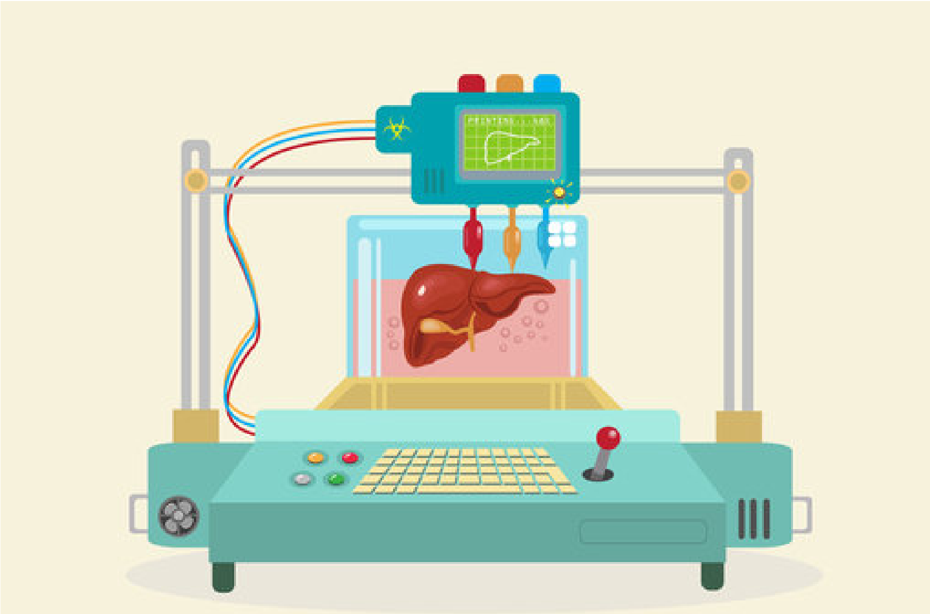


Adobe Stock

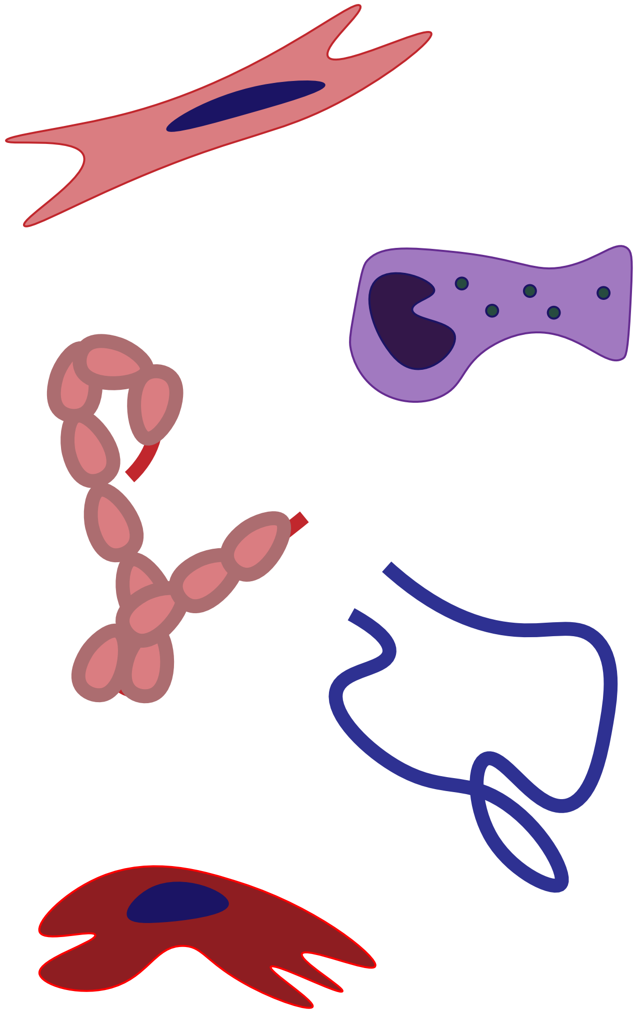
3D Printing with Cells & Biologics

Sarah Van Belleghem, PhD
3D Printing & Biofabrication Workshop
Fischell Department of Bioengineering

November 13, 2020

Center for Engineering Complex Tissues
University of Maryland

Cells & Biologics



<u>*Cells play a critical role in promoting tissue healing and regeneration*</u>

Variety of sources have been investigated: autologous, allogeneic, and xenogeneic

<u>*Biomolecules are an essential component of all tissue-engineered constructs*</u>

Play key roles to guide and regulate cell response, both in vivo and in vitro

Large number of biomolecules have been explored to induce tissue regeneration:

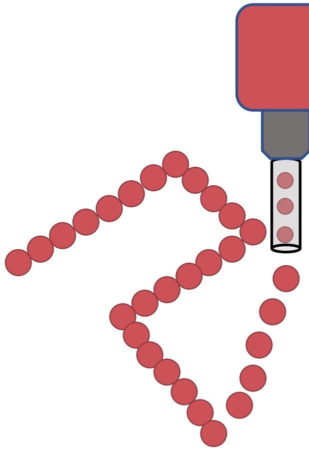
Small Molecules
Corticosteroids, hormones

Proteins/Peptides
Mitogens, morphogens, growth factors, cytokines

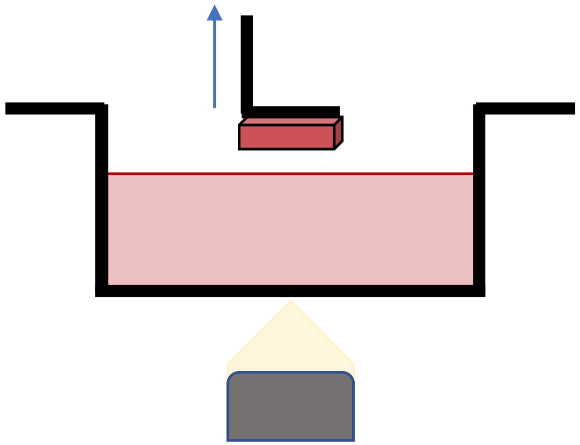
Oligonucleotides
DNA or RNA

3D Printing
Cells & Biologics

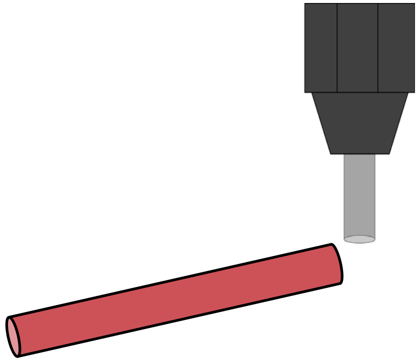
Inkjet
Small volume deposition



Stereolithography
Photopolymerizable vat



Extrusion
Continuous filament deposition



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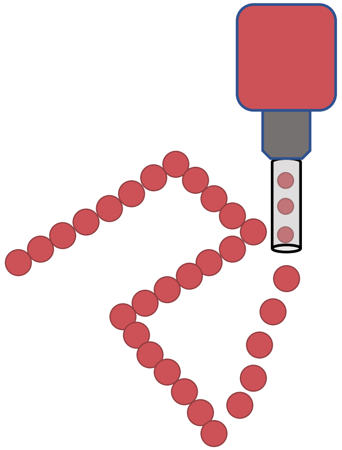
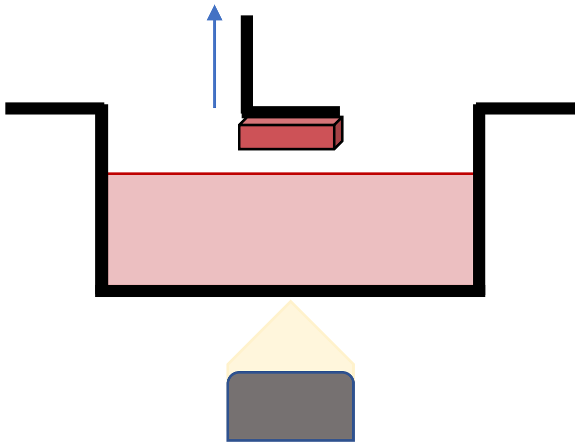
3D Printing
Cells & Biologics

Inkjet
Small volume deposition



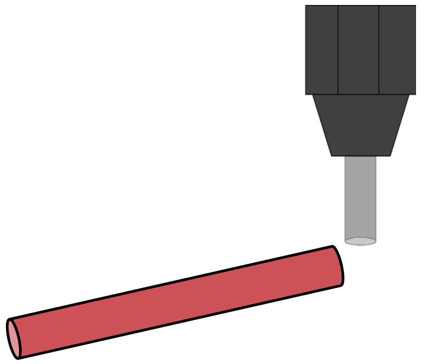
Instant heat exposure
Extreme shear stress
Cell damage
Newtonian Fluid
Limits cell density
Limited print size

Stereolithography
Photopolymerizable vat



Single material printing
Low material versatility
Excessive light exposure
Low cell viability
Waisted material
Lengthy print time

Extrusion
Continuous filament deposition

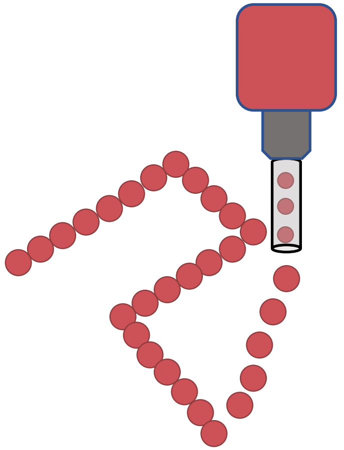


Limited printing resolution
Shear-thinning inks
Bioink Limitations

3D Printing
Cells & Biologics

Inkjet

Small volume deposition



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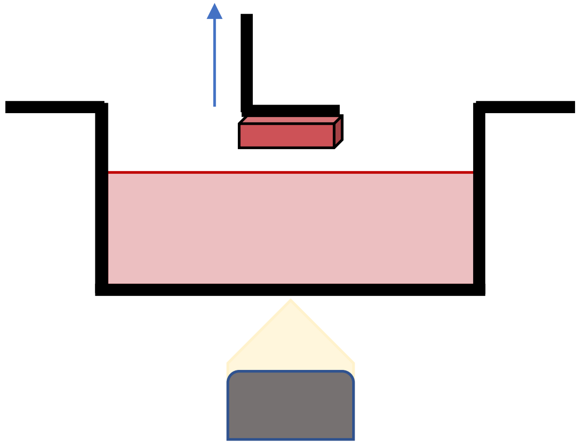

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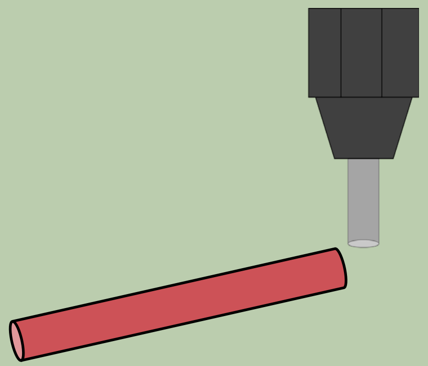

Limited printing resolution
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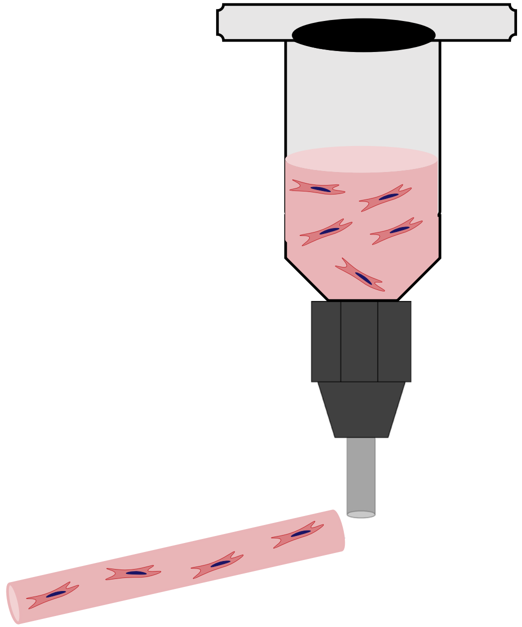
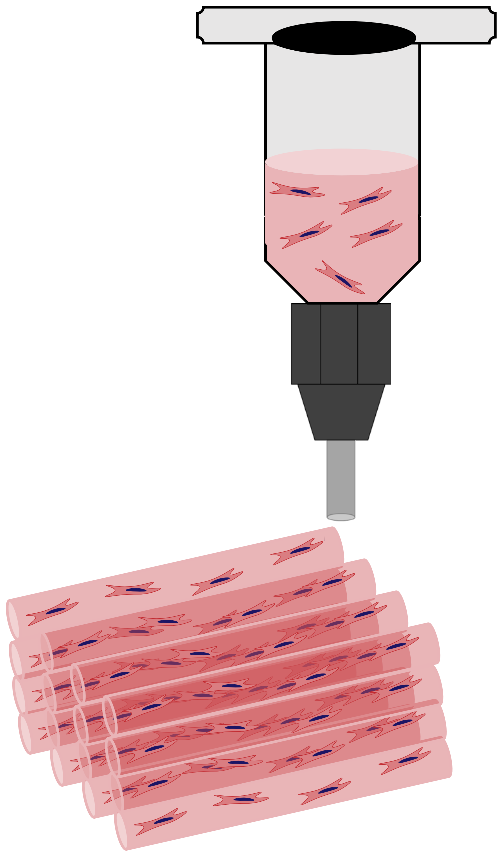
Extrusion Printing Cells & Biologics



Biological material can be encapsulated in hydrogels for extrusion printing

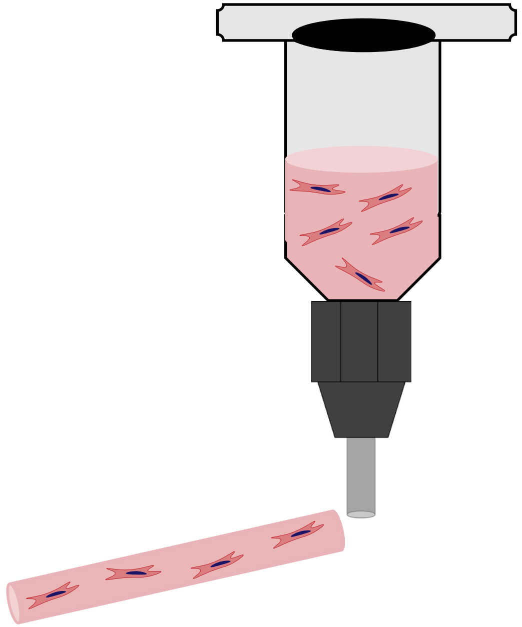
Shear-thinning, thixotropic material is necessary



For a 'successful' print, strands must maintain shape throughout the fabrication process

Temperature and/or light exposure can aid ink stabilization

Extrusion Printing Cells & Biologics



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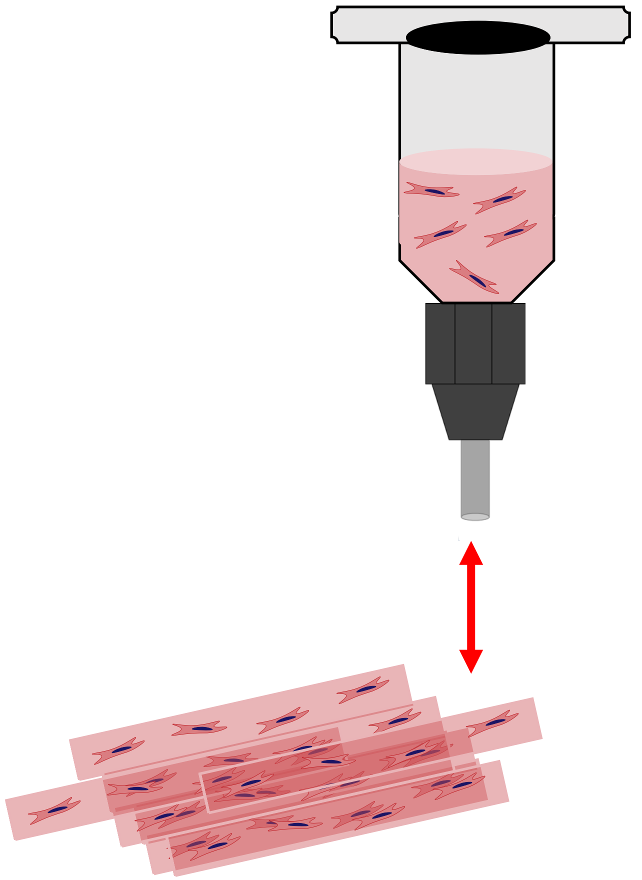

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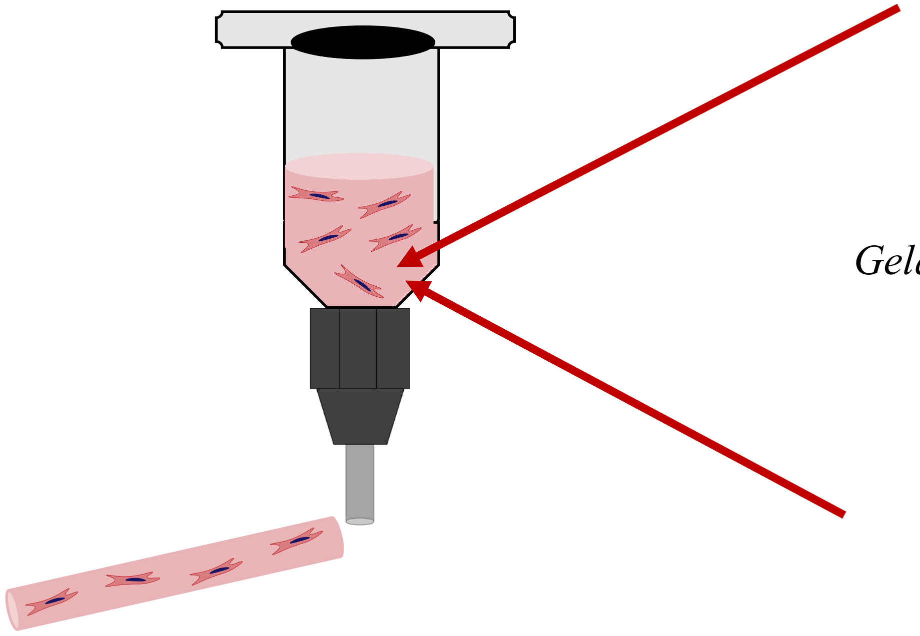
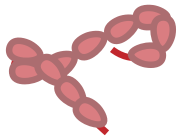
Extrusion Printing Cells & Biologics



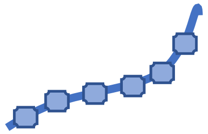
Wide range of hydrogel bioinks have been investigated

Natural Materials:



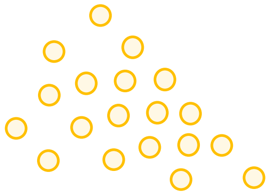
Proteins

Gelatin/collagen, fibrin, silk fibroin, elastin



Polysaccharides

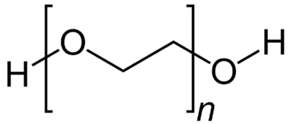
Hyaluronic acid, chitosan, alginate



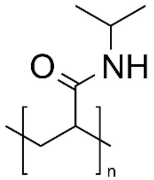
Decellularized Matrix

Lyophilized powders of major tissues

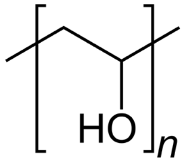
Synthetic Materials:



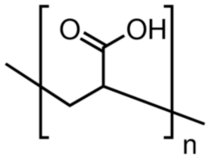
Poly(ethylene glycol)



Poly(N-isopropylacrylamide)



Poly(vinyl alcohol)



Poly(acrylates)

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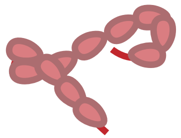
Extrusion Printing Cells & Biologics

Wide range of hydrogel bioinks have been investigated

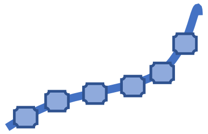
Natural Materials:

Natural Gelation Mechanism



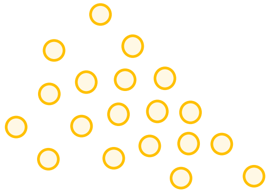
Proteins

Gelatin/collagen, fibrin, silk fibroin, elastin



Polysaccharides

Hyaluronic acid, chitosan, alginate



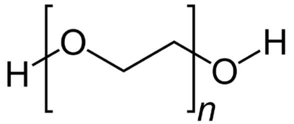
Decellularized Matrix

Lyophilized powders of major tissues

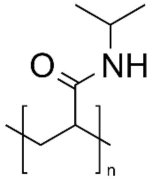
Synthetic Materials:

Additives Needed (Thickeners)

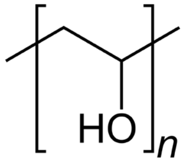
Nanosilicates, natural materials, pre-polymerization



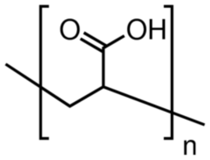
Poly(ethylene glycol)



Poly(N-isopropylacrylamide)



Poly(vinyl alcohol)



Poly(acrylates)

Hydrogel Bioink Pitfalls

Natural Materials

Proteins
Polysaccharides
Decellularized Matrix

- *Solubilize*
- *Rapid remodeling in vivo*
- *Weak material properties*

Synthetic Materials

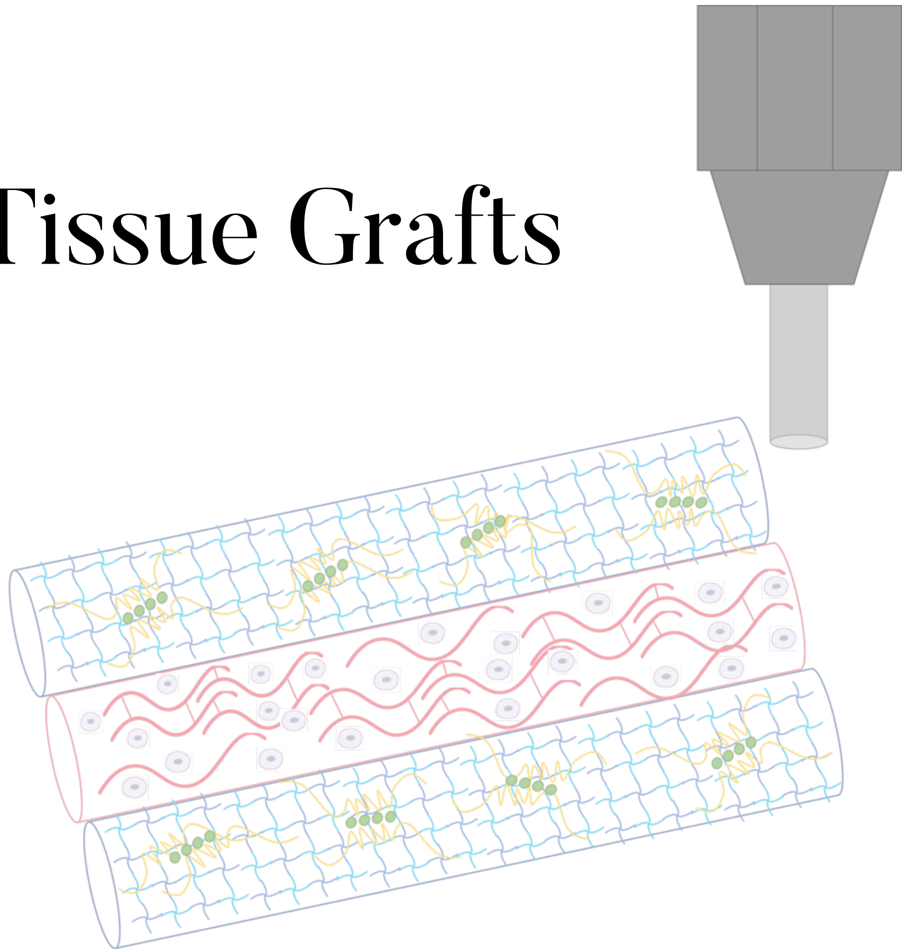
Poly(ethylene glycol)
Poly(vinyl alcohol)
Poly(N-isopropylacrylamide)
Poly(acrylates)

- *Difficult to extrude*
- *Lack bioactive moieties*
  - *Poor cell attachment and host integration*

Case Study:

Shape Retaining Soft Tissue Grafts

*DISSERTATION



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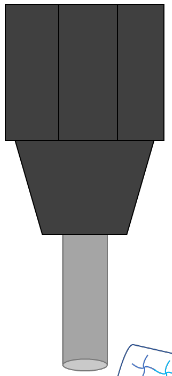
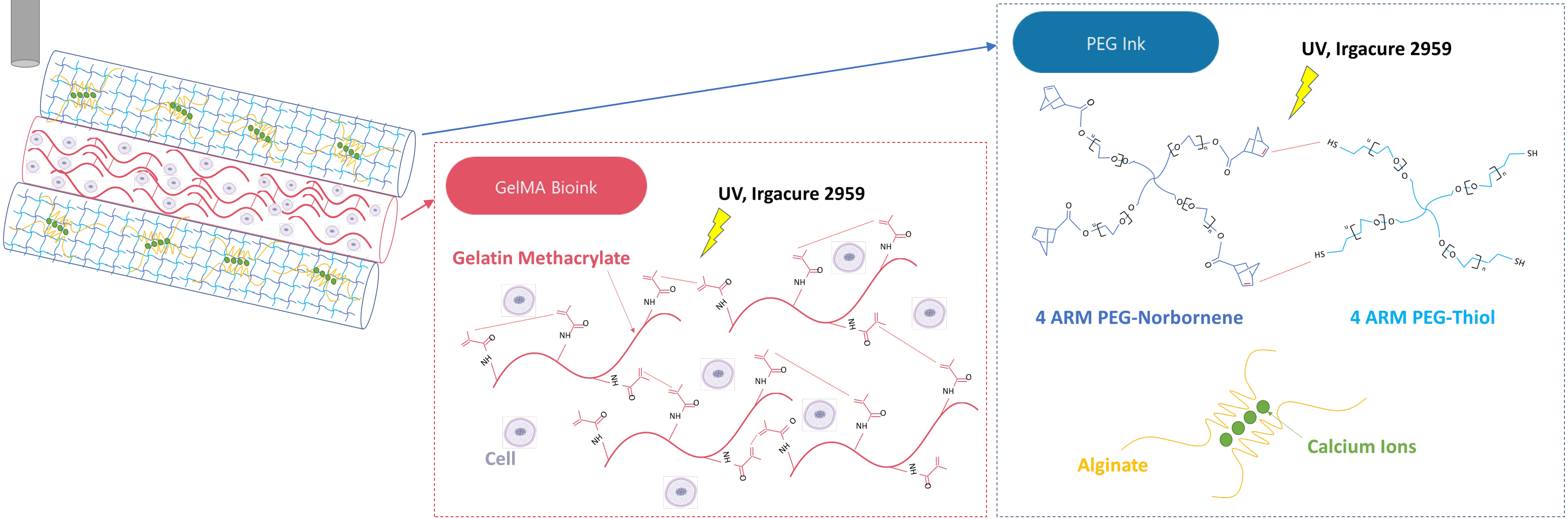
Hybrid Printing Synthetic and Biodegradable Bioinks

*Large soft tissue regenerative grafts can be 3D printed using a hybrid of two bioinks: <u>gelatin methacrylate</u> <u>**(GelMA)**</u> and <u>poly(ethylene) glycol</u> <u>**(PEG)**</u>*





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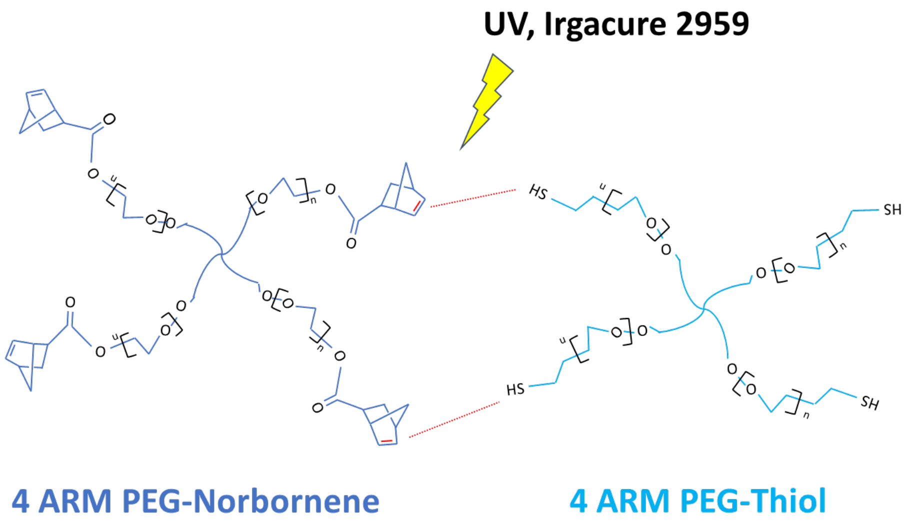
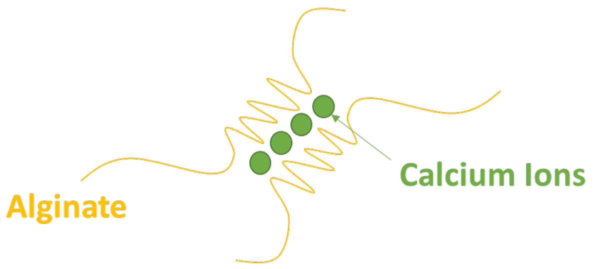
PEG Extrusion and Swelling tuned with Alginate

1 ***Poly(ethylene) Glycol (PEG) is a synthetic polymer known for its bio-inertness and resistance to common enzymes***



Thiol-Norbornene reaction is chosen due to this system's ***predictable swelling characteristics*** *and* ***low toxicity***

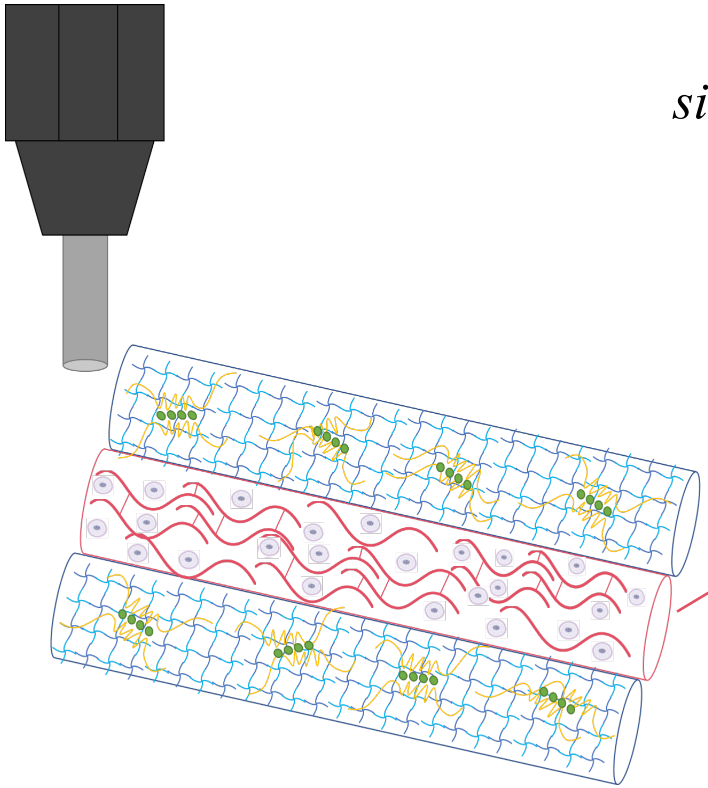
2 ***Alginate was chosen as a thickener to create extrudable hydrogels***

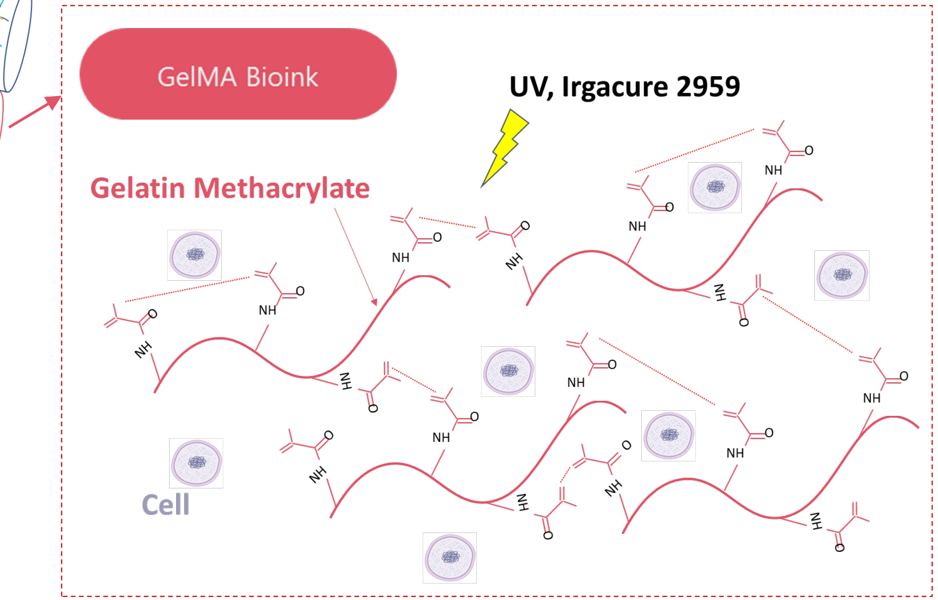


When calcium ions are present in solution, alginate can ionically interact with itself, which provides a second interlocking polymeric network within the ink

Hybrid Printing Synthetic and Biodegradable Bioinks

The implant is 3D printed with two inks simultaneously: one bioink called gelatin methacrylate (GelMA) and one novel synthetic ink composed of poly(ethylene) glycol (PEG)



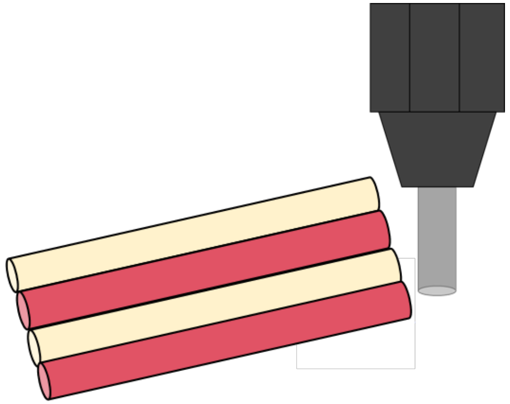


Gelatin Methacrylate (GelMA) is chosen for its resemblance to native extracellular matrix, and offers

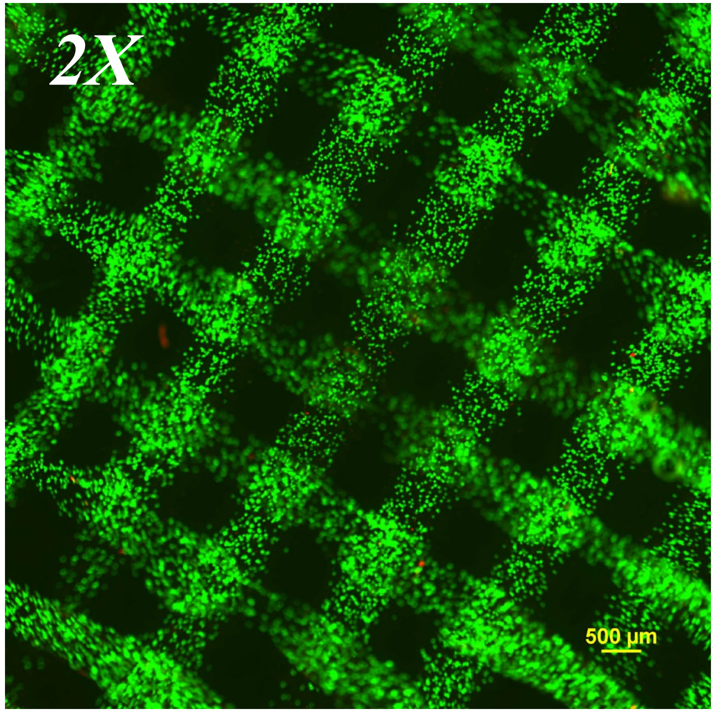
- ***Ease in printability***
- ***UV photopolymerization***
- ***Natural cell binding motifs***

It is a tunable biomaterial whose composition and crosslinking degree can be customized to match the rate in degradation to the regenerated tissue its replacing

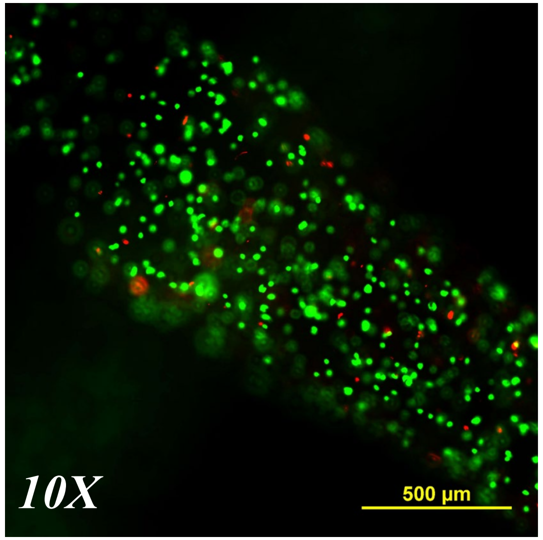
Hybrid Printing Synthetic and Biodegradable Bioinks



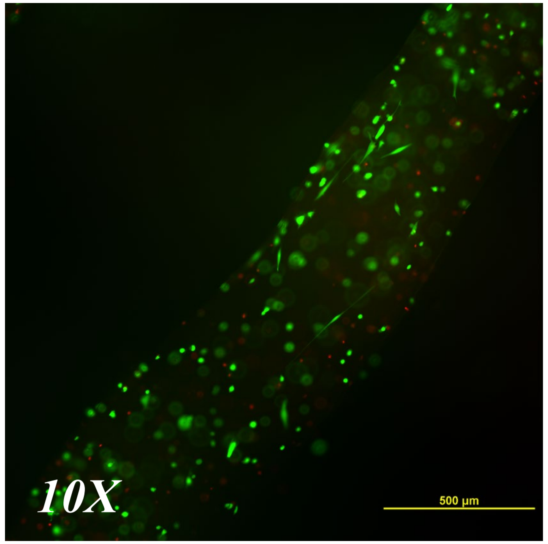
Accurate deposition of cell laden bioink and synthetic ink promotes healthy fibroblast proliferation



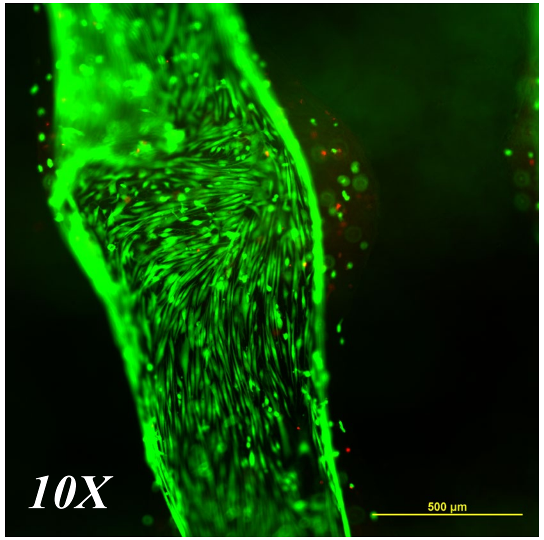
Day 0



Day 7

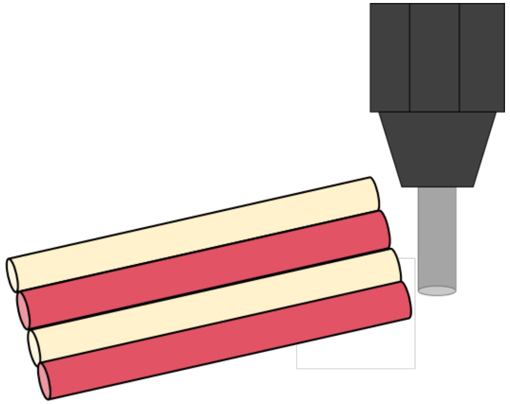


Day 14

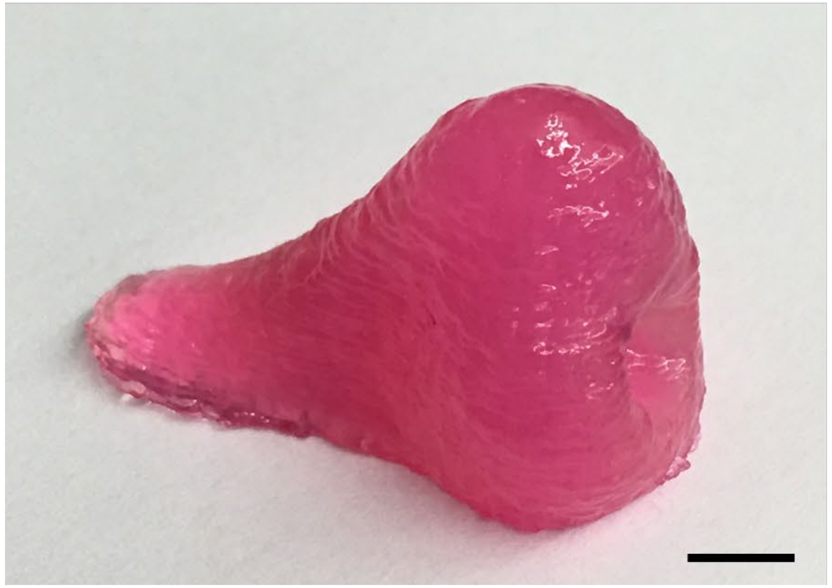


Spindle-like morphology is achieved after 14 days of submerged in vitro culture

Hybrid Printing Synthetic and Biodegradable Bioinks

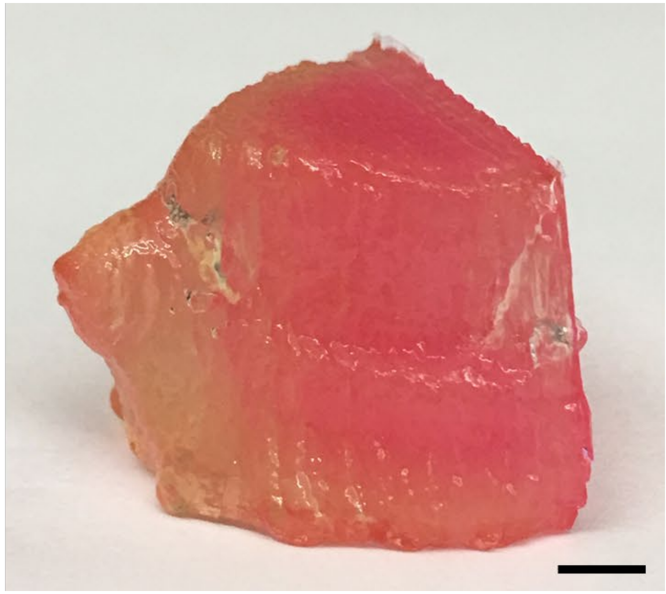


Complex architectures can be created using this printing technique



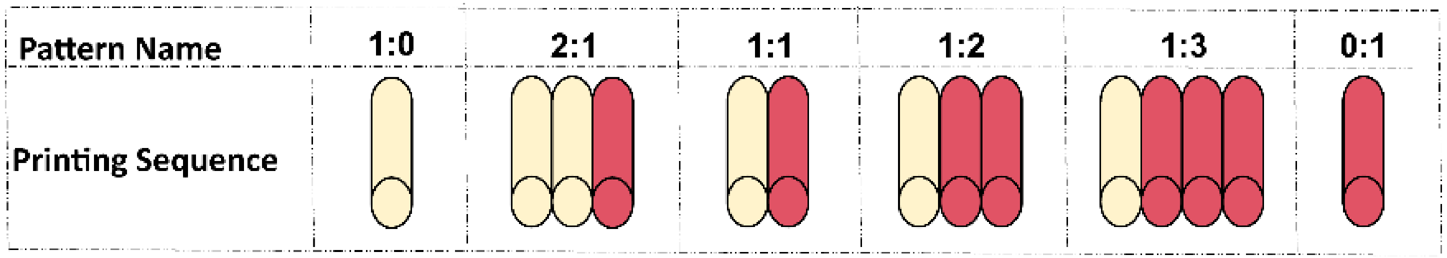
Nose

Ear

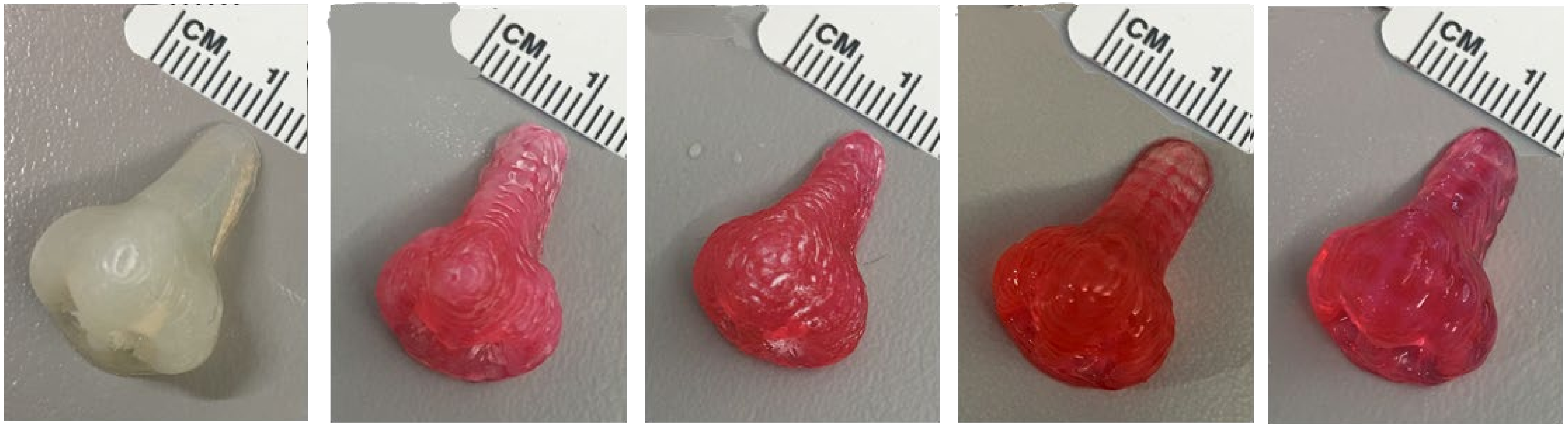


Thyroid Cartilage
(Adam's Apple)

Shape Retention after Enzymatic Degradation



1:0 Pattern **2:1 Pattern** **1:1 Pattern** **1:2 Pattern** **1:3 Pattern**



Shape Retention after Enzymatic Degradation

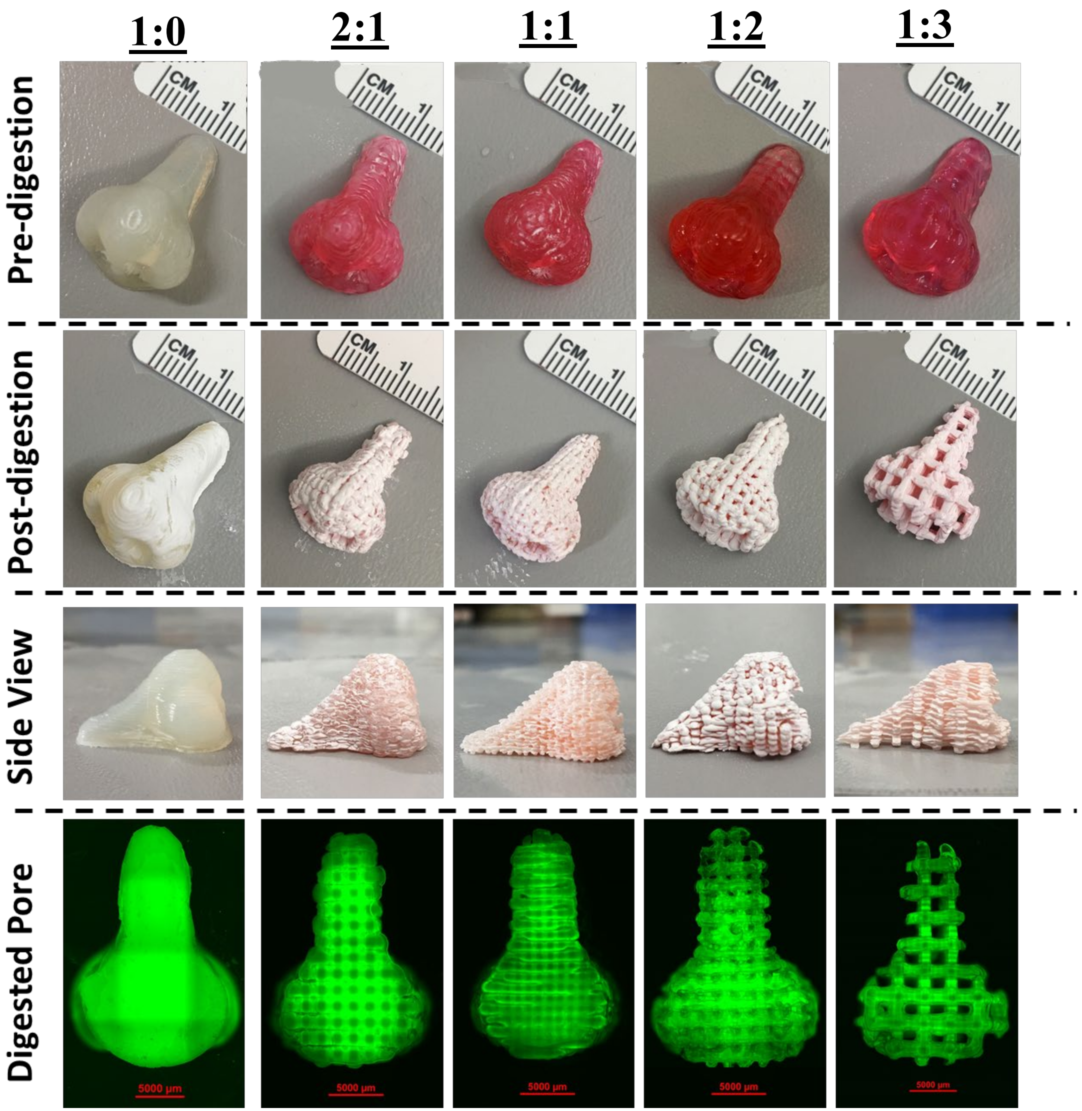
3D Scanned objects with a ROMER Arm Absolute Scanner

↓

Digested GelMA to represent 'extreme' or rapid dissolution of bioink (in vivo immune response)

↓

Compared 3D object point clouds in software CloudCompare

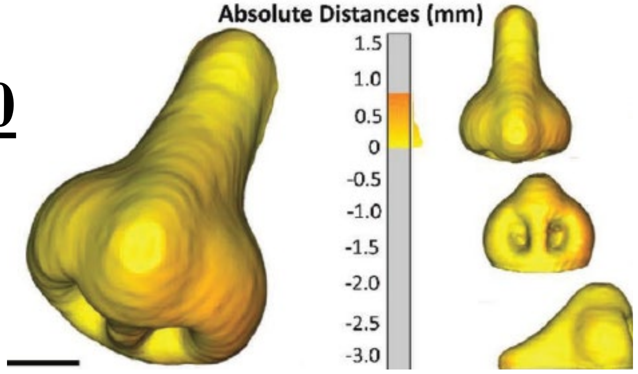


CloudCompare Analysis

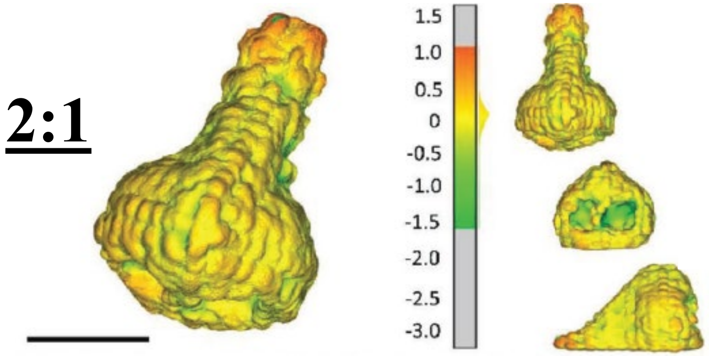
Generated Heat Maps of Shape Maintenance

Red/orange: positive deviation (expansion)
Yellow: no deviation
Blue/green: negative deviation (contraction)

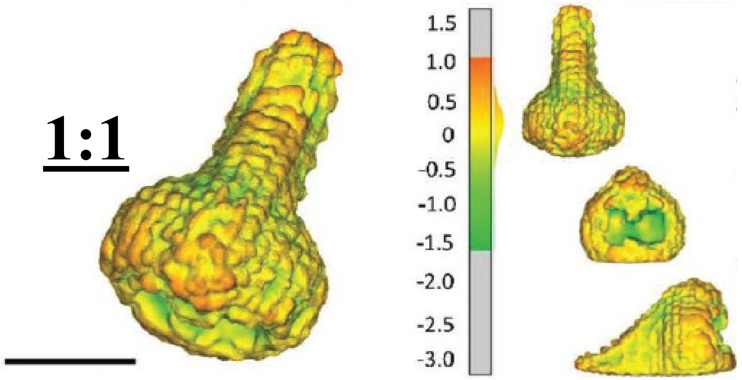
1:0



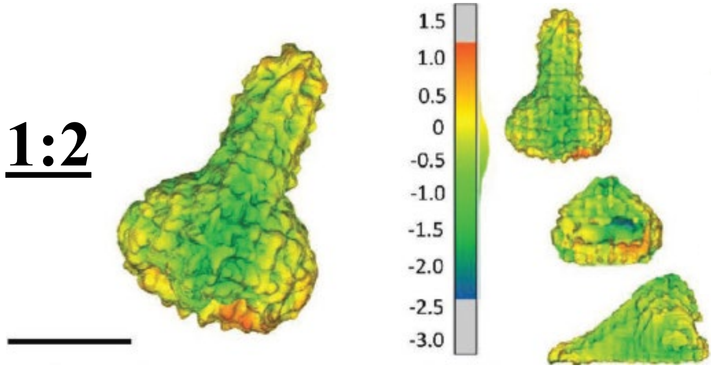
2:1



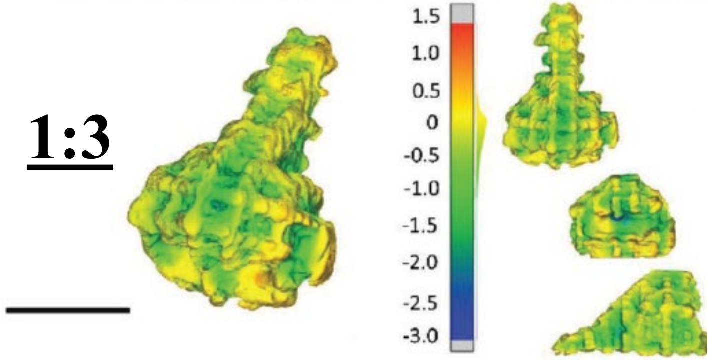
1:1



1:2



1:3



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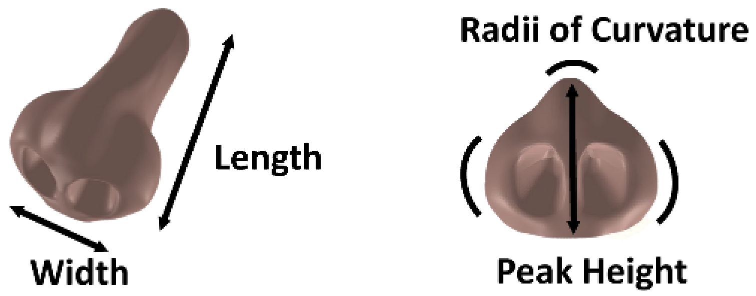
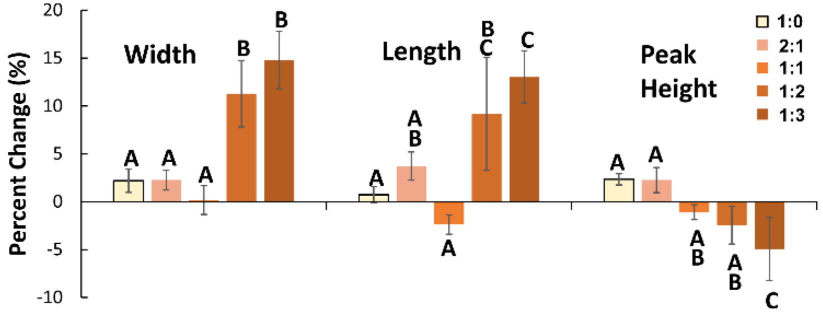
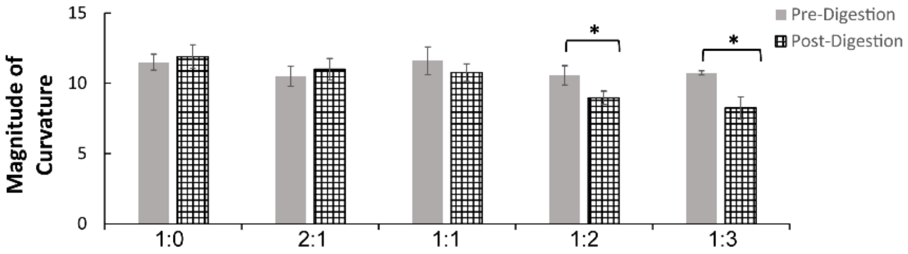
CloudCompare Analysis







__Shape is uniquely important for 3D printed products__

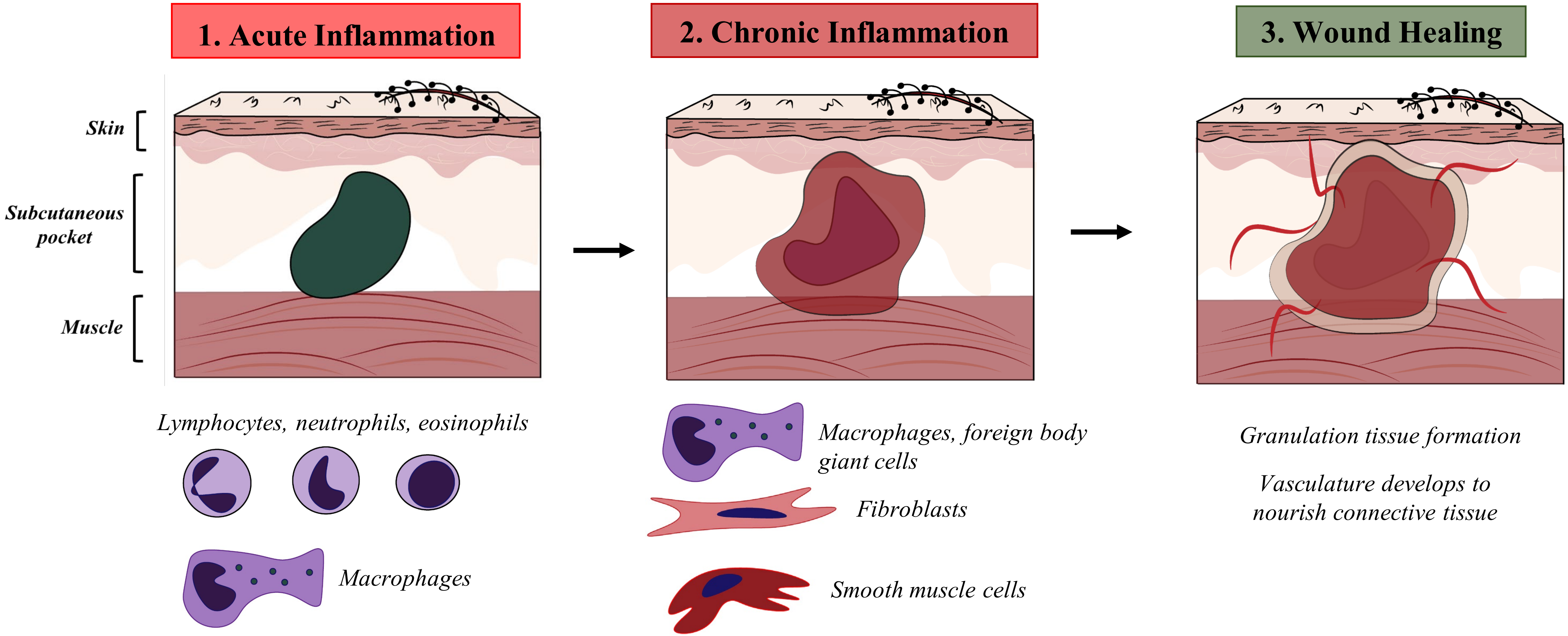
Data holistically shows PEG's ability to dictate scaffold shape during degradative remodeling processes

__Technical Advancement:__

Defined a new point cloud comparison method that describes the retention of the object's shape

Van Belleghem S, Torres Jr L, Santoro M, Mahadik B, Kofinas, P, and Fisher, JP, "**Hybrid 3D Printing of Synthetic and Cell-Laden Bioinks for Shape Retaining Soft Tissue Grafts**". *Adv Funct Mater*. 2019;30(3):1907145. doi:10.1002/adfm.201907145

Biocompatibility of implanted materials



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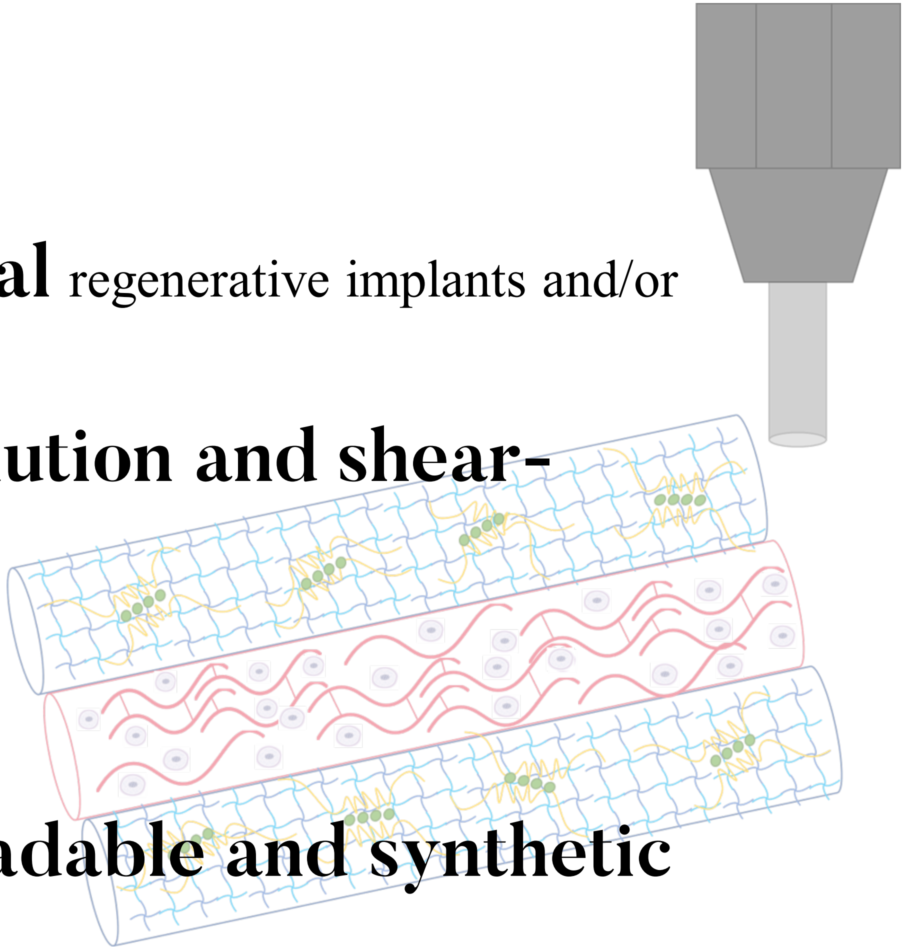
Conclusion

Extrusion 3D Printing allows:

- ❑ the fabrication of **complex, multi-material** regenerative implants and/or tissue models
- ❑ pitfalls exist, such as **limited printing resolution and shear-thinning bioink formulations**



We developed a fabrication strategy :

- ✓ that capitalizes on the strengths of both **biodegradable and synthetic** materials
- ✓ demonstrates the ability to **maintain shape** by providing a macro-support structure to the tissue and **high cell viability** (*in vitro*)

Thank you!

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