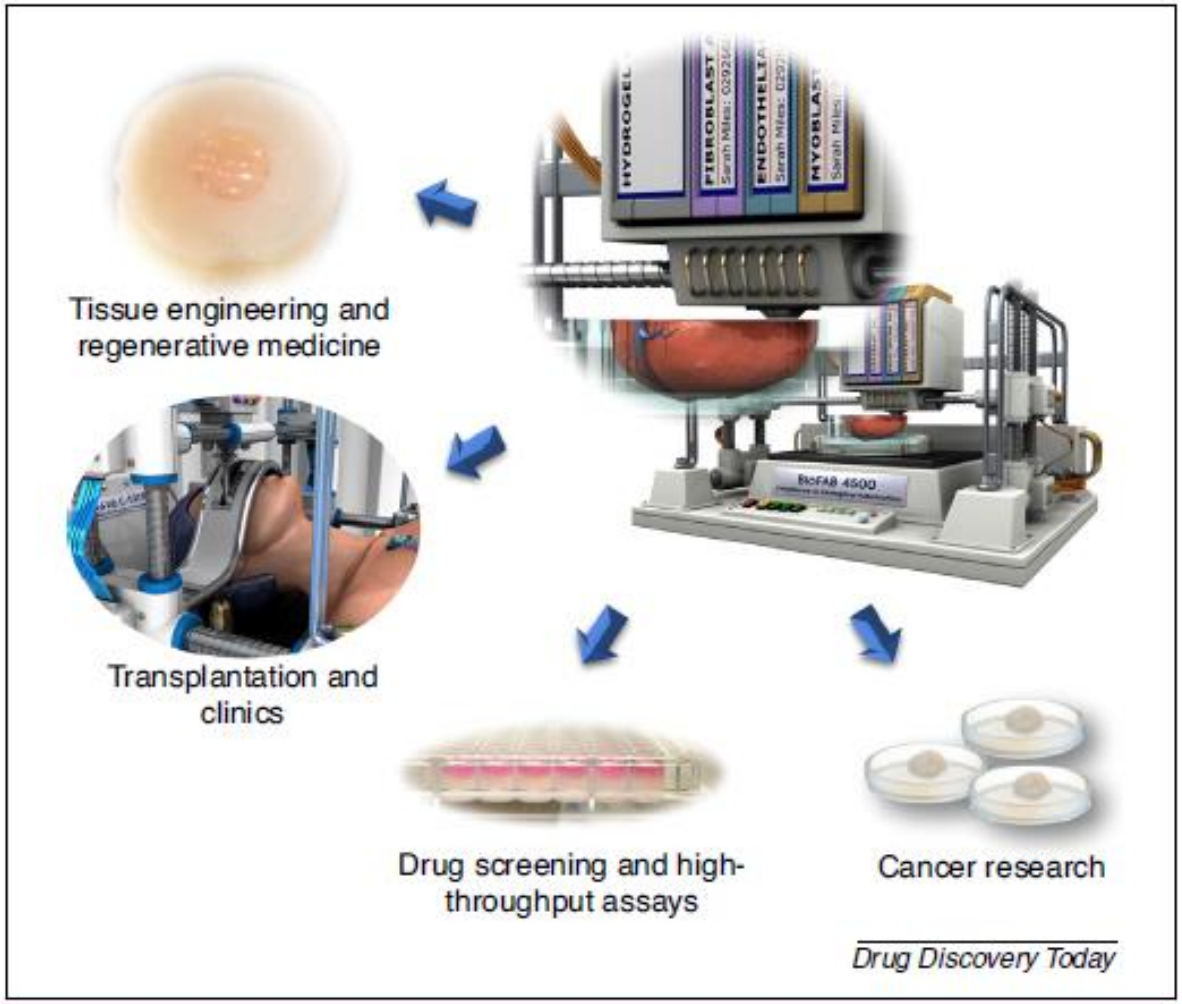
Possible applications



Ozbolat, I.T. *et al.* , 2016
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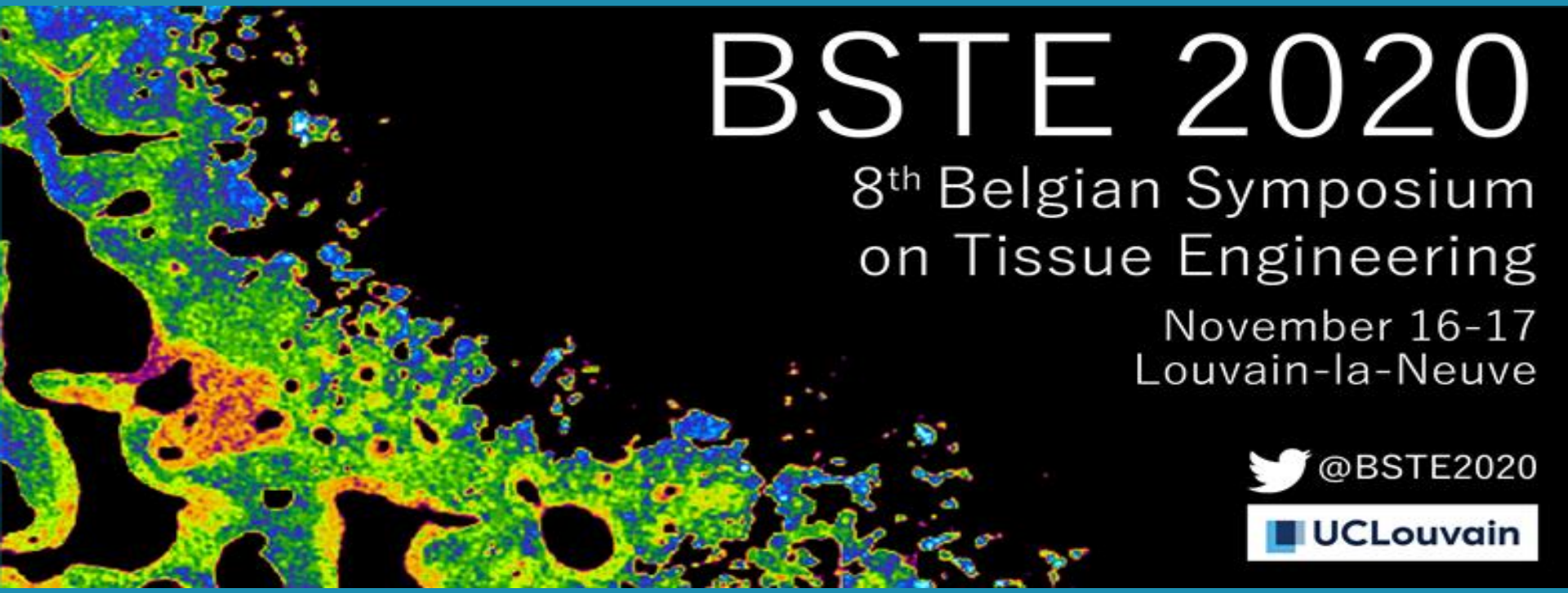
THANK YOU



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