Tissue Engineering and Regenerative Medicine

NIH Center for Engineering Complex Tissues (CECT)
June 7, 2019

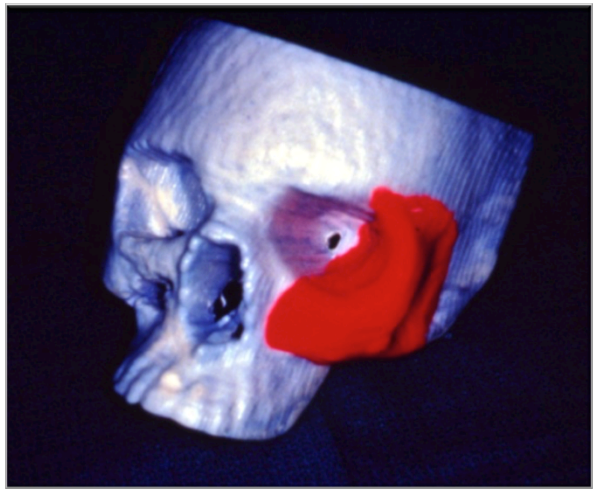
Bhushan Mahadik, Ph.D.
Assistant Director, CECT
University of Maryland

CECT Center for Engineering Complex Tissues
UNIVERSITY OF MARYLAND • RICE UNIVERSITY • WAKE FOREST UNIVERSITY

Addressing a biomedical need

- Large tissue defects
- Scar tissue formation
- Limited innate healing capacity
- Other pathologies that limit desired regeneration

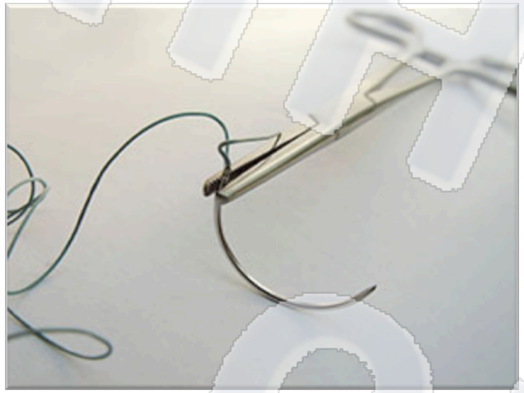
Source: Usatine RP, Smith MA, Mayeaux EJ, Chumley HS: *The Color Atlas of Family Medicine, Second Edition:* www.accessmedicine.com



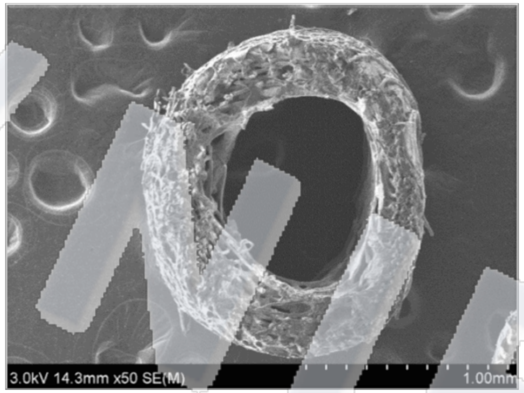
(Photo: Ann Surg Treat Res. 2014 Nov; 87(5): 253-259)

Advances in Biomaterial Applications

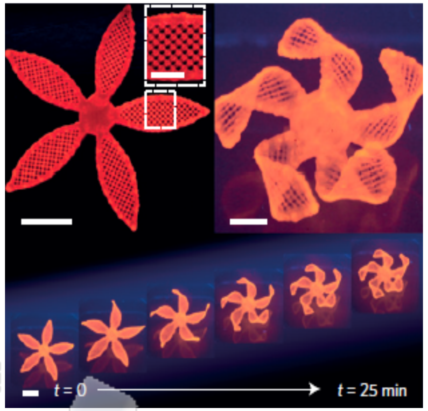
Contact Lens



Absorbable Sutures



Vascular Grafts



4D

Bioinertness
(1970's)

Bioactivity
(1980's)

Regenerative Properties
(2000's)

Responsive/Smart
(2010's)

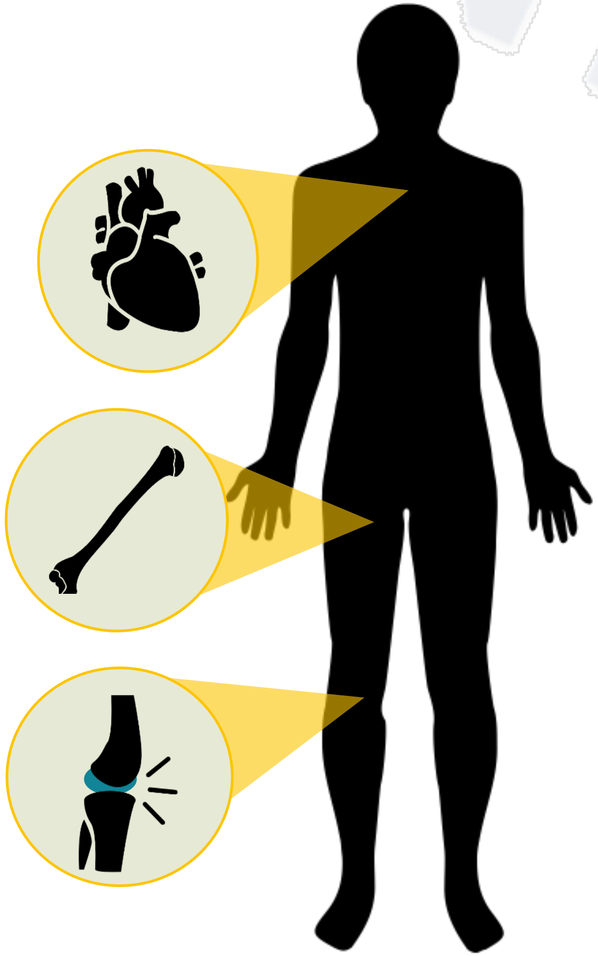
Tissue Engineering

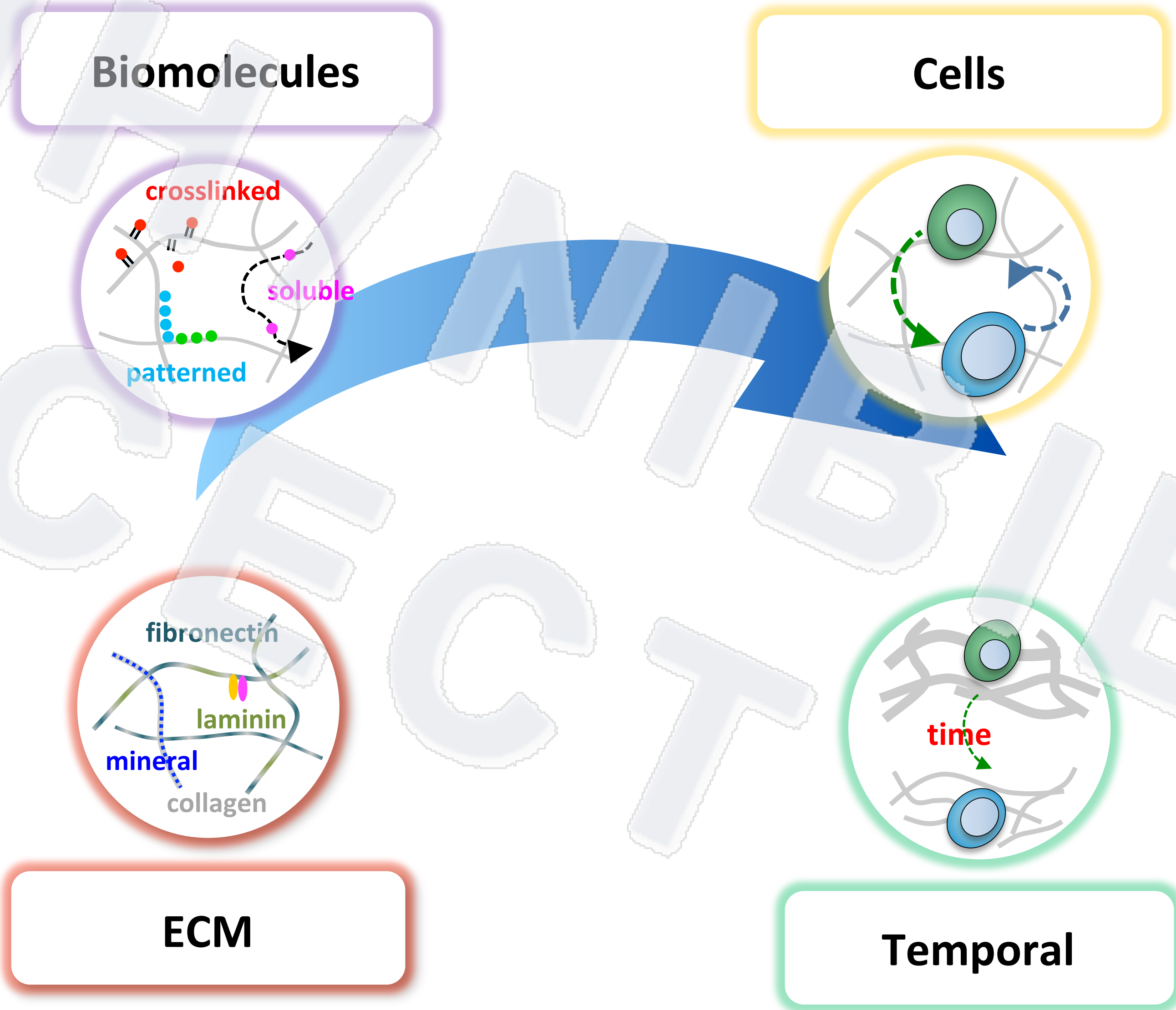
- **Regeneration**
  - Replacement of lost tissue with the tissue itself
  - Initiate regeneration where it is not normally observed
    - Cartilage defects
    - Large (critical size) bone defects

- **Repair**
  - Replacement of lost tissue with a functional substitute
  - Enhance the rate of repair where it is seen
    - Nearly any tissue defect

- **Replacement**
  - Replacement of a missing cell population
  - Red blood cells in a blood transfusion
  - Bone marrow cells in marrow replacement

Elements of Tissue Engineering

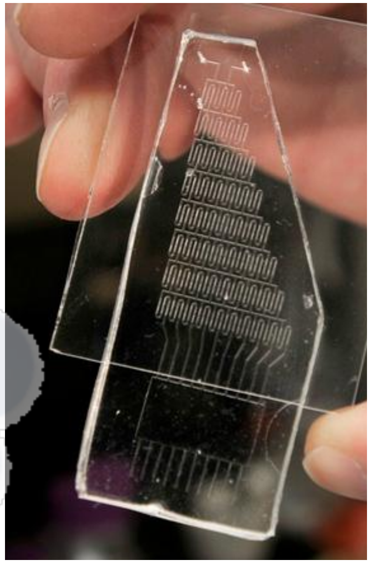



Biomolecules

Cells

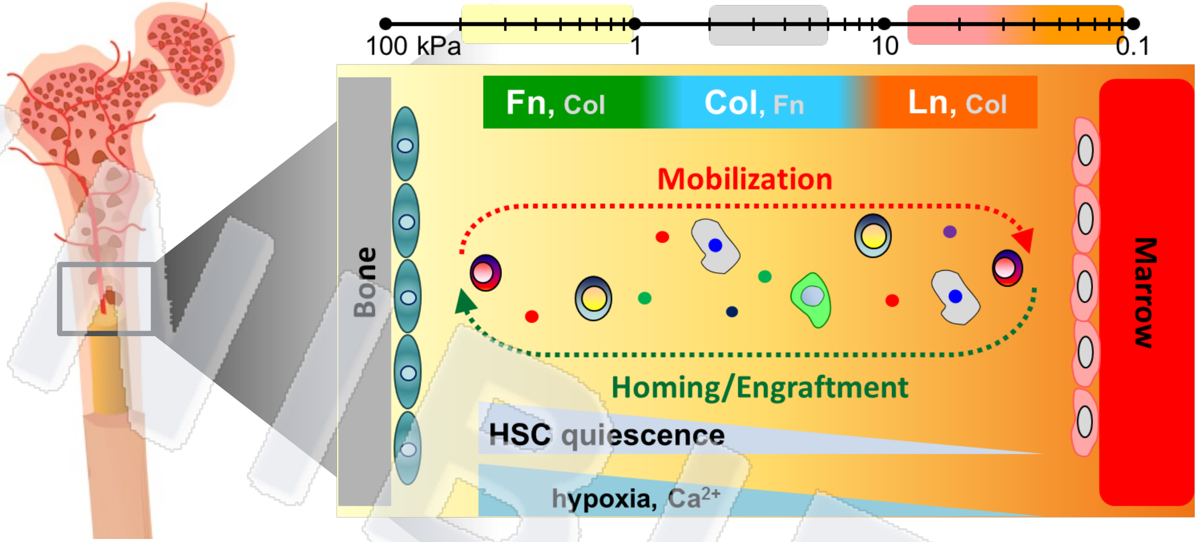
ECM

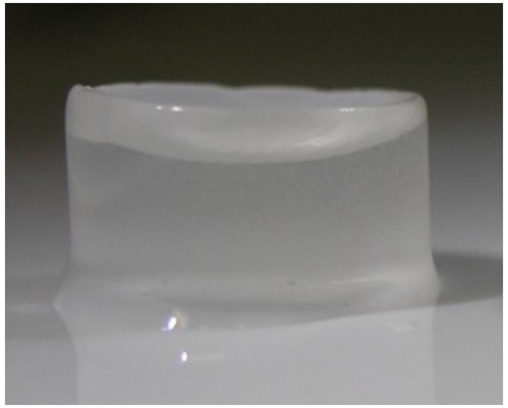
Temporal

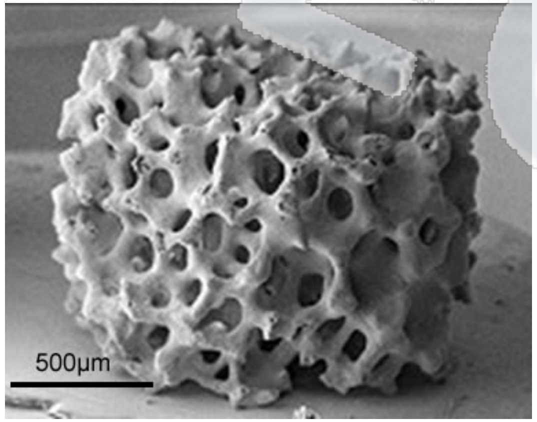


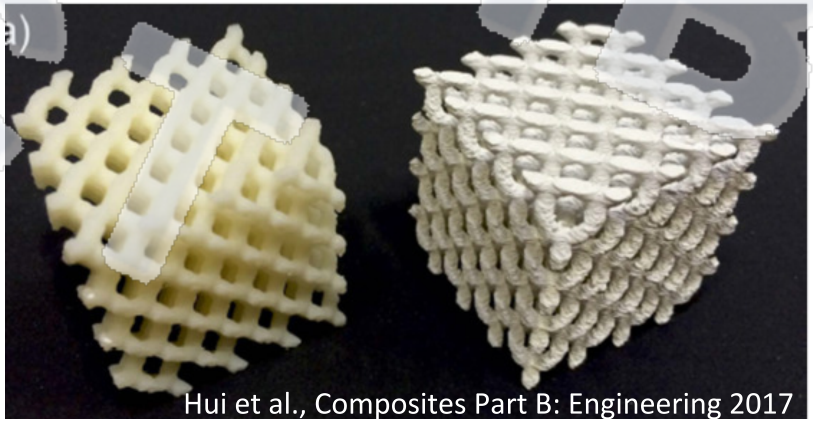
Tissue Engineering Challenge

- Capturing native complexity
- Biomaterial choice
  - Biocompatible, Biodegradable
  - Natural vs. Synthetic
  - Chemical, Biomechanical, Structural similarity



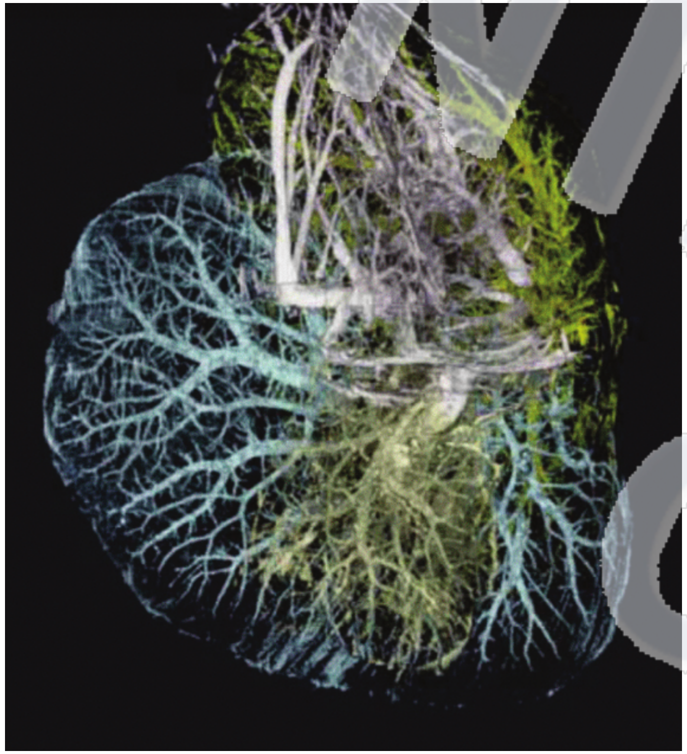






Hui et al., Composites Part B: Engineering 2017

Tissue Engineering Challenge

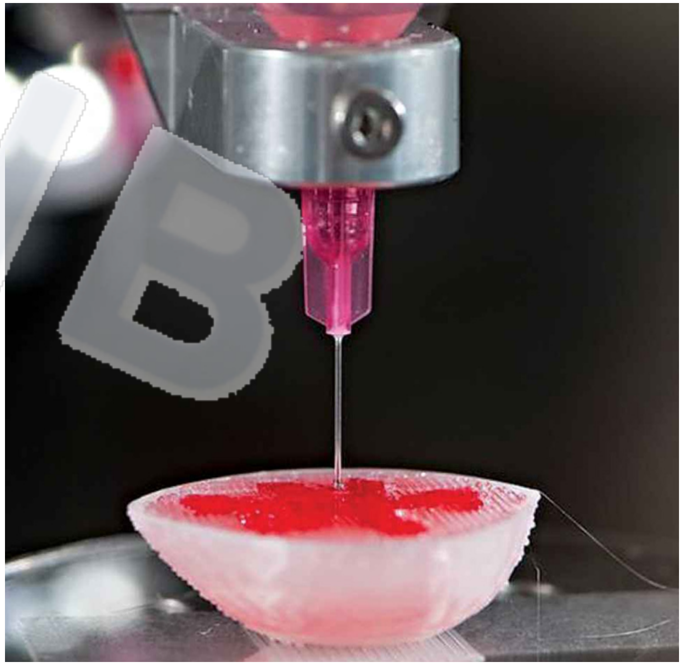


- Control over cell response *in vitro* and *in vivo*

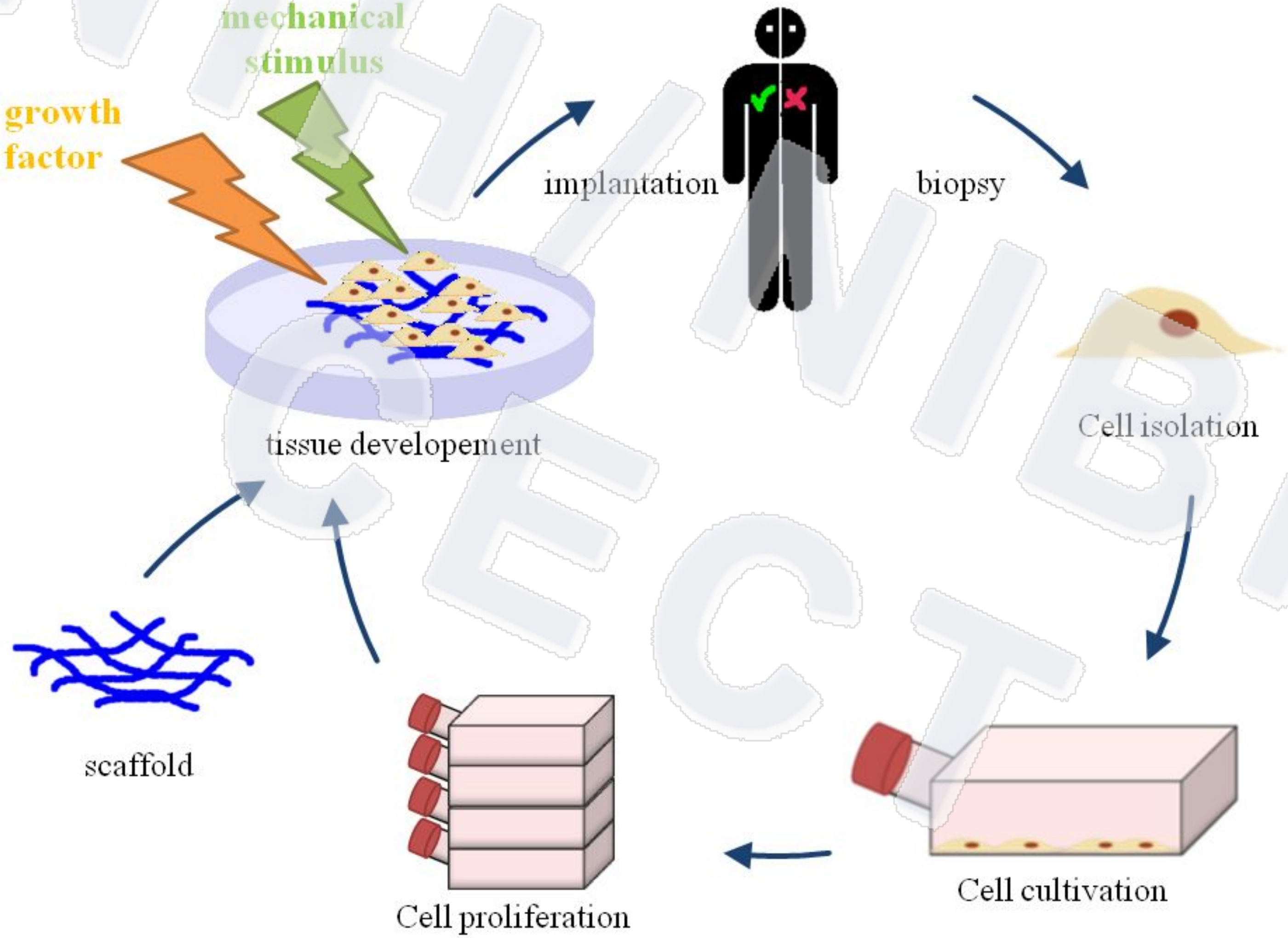
- Cell viability in larger constructs
  - Diffusion limitations
  - Vascularity

- Large constructs for critical-size defects

- Biomanufacturing limitations

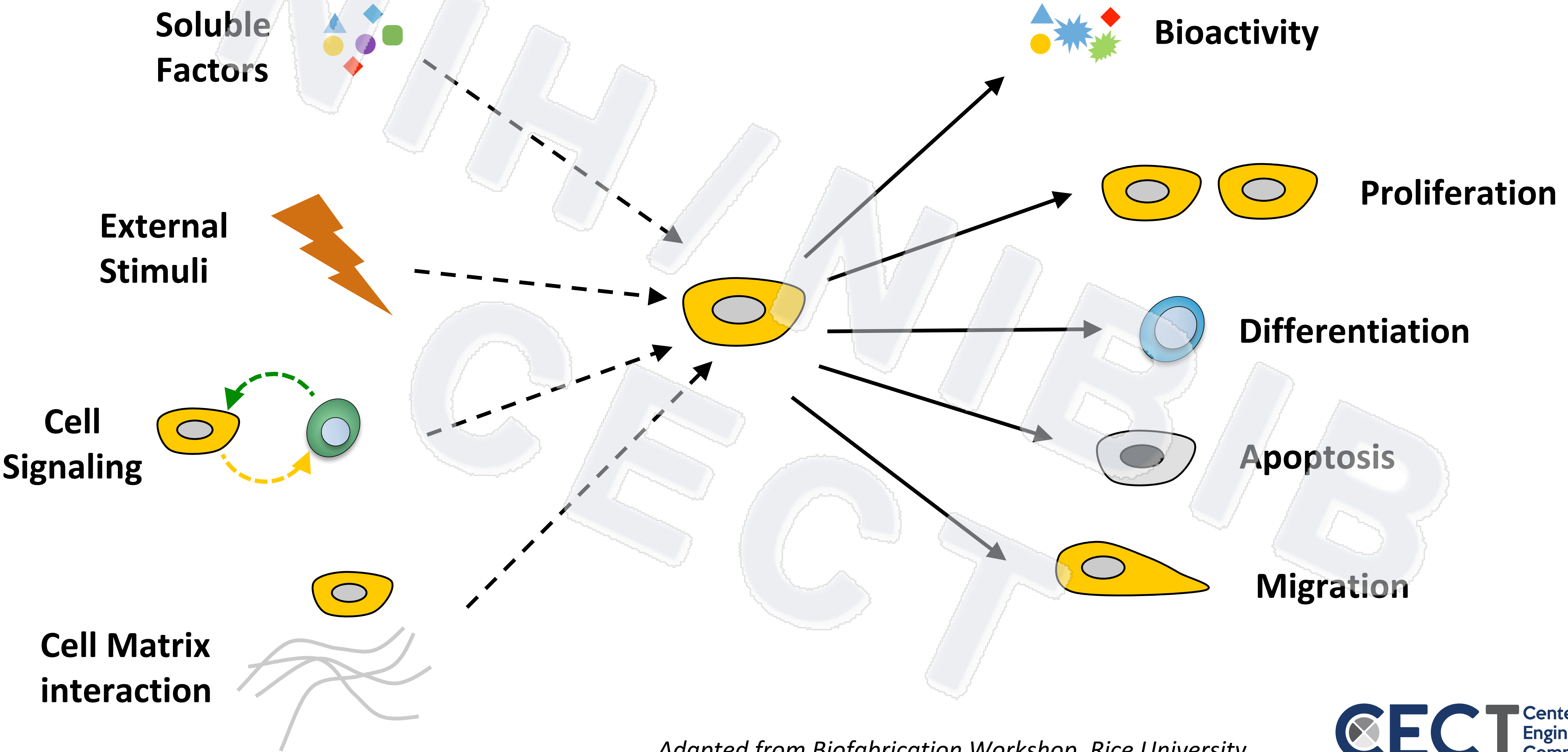


Tissue Engineering Approach



Illumin.usc.edu

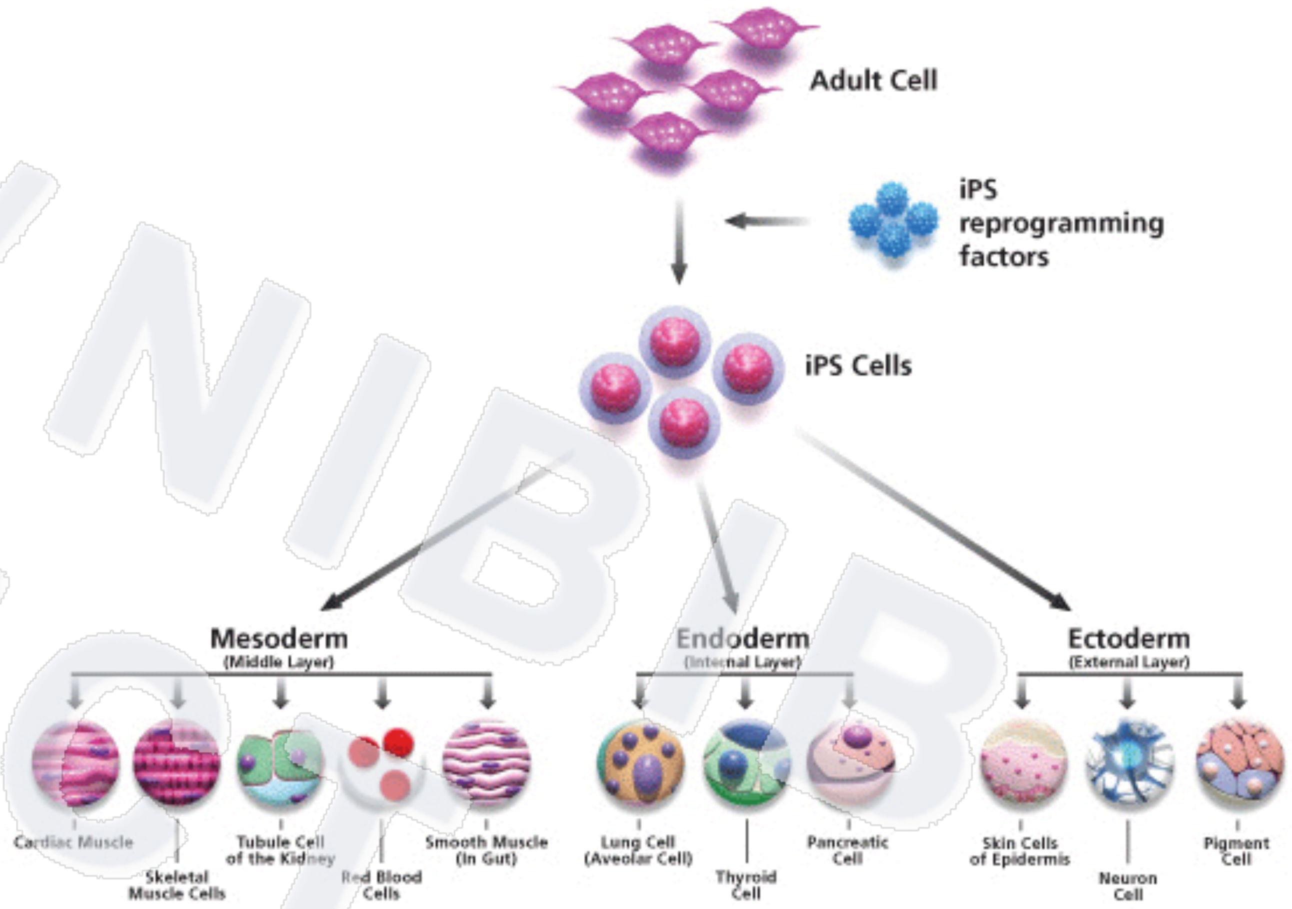
Cells



Adapted from Biofabrication Workshop, Rice University

Cells

- Cell types
  - Mature cells
  - Adult stem cells or somatic stem cells
  - Induced pluripotent stem cells
  - Embryonic stem cells
  - Totipotent stem cells
- Primary cells
  - Potential harvest challenges
  - Cells may be differentiated from patients
  - Age-related challenges
- Passaged cells
  - Serially expanded primary cells
  - May lose function or de-differentiate over passages
- Stem cells
  - Undifferentiated
  - Self-renewal
  - Source may be a challenge

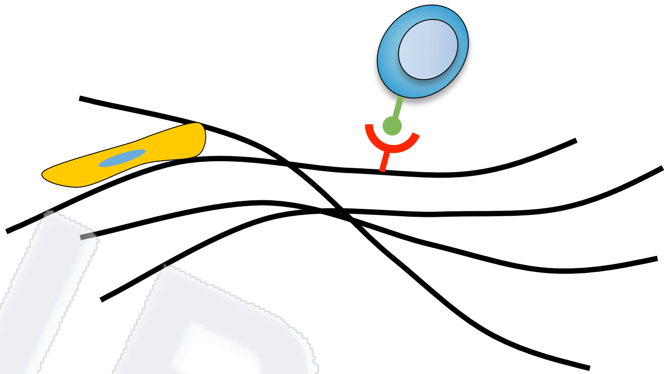


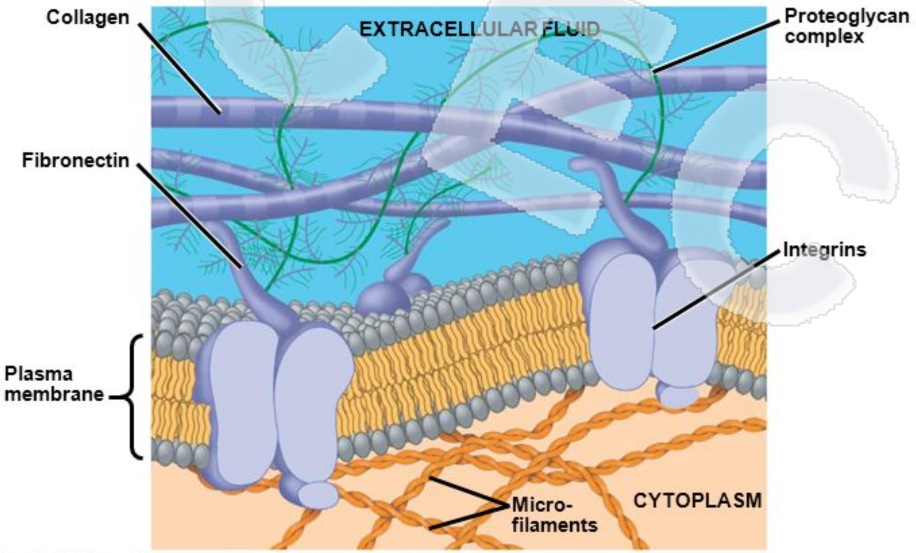
Adapted from Biofabrication Workshop, Rice University

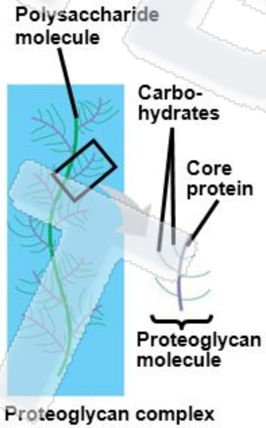
Extracellular Matrix (ECM)

- Macromolecules composed of
  - Proteins (collagen, fibronectin, laminin etc.)
  - Glycosaminoglycans linked to proteins (heparin sulfate, chondroitin sulfate etc.)

- Scaffold material that provides support for cell growth and function
  - Growth, differentiation, bioactivity



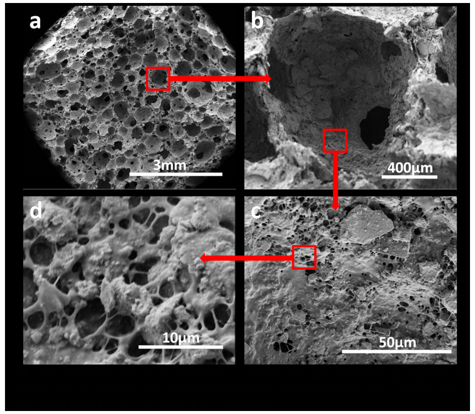




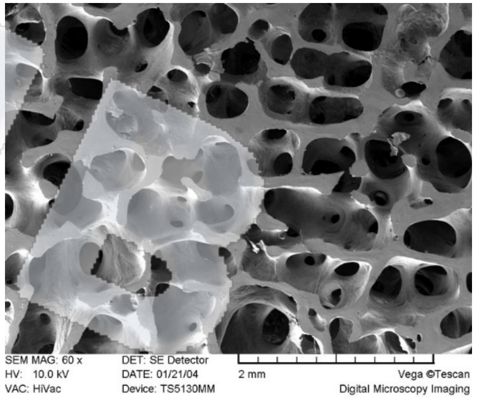
Scaffold Properties

- Bulk properties that correlate to the native tissue
  - Mechanical
  - Architectural
  - Chemistry

- Microstructural properties that dictate cell response
  - Pore size
    - Cell infiltration and surface mechanics
  - Porosity
    - Dictates mechanical properties, transport phenomenon
  - Fiber orientation
    - Dictates cell migration and growth



Above: porous hydroxyapatite/starch scaffold
Below: Human adult bone sample



Xu, et al. *World Biomaterials Congress*. 2016 and
Paul Hansma Research Group, 2018

Adapted from Biofabrication Workshop, Rice University

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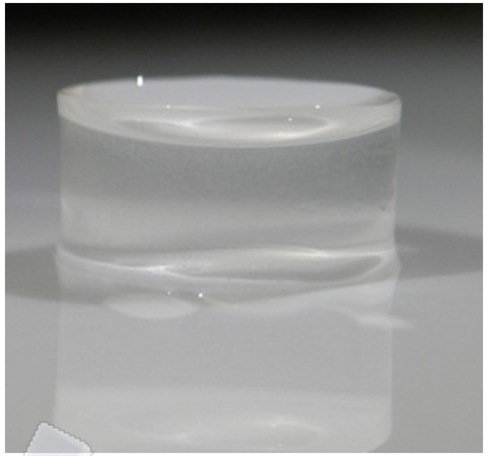
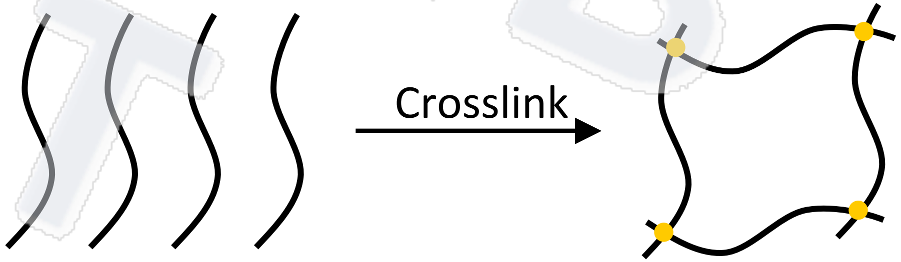
Hydrogels

- Polymeric chain network dispersed in an aqueous medium
  - Retains a high fraction of water compared to the polymer



- Individual polymer chains can be cross-linked to assemble and form a network
  - Thermal
  - pH
  - Chemical
  - Photo-sensitive

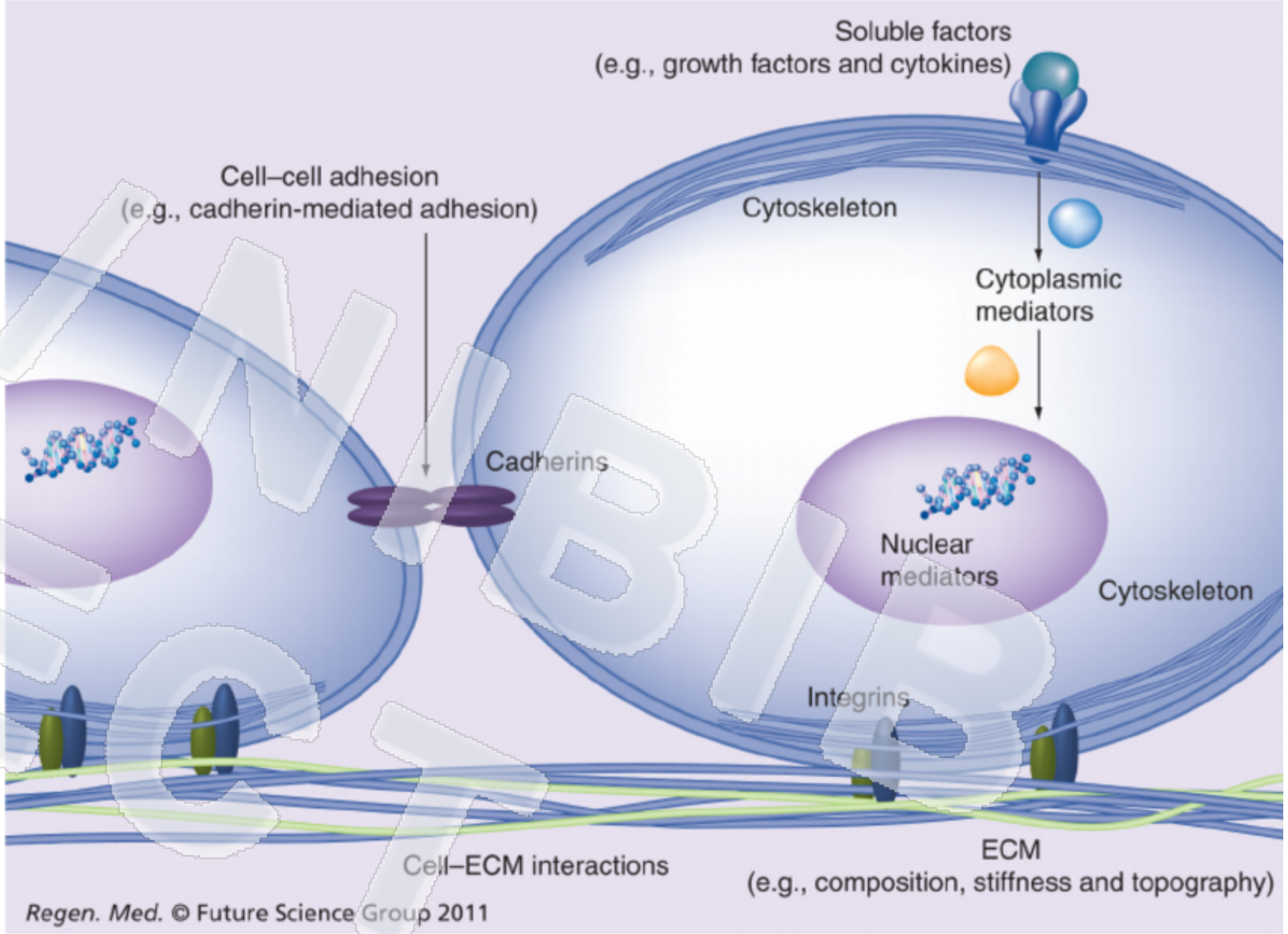


Scaffold examples

Natural		Synthetic	
Biomaterial	***Crosslinking Chemistry***	***Biomaterial***	***Crosslinking Chemistry***
Collagen	Thermal	Polyethylene glycol (PEG) and derivatives	Photo/chemical
Gelatin	Photo/chemical	Polycaprolactone (PCL)	Thermal
Alginate	Chemical (CaCl2)	Polylactic acid and derivatives	Thermal
Fibrin	Chemical (Thrombin)	Poly (propylene fumarate)	Photochemical
Hyaluronic Acid	Photo/chemical	Polyacrylamide	Photochemical
Decellularized ECM	Thermal		

Biomolecules

- Communication and molecular signaling conduit
- Cytokines
- Growth Factors and Receptors
- Cell adhesion molecules



Regen. Med. © Future Science Group 2011

Li et al., Regen Med. 2011 Mar;6(2):229-40

Biomolecules: Function-specific

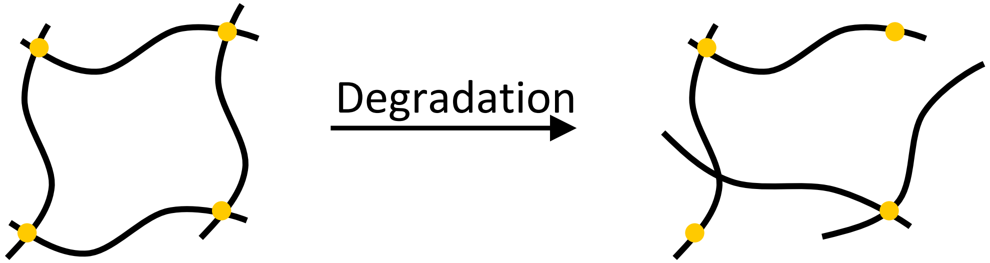
Abbreviation	Tissues treated	Representative function
Ang-1	blood vessel, heart, muscle	blood vessel maturation and stability
Ang-2	blood vessel	destabilize, regress and disassociate endothelial cells from surrounding tissues
FGF-2	blood vessel, bone, skin, nerve, spine, muscle	migration, proliferation and survival of endothelial cells, inhibition of differentiation of embryonic stem cells
BMP-2	bone, cartilage	differentiation and migration of osteoblasts
BMP-7	bone, cartilage, kidney	differentiation and migration of osteoblasts, renal development
EGF	skin, nerve	regulation of epithelial cell growth, proliferation and differentiation
EPO	nerve, spine, wound healing	promoting the survival of red blood cells and development of precursors to red blood cells.
HGF	bone, liver, muscle	proliferation, migration, differentiation of mesenchymal stem cells
IGF-1	muscle, bone, cartilage, bone liver, lung, kidney, nerve, skin	cell proliferation and inhibition of cell apoptosis
NGF	nerve, spine, brain	survival and proliferation of neural cells
PDGF-AB (or -BB)	blood vessel, muscle, bone, cartilage, skin	embryonic development, proliferation, migration, growth of endothelial cells
TGF-α	brain, skin	proliferation of basal cells or neural cells
TGF-β	bone, cartilage	proliferation and differentiation of bone-forming cells, anti-proliferative factor for epithelial cells
VEGF	blood vessel	migration, proliferation and survival of endothelial cells.

Adapted from Biofabrication Workshop, Rice University

Lee, et al. *J R Soc Interface*. 2011

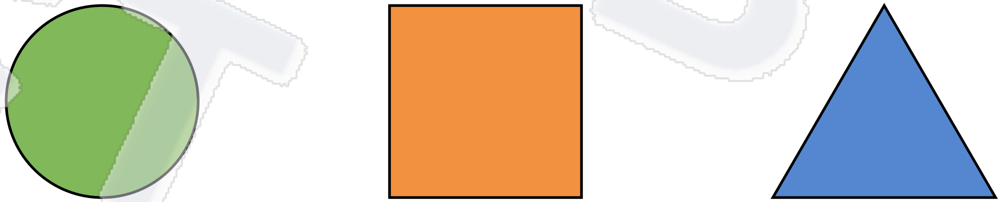
Other factors

- **Time**
  - Matrix degradation, remodeling



- **Physicochemical**
  - Shear forces, mechanical stresses, cyclic tension

- **Topography**
  - Curvature, roughness



Applications of TERM

- Promising *in vitro* platform to interrogate *in vivo* biology
  - Wealth of research exploiting TE capabilities

- Several clinical applications to date

Applications of TERM

- Dermal regeneration

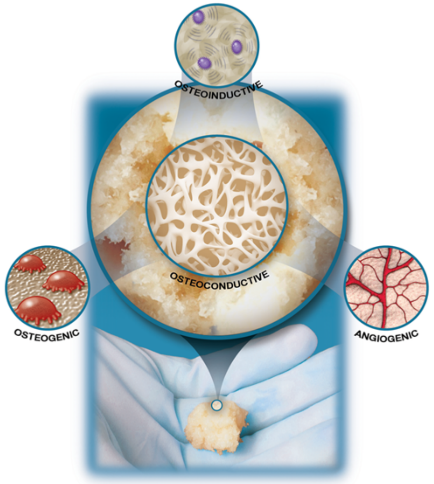
Integra ® Skin grafts

Brand	Scaffold material	Cells
Dermgraft® (Advanced Biohealing)	PGA, PLA, Silicon	Fibroblasts
Apligraf® (Organogenesis)	Collagen	Keratinocytes, Fibroblasts
Orcel® (Ortec Inc.)	Collagen sponge	Keratinocytes, Fibroblasts
Laserkin®, Hyalograft® (Fidia Adv. Bioploymers)	Hyaluronic acid	Keratinocytes, Fibroblasts

Applications of TERM

- Various bone/cartilage products
- Efforts to combine the right cellular, molecular and structural cues

Brand	Scaffold material	Application
Collagraft ® (Nuecoll Inc.)	Collagen, HA, B-TCP	Subchondral support
ChondroMimetic™ (TiGenix NV)	Collagen, calcium phosphate	Osteochondral
Gel-One® (Zimmer Biomet)	Hyaluronic acid	Osteoarthritis
TruGraft™ (Osteobiologics)	PLGA granulate	Bone void filler

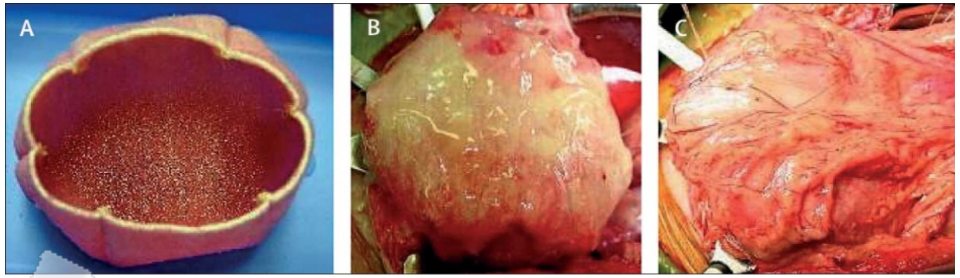


Osiris Therapeutics Bio$^{4®}$ bone matrix

Bond Apatite: bone graft cement (biphasic calcium sulfate and HA)

Applications of TERM

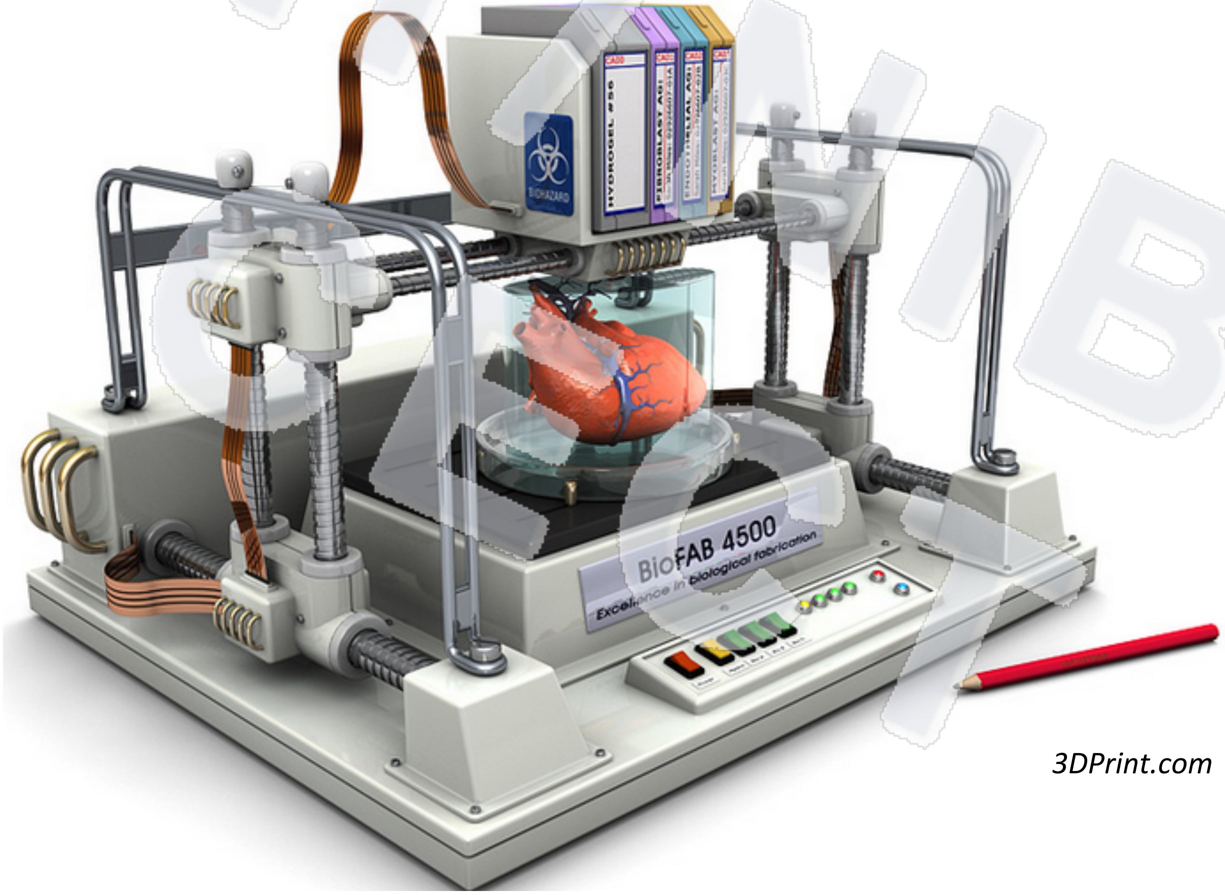
- Pioneering work by WFIRM on Bladder tissue engineering (2006)
  - Cells seeded on a biodegradable bladder-shaped scaffold made of collagen/PGA composite
- Tissue Engineered Tracheal replacement (2012)
  - Donor trachaeal scaffold with multiple cell/biomolecule stimulations
- On-going work with various other organs: cornea, blood vessels, liver etc.
- In a lot of cases, despite initial success, there was no long-term improvement



Atala et al., Lancet., 367 (9518) (2006), pp. 1241-1246

Elliott et al., Lancet, 380 (9846) (2012), pp 994-1000

3D Printing and Biofabrication



3DPrint.com

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Emergence of 3D Printing in Healthcare and Medicine

- **Dentistry** (restorations, dental models)
- **Tissue models** (implantation, drug testing)
- **Surgery** (maxillofacial, cranial, cardiovascular)
- **Medical devices** (surgical instruments, prostheses, hearing aids)
- **Drug formulations** (drug delivery, personalized medicine)

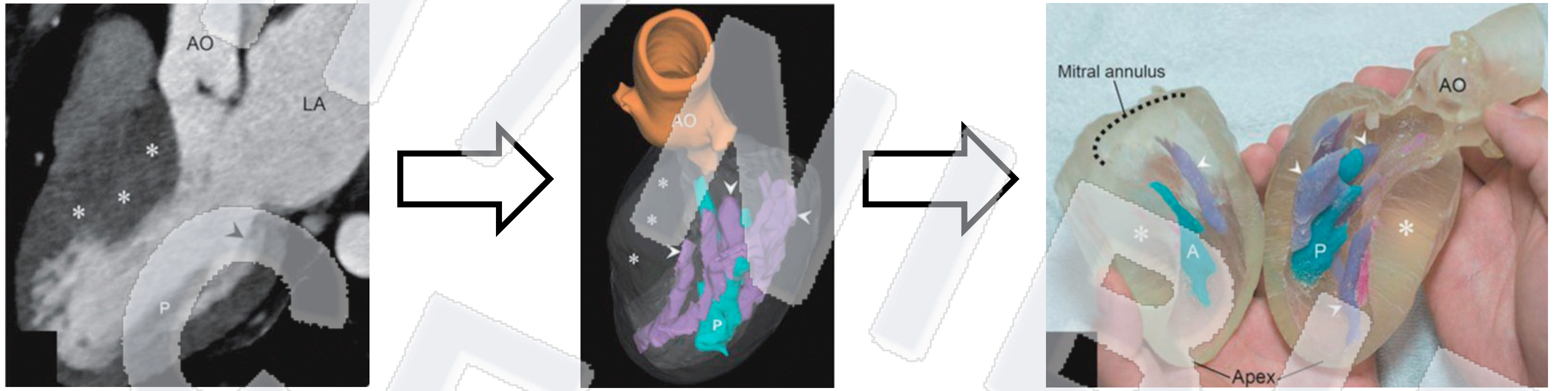
Biofabrication 9:024102 (2017)

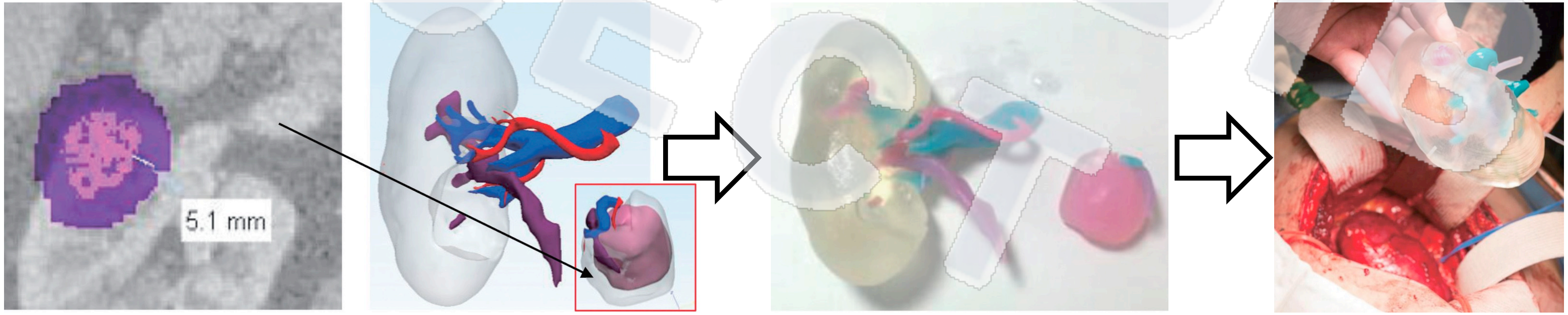
3D Printing & Patient Specific Treatments

Enable **personalized treatments** and account for **patient-specific anatomies**

3D phantoms for surgical planning and to improve outcomes

CT image
(cardiac)





CT image and model of renal carcinoma
and safety margin

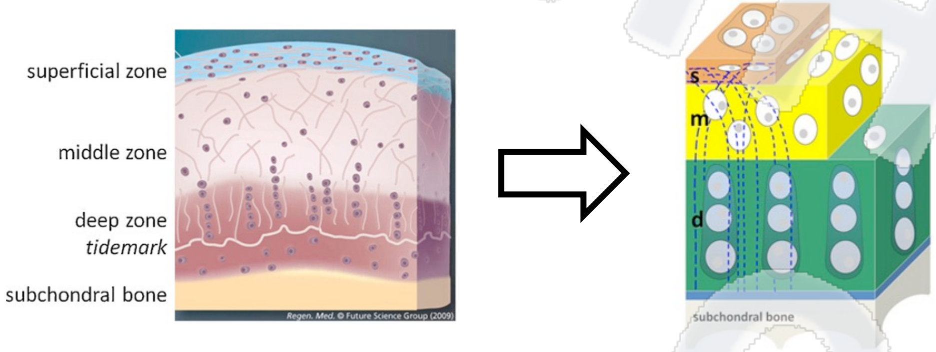
Images adapted from Korean J Radiol
2016;17(2):182-197

26% Growth
Over 27
Years Into A
$5 Billion
Market

3rd Largest
Sector In
Additive
Manufacturing

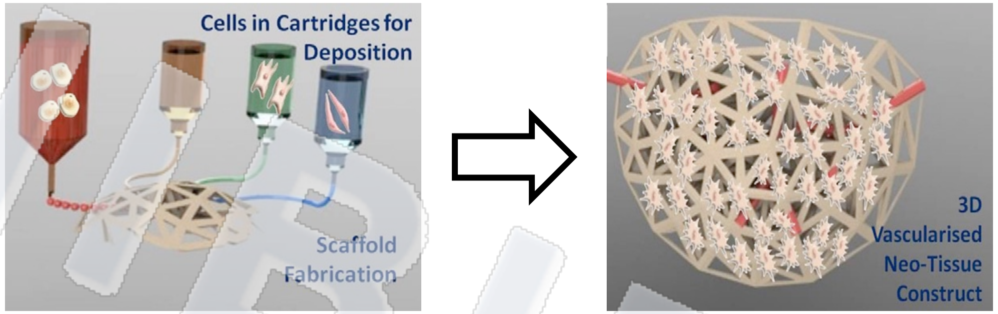
3D Printing & Tissue Engineering

Fabrication of heterogeneous native cellular environments

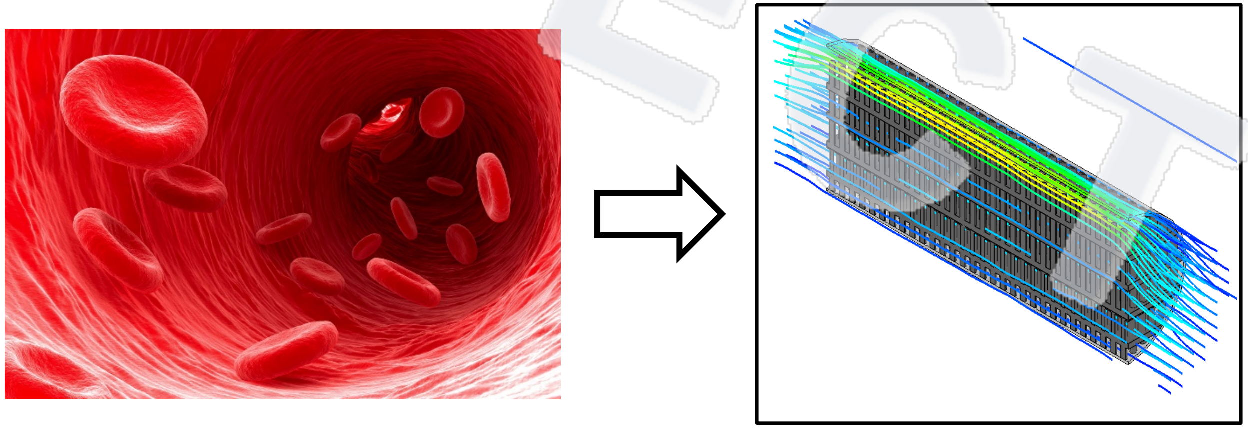


Melchels et al., Progress in Polymer Science 27 (2012) 1079-1104

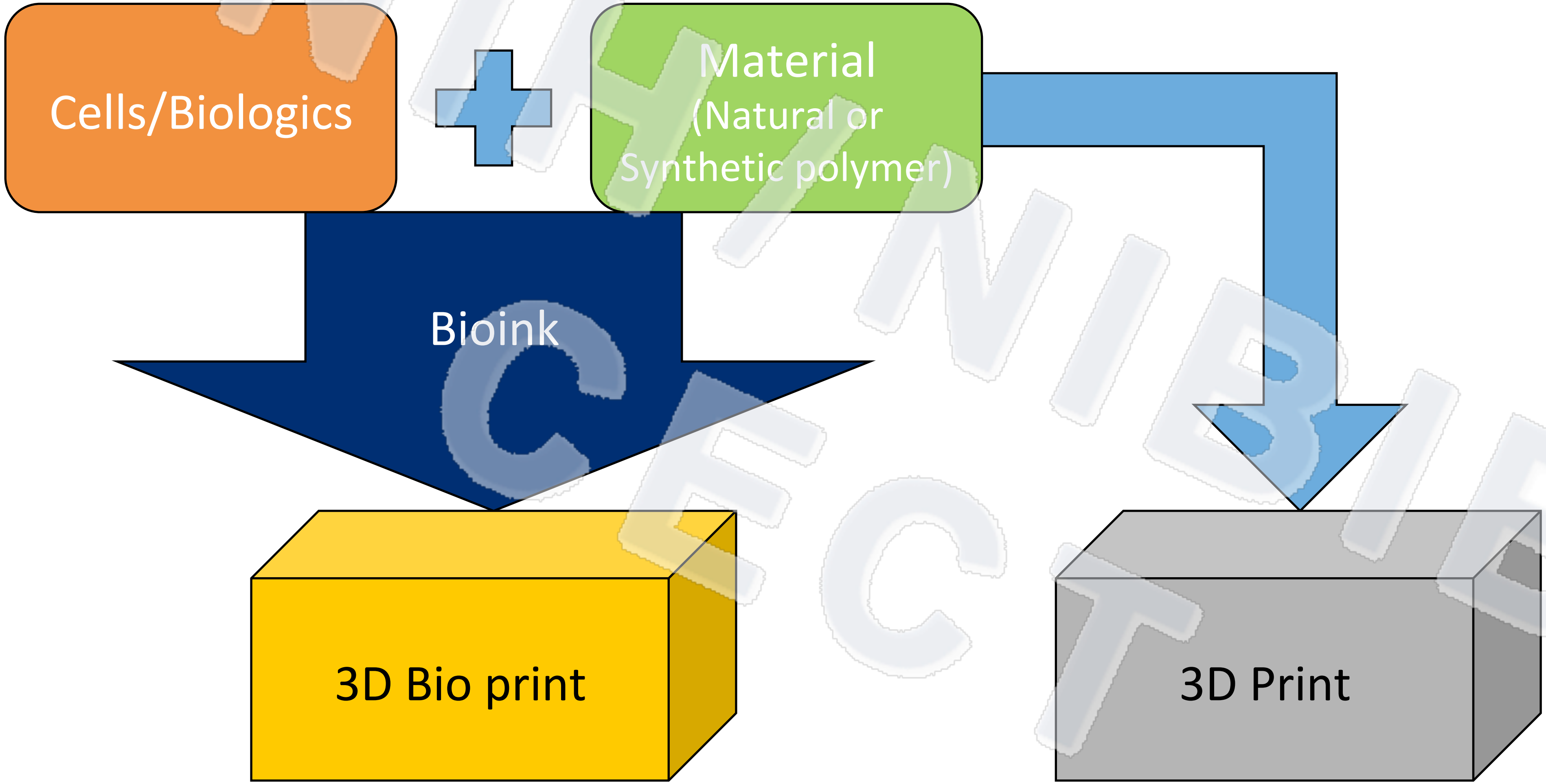
Tissue models for investigative biology



Recapitulate native microenvironment



Bioprinting



Cells/Biologics

Material
(Natural or
Synthetic polymer)

Bioink

3D Bio print

3D Print

3D Printing techniques

Light-based

- Stereolithography (SLA)
- Digital Light Projection (DLP)
- Laser-Induced Forward Transfer (LIFT)
- Selective Laser Sintering (SLS)

Extrusion-based

- Fused Deposition Modeling (FDM)
- Microextrusion printing

Inkjet

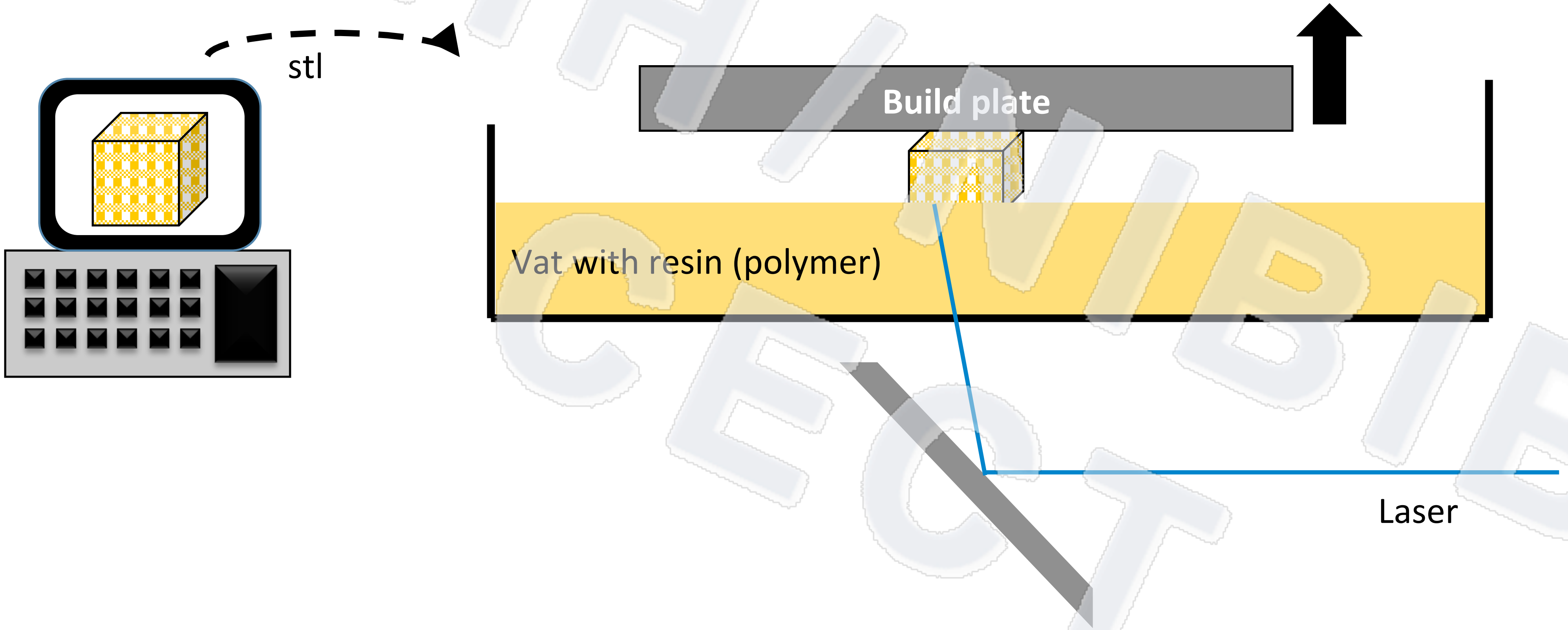
- Drop-on-demand
  - Piezo electric
  - Thermal
- Continuous

Others

- Electrospinning
- Scaffold-free

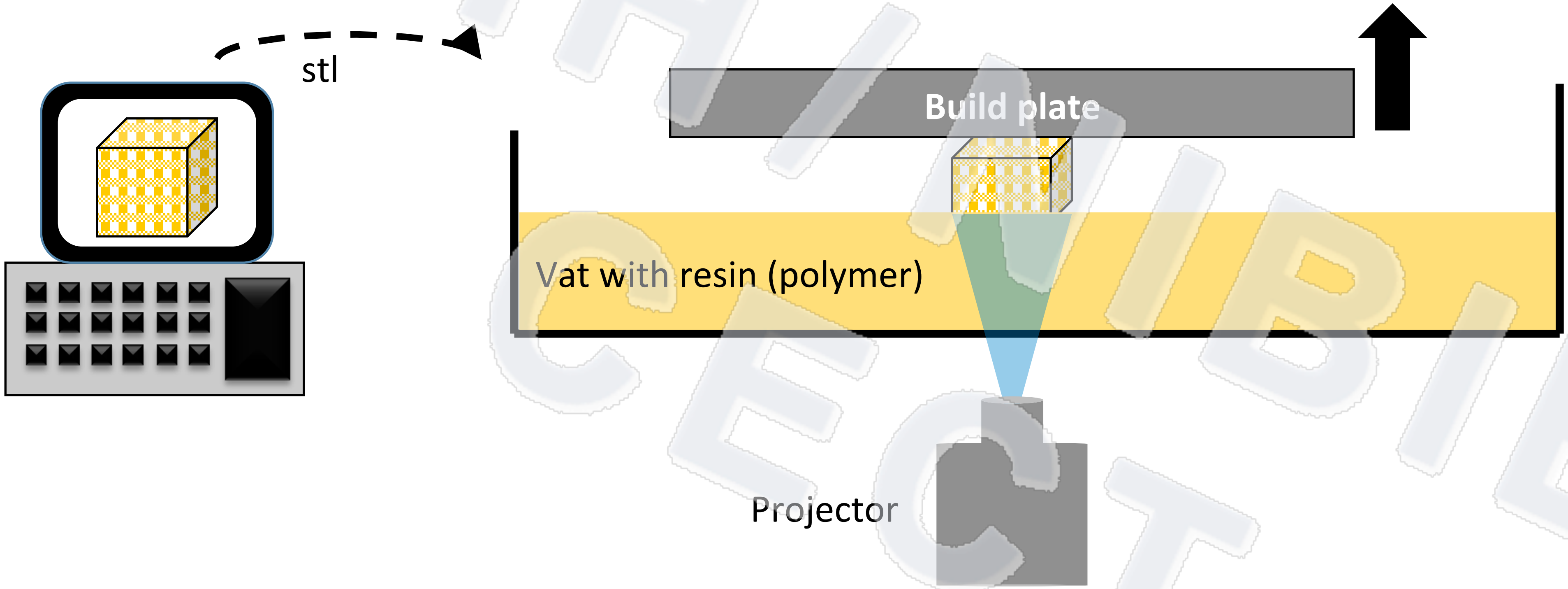
Vat Photopolymerization

Stereolithography (SLA)



Vat Photopolymerization

Stereolithography (SLA)



Vat Photopolymerization

Advantages

- High resolution (~20 µm)
- Controllable crosslinking to tailor mechanical properties
- Compatible with photopolymerizable materials

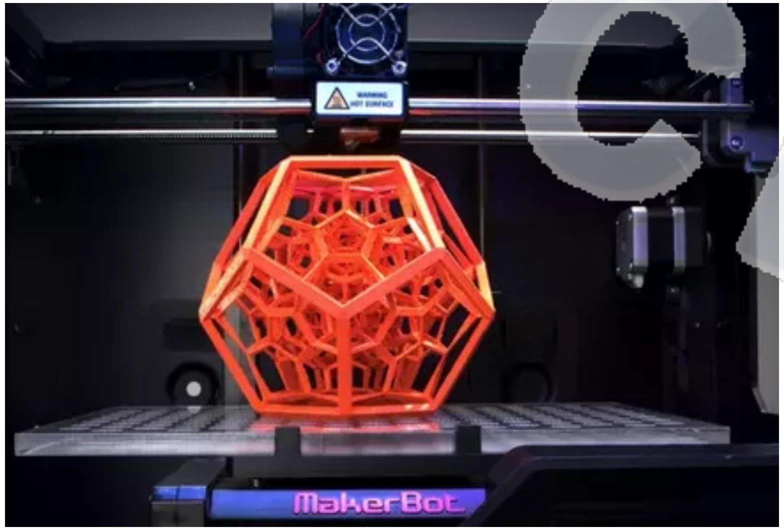
Disadvantages

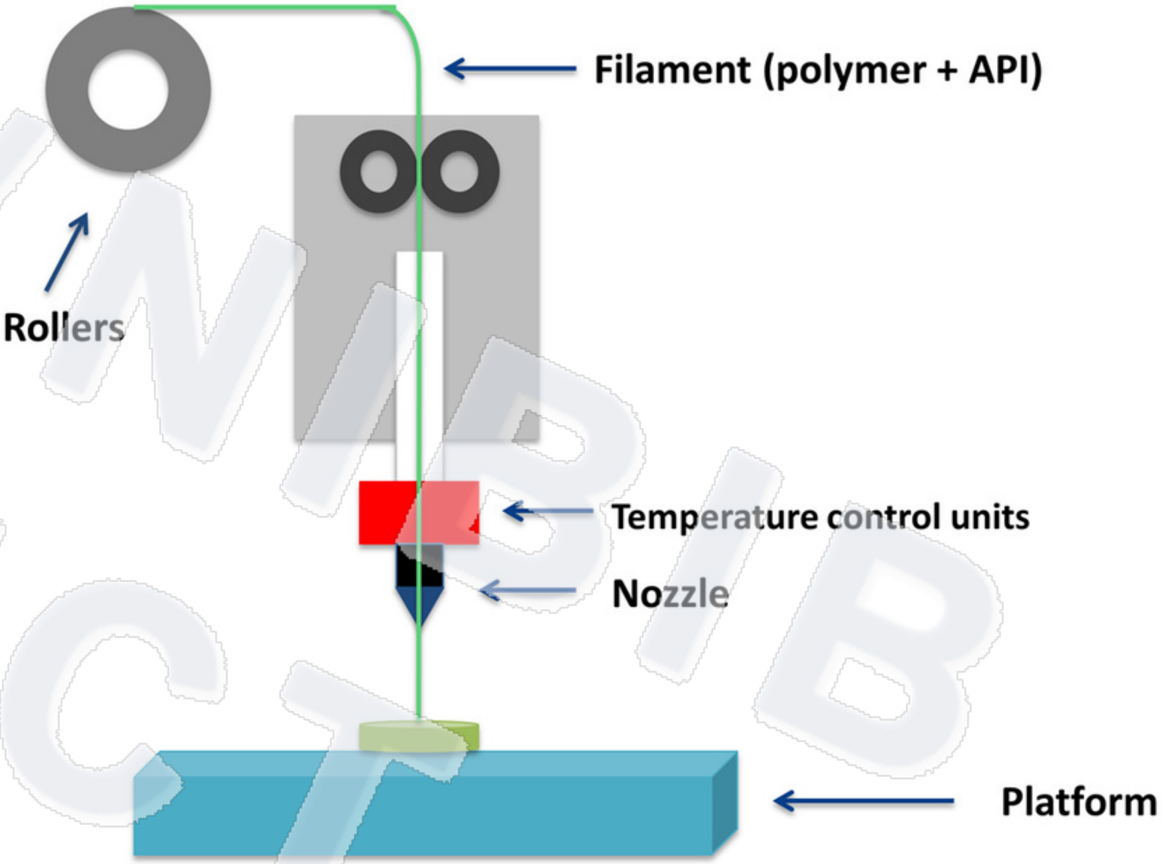
- Slow fabrication (hours) and requires support structures
- Photoinitiators/inhibitors are detrimental to cell viability
- Not always cell compatible
- Typically single-material
- Requires post-fabrication processing

Extrusion-based printing

Fused Deposition Modeling

- Thermoplastics (T_m ~ 200 °C)
- Layer-by-layer print



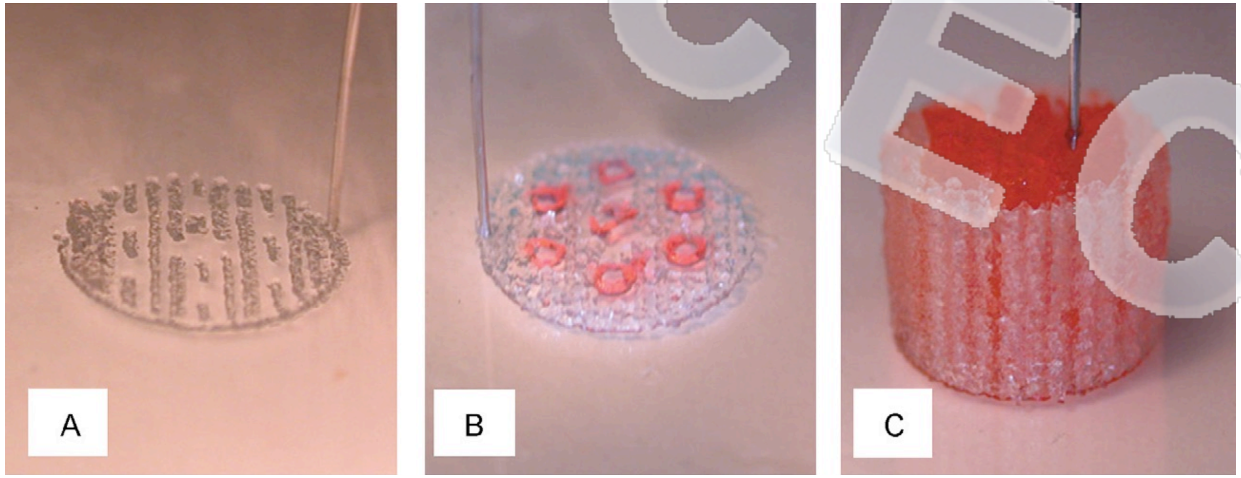


Konta et al., Bioengineering 2017, 4, 79

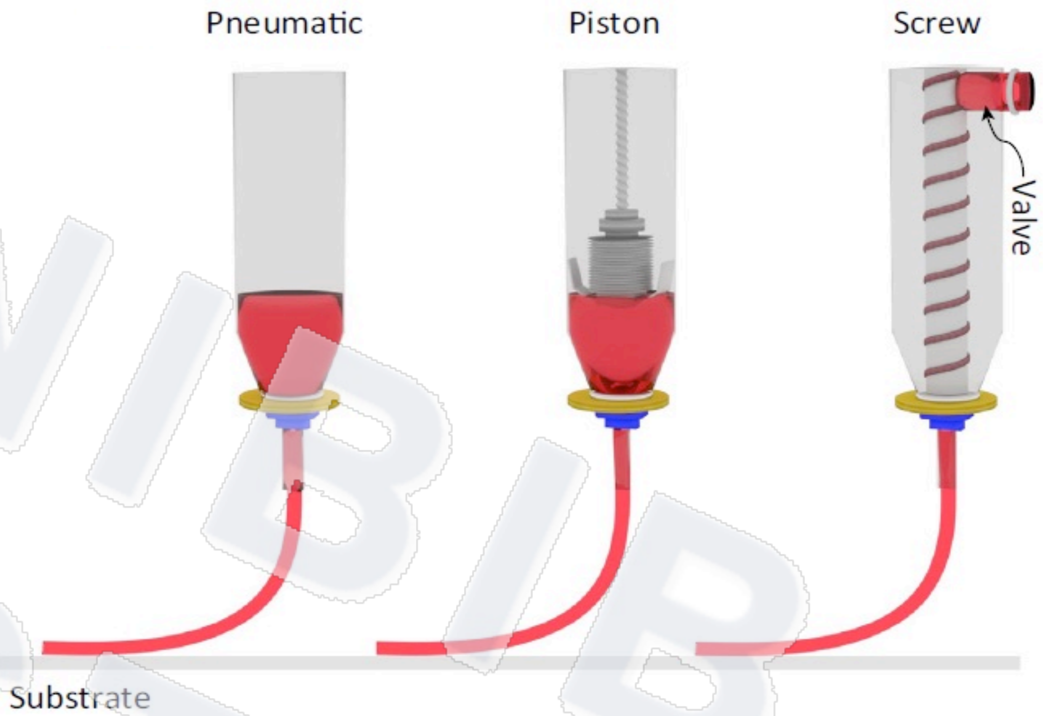
Extrusion-based printing

Microextrusion Printing

- Varying needle diameters
- Wide range of materials
  - High viscosity but ideally shear thinning



F.P.W. Melchels et al. / Progress in Polymer Science 37 (2012) 1079– 1104

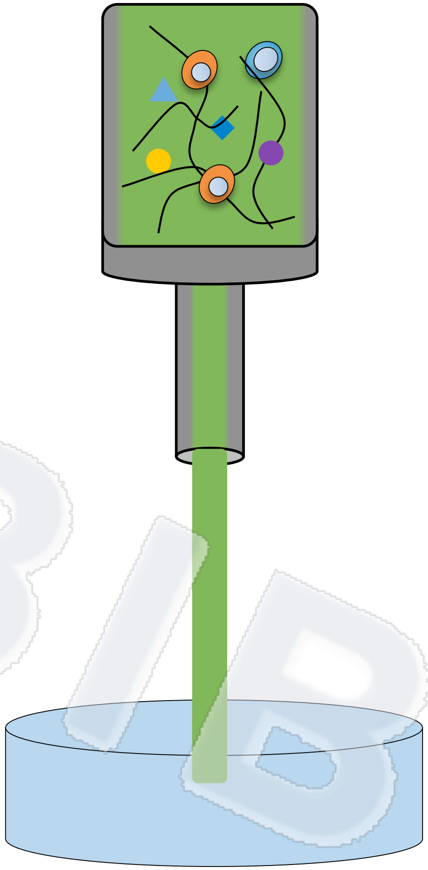


Knowlton et al., Trends in Biotechnology, 2015, Vol. 33, No. 9

Extrusion-based printing

Microextrusion Printing

- A method of crosslinking is essential if the individual polymer strands will not fuse
- Photo-induced
  - GelMA – photoinitaitor
- Chemical
  - Alginate – CaCl2
  - Gelatin – Transglutaminase
- Thermal
  - Collagen



Extrusion-based printing

Advantages

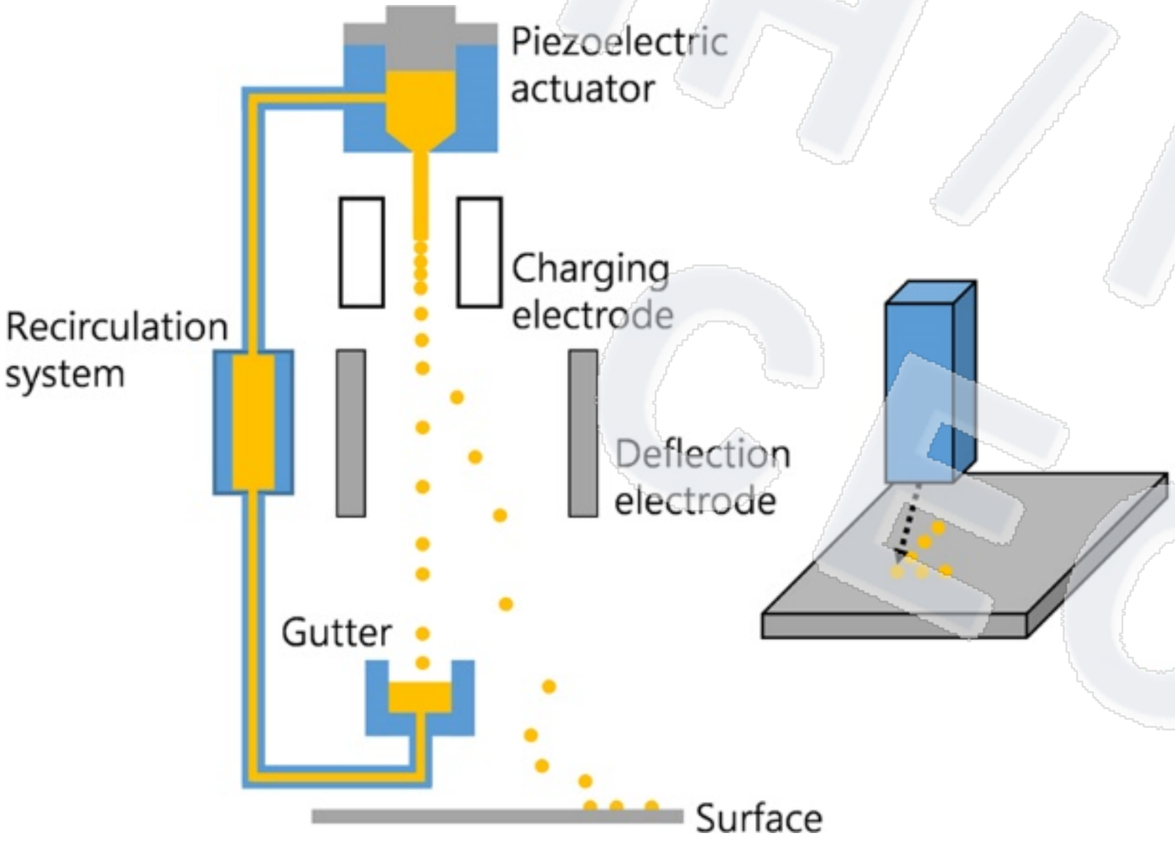
- Ability to deposit large cell populations in a spatially controlled manner
- Very fast fabrication (minutes) and broad range of possible materials
- Capable of different crosslinking techniques: access to larger library of materials
- Multi-nozzle printing enables multi-material printing with varying properties

Disadvantages

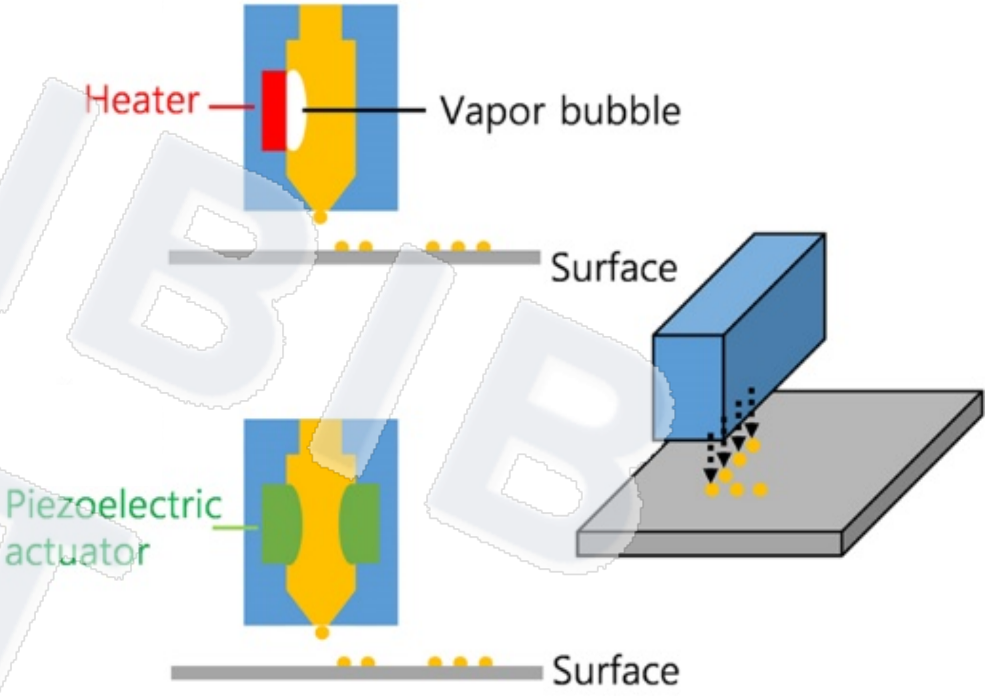
- Modest resolution (~100 µm)
- Limited viscosity range of materials
- The high shear stresses within the printing nozzle can be deleterious for cells
- Customization required for each material type

Inkjet Printing

Continuous



Drop on demand



Cho at el., Inkjet-based 3D printing, IOP Science, 2015

Inkjet Printing

Advantages

- Typically low cost
  - Commercial inkjets modified
- Bioinks with low viscosity or cells w/media can be printed with reliable accuracy
- Cell-friendly

Disadvantages

- Cell membrane damage
- Bioinks require low viscosity
- Requires rapid gelling or support substrate

Summary

	Biomaterials	Cell viability / resolution	Speed	Cost	Advantages	Disadvantages
Inkjet	Low-viscosity suspension of biologics	~90% 20 – 100 um	Fast	Low	• High resolution, speed • Concentration gradients	• Poor vertical structure incorporation • Limited bioinks
Pressure-driven	Hydrogels, select thermoplastics	40 – 80% >100 um	Slow	Medium	• Many bioinks available • Broad operating ranges	• Gelation limitations • Shear stress
Laser-assisted	Hydrogel, media, cells, proteins	>95% >20 um	Medium	High	• Nozzle-free, non-contact • High precision	• Slow • Requires metal film • Limited materials
Stereolithography	Light-sensitive polymers, curable acrylics	>90% ~12 – 200 um	Medium	Low	• High accuracy • Many available resins	• Photopolymerizable-only • Issues with cell viability • Post-processing

Li et al. J Transl Med (2016) 14:271

Other methods

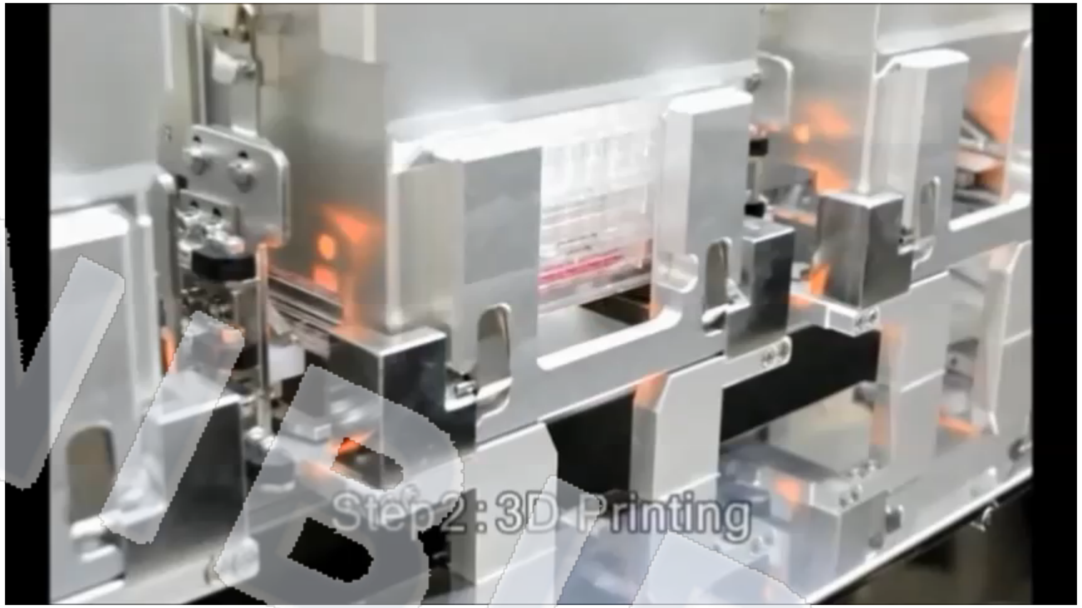
LIFT (Laser Induced Forward Transfer)

Laminate Object Manufacturing (LMO)

Micro and Nano-scale printing (**Nanoscribe**)

Scaffold-free fabrication

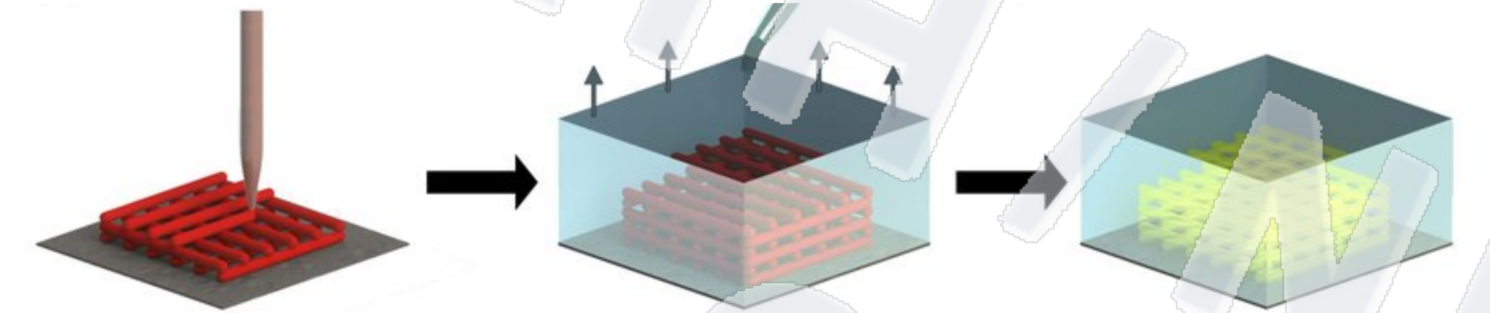
- No biomaterial/ECM for cell support
- Kenzan method for spheroid-based 3D Printing
  - Fusion of cell spheroids on a needle array Cell-secreted EXM and biomolecules
- High cell density applications



Sacrificial templating

A material that is 3D printed (either for support or as a feature), along with the bioink of interest, only to be removed upon completion of the print

- Pluronic F-127, Glass carbohydrate

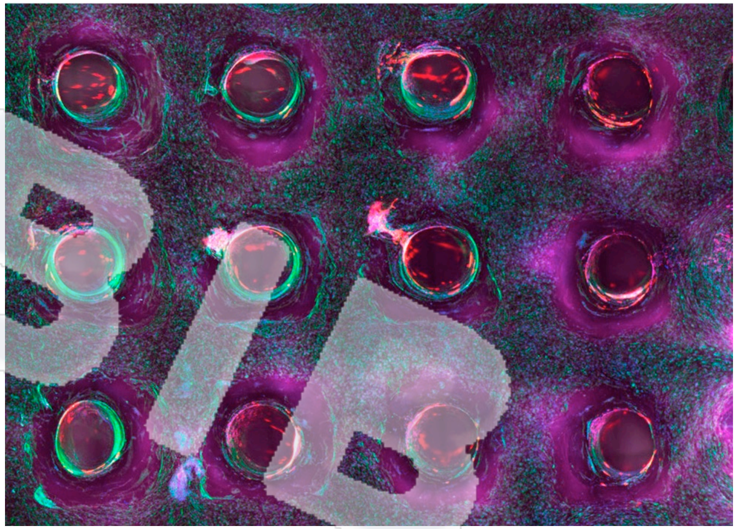


self-supported structures printed from sacrificial inks

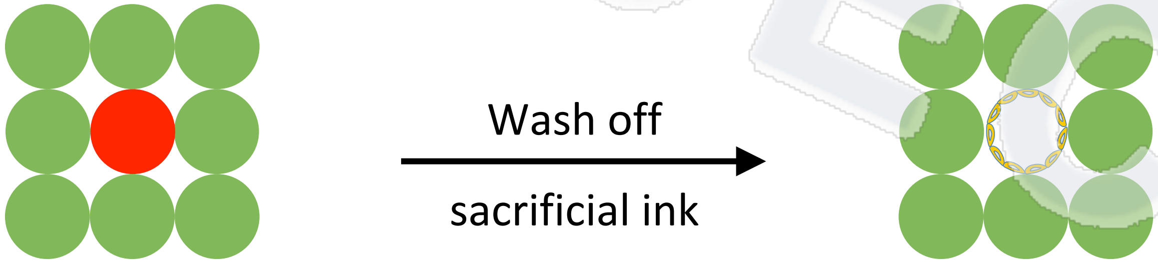
liquid precursor of permanent structure permeates and surrounds sacrificial structure

permanent structure solidifies and sacrificial structure removed

O'Bryan et al., *MRS Bulletin, 42(08):571-57*



Kolesky et al., PNAS 113 (12); 3179-3184 (2016)



Print desired bioink/sacrificial ink

'Cavity', infused with cells

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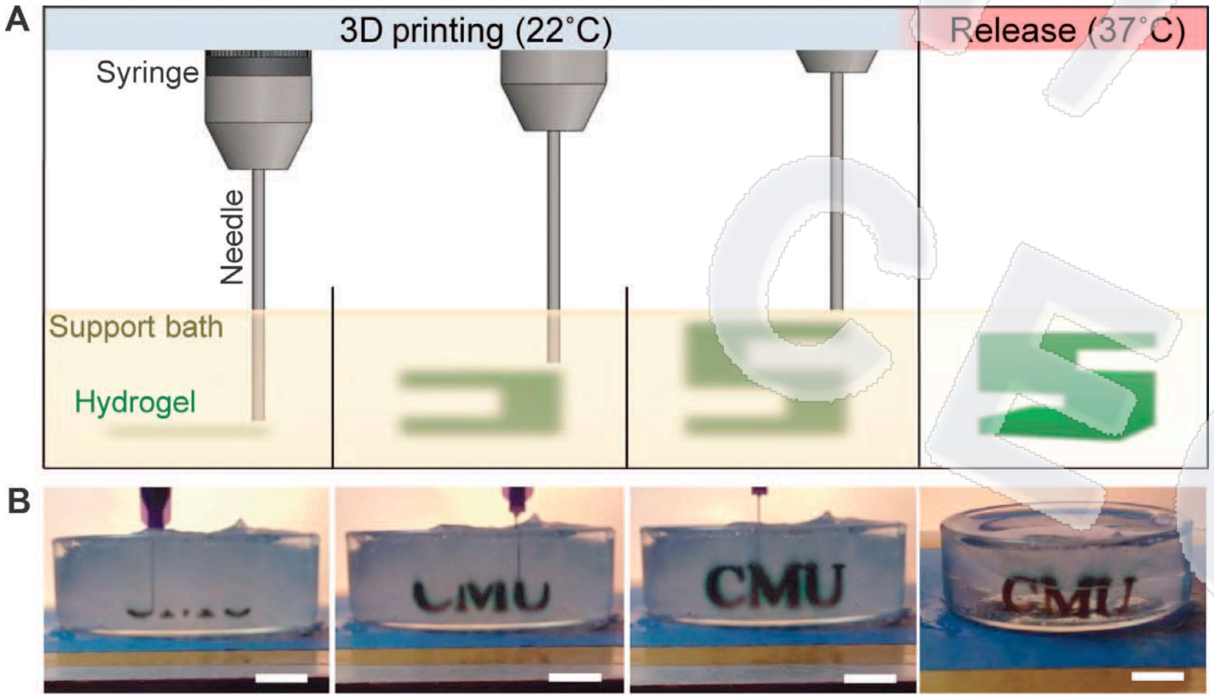
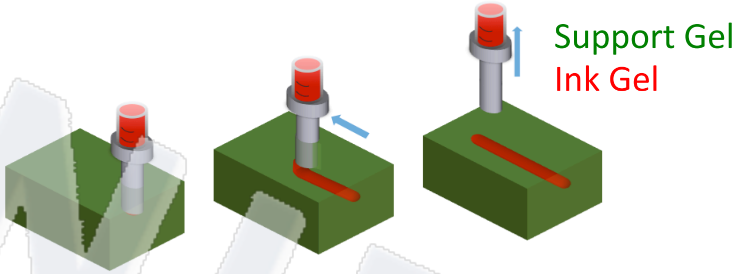
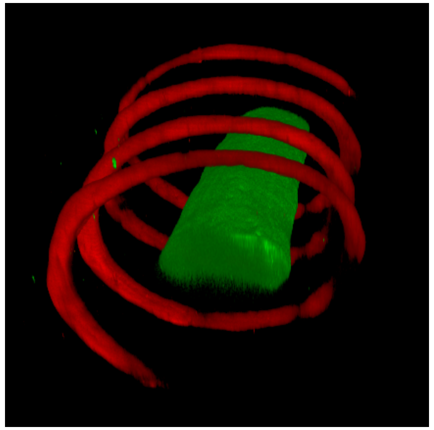
Gel-in-gel printing

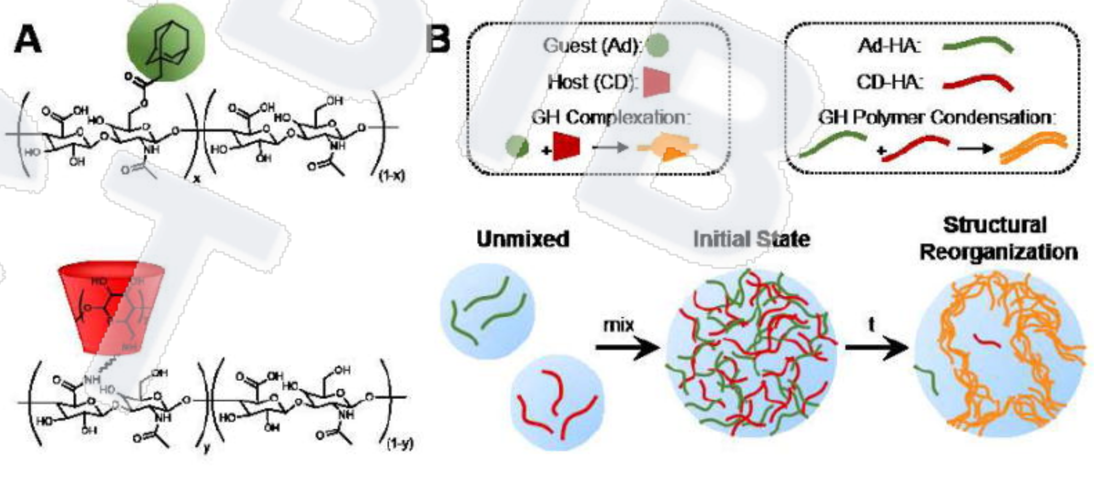
- Extruding a bioink into another support bioink/bath for structural stability
  - Gelatin microparticles
  - Guest-host complexes



Hinton et al. Sci. Adv. 2015;1:e1500758

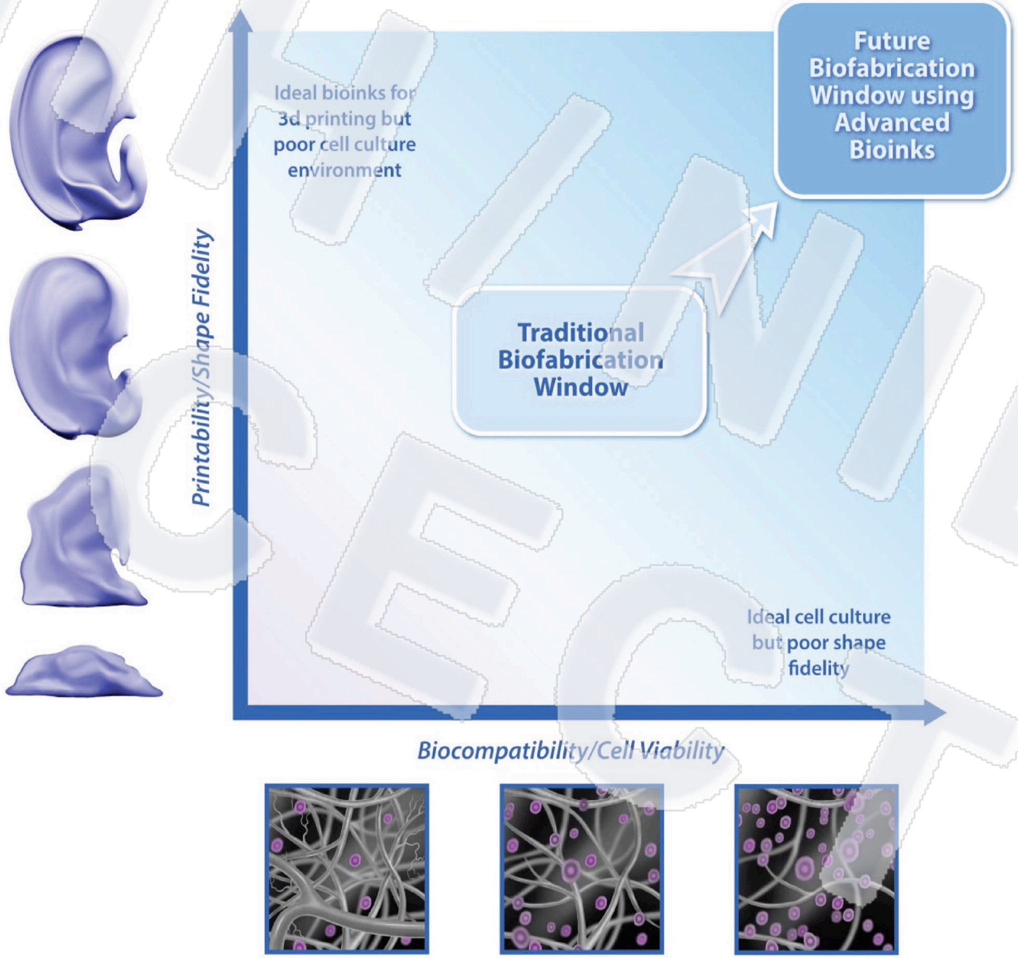






Soft Matter, 2016, 12, 7839

Tradeoffs while printing



Kyle et al., Adv. Healthcare Mats. 2017, 6, 1700264

Tissue Engineering & Biomaterials Laboratory

3D Printing & Bioprinting for TE/RM

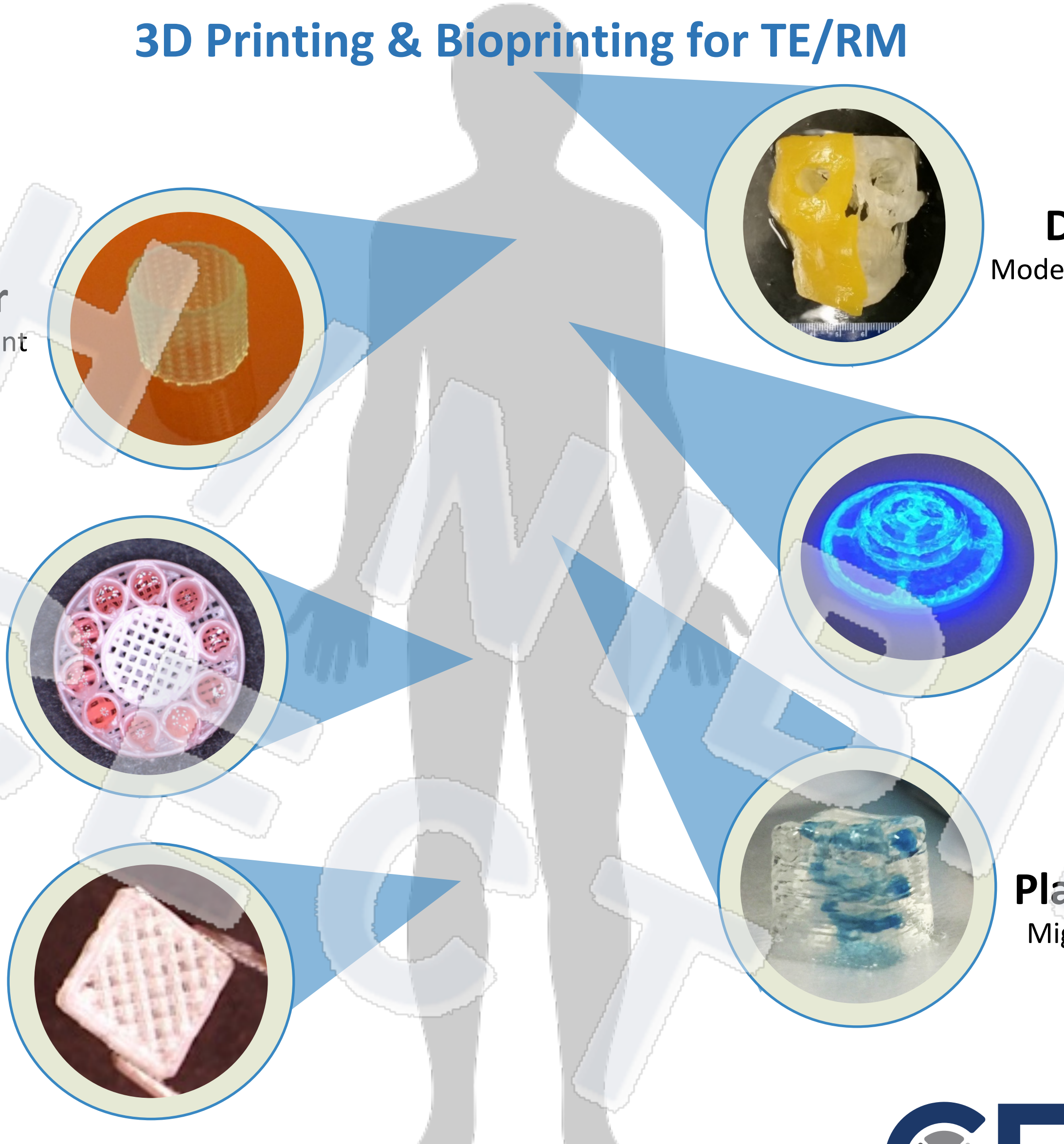
3D Printers


Bioplotter


Perfactory


6-axis BioBot

Tissue Regeneration & Disease Models



Cardiovascular
Grafts & Cell Recruitment

Bone
Bioprinted Osteons

Cartilage
3D Printed
Acellular Scaffolds

Dermal Tissue
Model System & Replacement

Nipple-Areola Complex
Bioprinting with Defined,
Dynamic Structure

Placental Tissue
Migration & Transport
Models

Selected References
Biotechnology & Bioengineering 116, 181-192 (2019)
Biomaterials 185, 219-231 (2018)
Tissue Engineering Part A 24, 1715-1732 (2018)
Biomacromolecules 18, 3802-3811 (2017)
Advanced Healthcare Materials, 319-325 (2016)
Advanced Materials 27, 138-144 (2015)

John P. Fisher
Fischell Family Distinguished Professor & Department Chair
Fischell Department of Bioengineering, University of Maryland

Center for Engineering Complex Tissues
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Questions?