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Diversity, Equity, Inclusion, and Respect
*Modified from definitions from the National Institute for Work Life

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**Respect**

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Biomedical 3D Printing: Research Landscape, Applications, and New Innovative Materials

CHIA-WEI HSU, PhD
Information Scientist, CAS, a division of the American Chemical Society

SHRIKE ZHANG, PhD
Assistant Professor, Harvard Medical School

AXEL GUENTHER, PhD
Professor and Co-Director, Collaborative Centre for Research and Applications in Public Technologies (CRAFT), University of Toronto

ADAM W. FEINBERG, PhD
Professor, Regenerative Biomaterials and Therapeutics, Carnegie Mellon University

GILLES GEORGES, PhD
Vice President & Chief Scientific Officer, CAS, a division of the American Chemical Society

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Breakthroughs in biomedical 3D printing

Decades in the making, recent advances have accelerated innovation

<table>
<thead>
<tr>
<th>Tissues/organs</th>
<th>Pharmaceuticals</th>
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<td>First printed lung</td>
<td>Customized 3D printed drugs</td>
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<th>Bioprinting</th>
<th>Orthopedics</th>
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<td>3D bioprinting of heart valve</td>
<td>Hybrid biomaterials</td>
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Advancements in materials and techniques
Have enabled faster progress across the entire biomedical landscape

Materials

- **Natural**: gelatin, alginic acid, hyaluronic acid
- **Synthetic**: polycaprolactone, poly(lactic acid), polyethylene glycol
- **Inorganic**: titanium, hydroxylapatite

Techniques

- Powder bed fusion
- Jetting
- Extrusion
- Photopolymerization

Technique and materials are crucial drivers
Use cases and applications may dictate different prioritization across categories

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<td>Photopolymerization</td>
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Biomedical 3D printing application trend

Growth in materials is evident in all three classes
With key players emerging as publication trends
Growth in technique is driven by
Lower costs and material advancements

Global participation in biomedical 3D printing
Journals and patents

<table>
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<tr>
<th>Country</th>
<th>Publications</th>
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<tr>
<td>China</td>
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<td>United States</td>
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<td>Rep. of Korea</td>
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<td>Germany</td>
<td>1,102</td>
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<tr>
<td>India</td>
<td>857</td>
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Handheld Skin Printer:
Rapid, wound-conformal delivery of skin precursor sheets improves healing in full-thickness burns

CAS-ACS webinar, 3D Printing Materials in Biomedical Applications

May 4, 2023

Axel Guenther
University of Toronto

axel.guenther@utoronto.ca
Multinozzle Microfluidic Printhead for Biomaterial Sheet Extrusion

- One-step
- Continuous
- Dynamic control over material composition
- High throughput
- Scalable to 3D

Payload
Planar Organization
Coded Fluid
Coded Soft Material
Solidification
Time Dependent Molecular Transport
Stacked Layers
Tubular or Rolled-Up Multilayers


Microfluidic Printhead for Biomaterial Sheet Extrusion

Payload
Coded Fluid
Coded Soft Material
Solidification
Time Dependent Molecular Transport
Stacked Layers
Tubular or Rolled-Up Multilayers

Architected Biomaterial Sheets

Manual Stacking of Heterogeneous Biopolymer sheets

Rolled-Up Multilayered Bulk Soft Material

Tubular Soft Material


Architected Biomaterial Sheets with Cellular Payloads

Single Cell Patterning
- 1.2%w.v. alginate
- 19%v.v. Matrigel™
- 1.8mg/ml Collagen Type I
- 0.46mg/ml RGDs-functionalized
- 10million cell/ml

1Plouffe et al. Lab Chip 9, 2009.

Co-Culture

Cell Distribution

Leng et al. Adv. Mat. 2012, 24, 3650–3658 (with Milica Radisic)
Skin

- Largest organ of the body.

- Layered organization
  Epidermis, 0.2-0.5mm (dense barrier)
  Dermis, 0.5-20mm (fibrous collagen network)

- Total body surface area: 2.0-2.5m². Severe skin injuries (e.g., full-thickness burns) up to 80% skin loss

Handheld Skin Printer

Hakimi et al. Lab Chip (2018) 18, 1440-145
Characterization of Biomaterial Sheets

In-Situ Formation of Skin Tissues in vitro

In-Situ Delivery of Biomaterials in vivo

Hakimi et al. Lab Chip (2018) 18, 1440-145. (with Marc G. Jeschke)
in-vivo deposition on a porcine excisional wound model

Cheng, et al. (2020) *Biotfection* **12** (2) 025002
Cheng, et al. (2020) *Biofabrication* **12** (2) 025002

Cheng, et al. (2020) *Biofabrication* **12** (2) 025002
Summary

• Rapid in-situ bioprinting strategies based on multinozzle microfluidic printheads

• Formation of ECM-based biomaterial sheets and precursor tissues conformal to wound surface accelerates wound healing

• Current work: ECM-based granular bioinks with tailored printability and wound contraction, rapid biofabrication strategies for load bearing tissues.

axel.guenther@utoronto.ca
3D Bioprinting Human Tissues and the Path Towards Translation

Adam W. Feinberg, Ph.D.
Professor
Regenerative Biomaterials & Therapeutics Group
Department of Materials Science and Engineering
Department of Biomedical Engineering
Carnegie Mellon University

CTO and Co-Founder
FluidForm Inc

May 4, 2023

How do we get to therapeutic tissues & organs?

Volumetric Scaffolds with Patient-Specific Anatomical Structure

Heart Valves

Multiscale Vasculature from Coronary Arteries to Capillaries

Dense Ventricular Myocardium

Lee et al, Science (2019)
3D Printing → A Problem for Hydrogels & Cells

Fidelity is lost when printing hydrogels

3D Bioprinting of Soft Materials - SUPPORT

Tony Atala

Gabor Forgacs

Christopher Chen

Jennifer Lewis

Jordan Miller

Printed within a custom-made housing, this method can be used to create tissue of any shape.
**FRESH → Supports Soft & Living Materials**

- **Freeform Reversible Embedding of Suspended Hydrogels** (FRESH)
- Gelling fluid bioink is embedded into sacrificial support material
- Bath behaves as a yield stress fluid
- BioInk is uniformly supported during printing while it gels
- Support is melted to retrieve print

**Embedded printing leaders**
- Adam Feinberg (CMU)
- Jennifer Lewis (Wyss)
- Jason Burdick (UC)
- Tommy Angelini (UF)


---

**FRESH → An Advanced Biofabrication Platform**

**Biologics**
- Alginate
- Cell-Laden
- Cell Slurries
- Collagen
- Decell-ECM
- Fibrin
- GelMA
- Hyaluronic Acid
- Matrigel
- Silk Fibroin

**Crosslinking**
- pH-driven
- Ionic
- Photo-crosslinking
- Enzymatic
- Thermal
- “Click” chemistry

**Synthetics**
- Epoxies
- Photoresists
- Silicones
- Urethanes

**Support Baths**
- Gelatin
- Alginate
- Carbopol
- Agarose
- Cell/spheroid slurry
- Cell-laden

Print Pathing
- Layer-by-layer
- Non-planar layer-by-layer
- Freeform

Shiwariski et al, APL Bioengineering (2021)
Engineering a Contractile Human Ventricle

Cardiomyocyte alignment in printed ventricle wall

Calcium Imaging of Ventricle

Lee et al, Science (2019)

Building Multiscale Vasculature – Printing / Self-Assembly

Coronary Vasculature

Microvasculature Through GFs and Microporosity

CD31 Casted + VEGF

Lee et al, Science (2019)
Now that we can 3D bioprint cells and ECM, how exactly do we create tissues & organs?

- Tri-leaflet Heart Valve
- Organ-Scale 3D Printing
- ECM in the Developing Heart
- Bioinspired Matrix Design
- EHTs w/ Preload and Afterload
- Contractile Heart Tubes
Building a Functional Human Heart Tube

High-density Cardiac Syncytium

Contractile Function

Bead Tracking

Calcium Handling

Pumping

Collagen Scaffolds Guide Muscle Organization

Stang et al (Unpublished)
Patient-specific Decellularized ECM Scaffold for VML

- De-identified human CT image showing volumetric muscle loss (VML) injury with contralateral uninjured leg
- The scaffold (blue) was created by isolating the vastus lateralis muscle from the uninjured leg and overlaying it onto the injured leg
- The ECM scaffold was FRESH printed using decellularized ECM bioink with a length of ~14 cm
- Dimensional analysis of the 3D printed scaffold shows excellent fidelity with <1.5 mm mean deviation

Driving Tissue Biofabrication Forward

- Advanced tissue engineering applications, including disease models
- Multiscale solutions to vascularization
- Biomanufacturing platforms w/ advanced 3D imaging
- Translation to large animal pre-clinical models
- Supporting the research community through education and open-source technologies
- Development of commercial applications including biomanufactured medical devices & in vitro disease models
Acknowledgements

We are actively recruiting graduate students & postdocs to build human tissue

@RegenBio
http://regenerativebiomaterials.com

Collaborators

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• Jana Kainerstorfer (CMU)
• Steve Badylak (Pitt)
• Peter Van der Meer (UGMC)
• Daniel Pijnappels (Leiden)

Disclosures

• FluidForm, Inc.
CTO & Co-founder

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Marcelle Machluf, Technion
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Kent Leach, UC Davis
Monica Laronda, Northwestern
Reuben Govender, Univ. of Cape Town
Kris Killian, Univ. of New South Wales
Chelsea Magin, UC Denver
Christian Franck, Univ. of Wisconsin
Shelly Peyton, UMass Amherst
Riccardo Gottardi, U Penn
Jonathan Vande Geest, U Pitt
Ritu Raman, MIT
Brenden Baker, Univ. Michigan
Chris Highly, Univ. Virginia

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