Bioprinting at the Naval Research Laboratory

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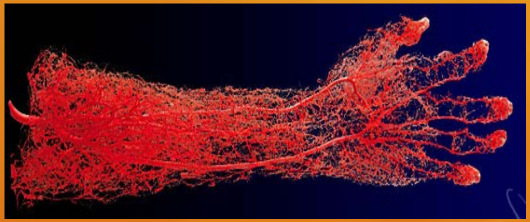
What is bioprinting?

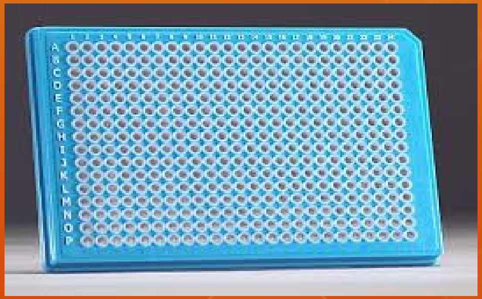
- Bioprinting is the controlled deposition of any living or non-living biological (DNA, protein, bacteria, mammalian cells, viruses) material into a computer-aided design or pattern
- Depending on the printer technology, pattern resolution (spot or line size) can vary from a continuous sheet (macroscale, e.g. skin) to 10's of microns (microscale, e.g. microcapillaries & vessels)
- Deposition speeds can exceed 1000 droplets per second (high throughput)
- Deposition volumes range from 10^{-3} mL to 10^{-10} mL per printed droplet
- 2D (single layer) and 3D (multi-layer) applications

Potential for Bioprinting to Have Broad and Far-Reaching Effect Across Biotechnology Sectors

- Healthcare
  - Transplants and regenerative medicine applications (pancreatitis, type I diabetes, parathyroid transplant, hepatic tissue transplantation, renal transplants, lung transplants, heart transplants, skin)
  - Infectious disease modelling *in vitro*



- Pharmaceutical
  - Drug testing/screening (toxicity, efficacy)
  - Biomarker discovery (diagnostics, vaccine and therapeutics)
  - Oncology (tumor models, efficacy, biomarker testing/screening)
  - Replace and/or supplement (pre-screen) animal models



- Medical Devices
  - Hybrid devices (living/non-living)
  - Prosthetics/implants
- Defense and Homeland Security
  - Chem/Bio assessment in vitro tissue models (human clinical trials impossible)
  - Agnostic chemical threat sensors (based on 3D bioprinted cellular systems)
  - Skin replacement; bio-artificial limbs
- Cell Sourcing
  - Automate and scale up cell production for in vitro/in vivo tissue/organ applications

Scientific Challenges Related to Bioprinting

- Expanding the diversity and mechanical properties of printed 3D human tissue constructs
  - Limited primarily to soft structures (hydrogel)
  - <u>Potential Solution</u>: Stacking thin biopapers fabricated using a variety of micro- and nano-scale tools/materials
- Balancing speed/throughput and print resolution
  - Certain bioprinters lay down large volumes of material with very poor resolution (~mm) while others deposit smaller amounts of material with micro-scale resolution
  - <u>Potential Solution</u>: Multi-tool approaches and/or tool improvement
- Develop and test regulatory pathways for *in vitro* and *in vivo* bioprinted tissues
  - Start with systems that can be well characterized and compared to both animal and human trials
- Controls, controls, controls!
  - Determine when bioprinting is required/advantageous and when it is NOT
  - This will require control experiments that try to accomplish the same cellular construct without printing (pipette, spray, molding?)
- Define what bioprinting really means
  - Robotic dispensing of 3D cell organoids and/or printing at meso- and micro-scale?

Cell and organ printing

MRS BULLETIN OCTOBER 2013 ISSUE

TECHNICAL FEATURE

CELL AND ORGAN PRINTING TURNS 15: DIVERSE RESEARCH TO COMMERCIAL TRANSITIONS

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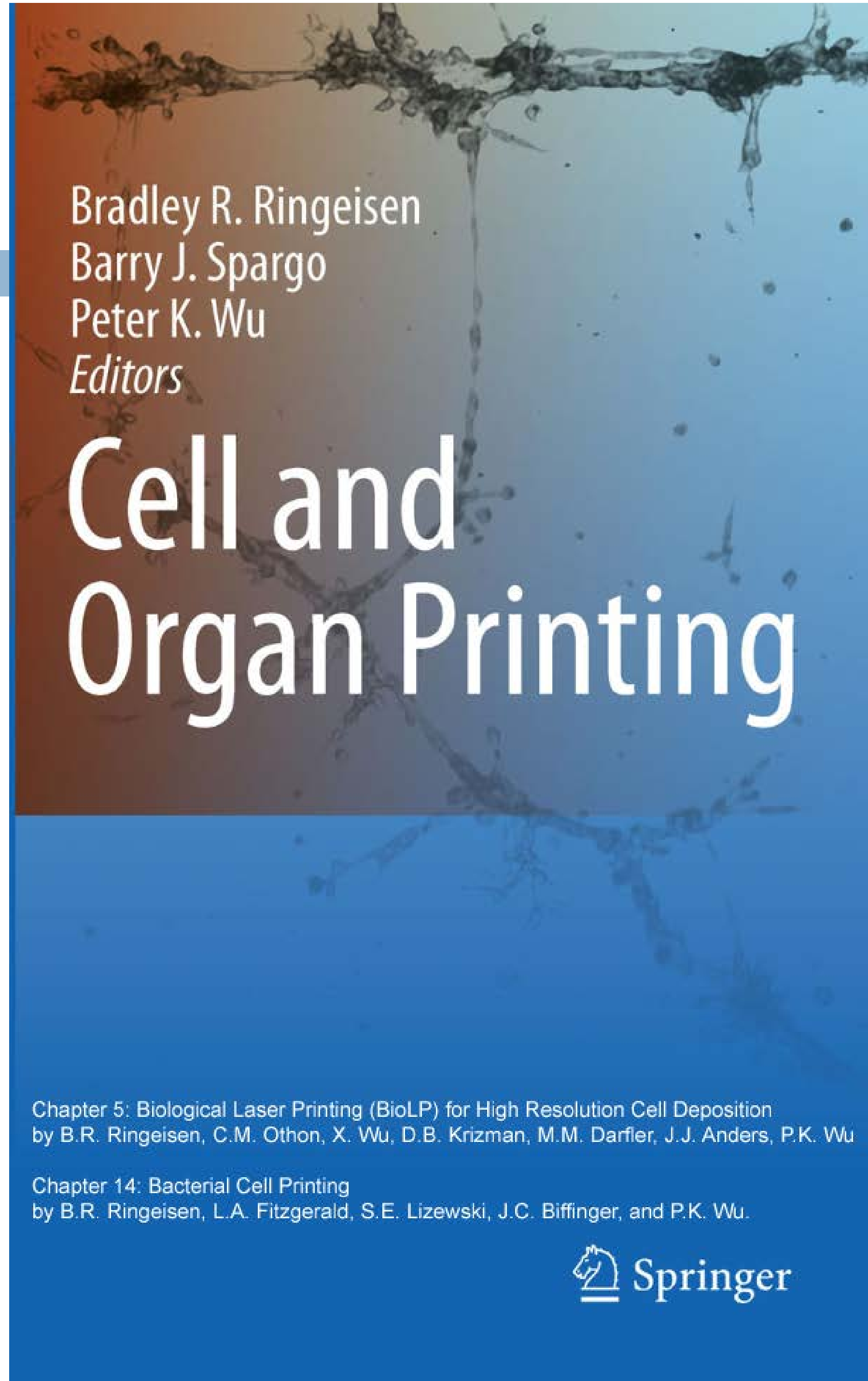
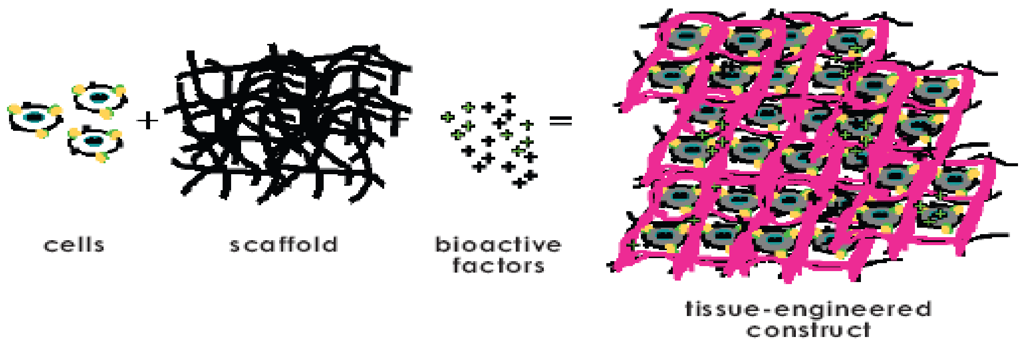
MRS Bulletin, **38**, pp. 834-843 (2013)


Bradley R. Ringeisen
Barry J. Spargo
Peter K. Wu
Editors
Cell and Organ Printing
Chapter 5: Biological Laser Printing (BioLP) for High Resolution Cell Deposition
by B.R. Ringeisen, C.M. Othon, X. Wu, D.B. Krizman, M.M. Darfler, J.J. Anders, P.K. Wu
Chapter 14: Bacterial Cell Printing
by B.R. Ringeisen, L.A. Fitzgerald, S.E. Lizewski, J.C. Biffinger, and P.K. Wu.
Springer

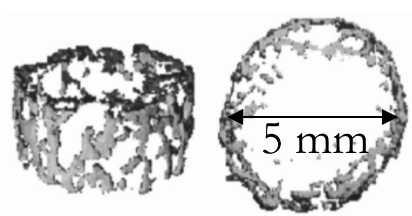
Trarditional Tissue Engineering Strategies

- Tissue Engineering
  - Combine cells, materials, and biochemical factors to generate complex, 3D structures that replicate nature tissues.



  - Impossible to culture 3D cell constructs beyond 100-200 μm
    - Nutrient and oxygen diffusion low at large thicknesses
    - A preformed vasculature network is absent

Mineralization in Bone Scaffold



VS

Mineralization in Freeze-Dried Bone

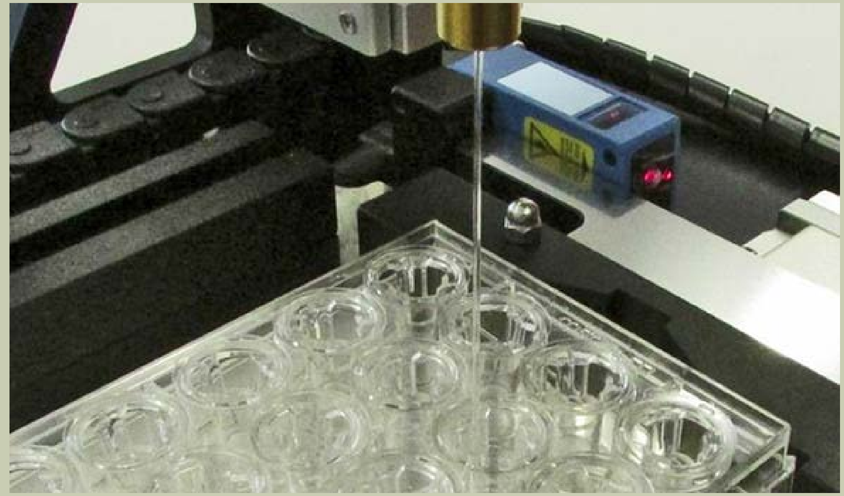
S.H. Cartmell, *et al*. 2003

Table I. Comparison between different cell printing tools.

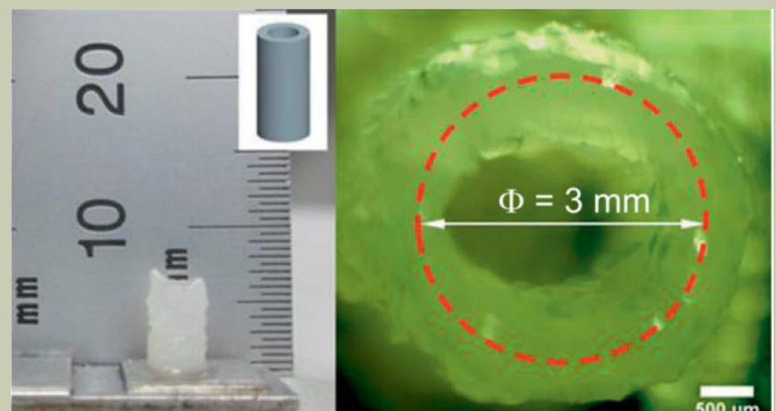
Printing Tool	Capabilities
Laser-Induced Forward Transfer (Laser)	Layer-by-Layer; Scaffold + Cells
Ink Jet	Layer-by-Layer; Scaffold + Cells; Cell Aggregates
Electrospray	Layer-by-Layer; Scaffold + Cells
Extrusion Pen	Cell Aggregates; Scaffold + Cells
Photo-Polymerization (Light or Laser)	Scaffold Only

MRS Bulletin, **38**, pp. 834-843 (2013)

The NovoGen Bioprinter™ fabricating tissue into a 24-well plate (organovo.com)

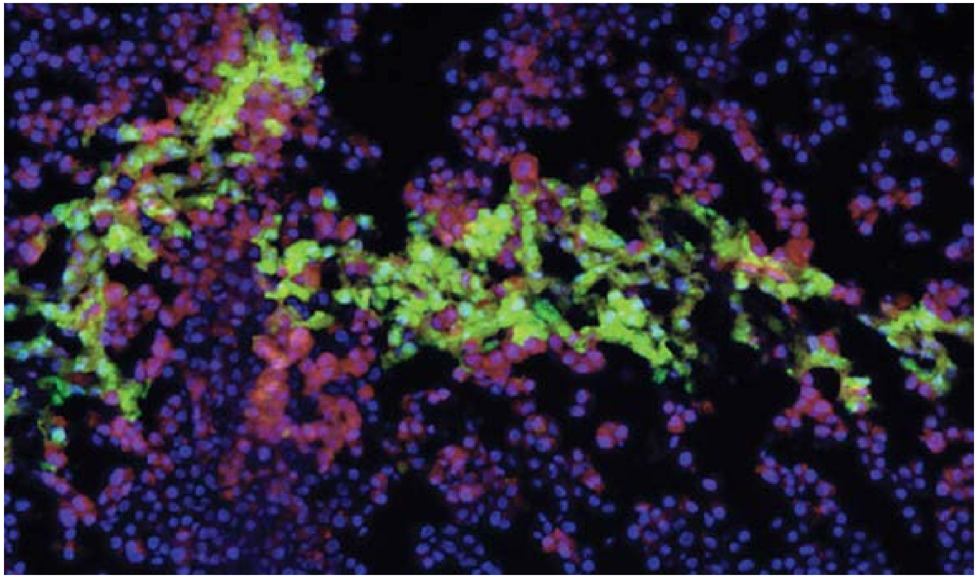


Laser Printed 3D Vessel Scaffold (MRS Bulletin, 38, pp. 834-843 (2013))

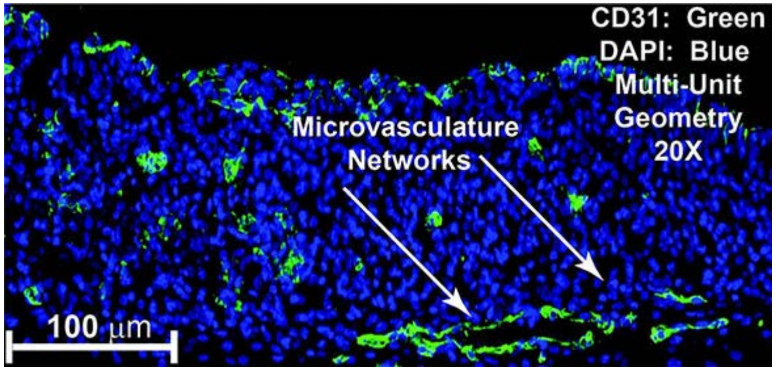


Examples of 3D Bioprinting (Extrusion)

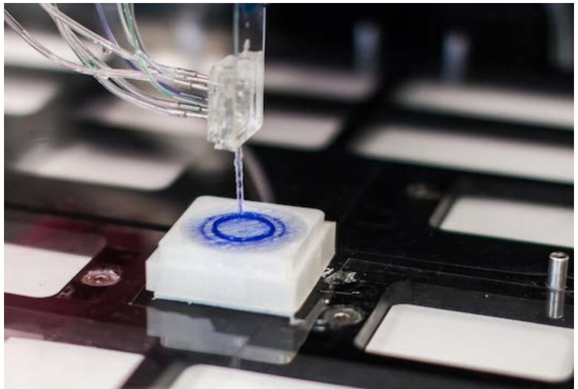
- Organovo, Inc.



3D "Liver" Tissue showing some heterogeneity and vasculature



http://www.organovo.com/tissues-services/3d-human-tissue-models-services-research/tissue-models/3d-human-liver-tissue-model



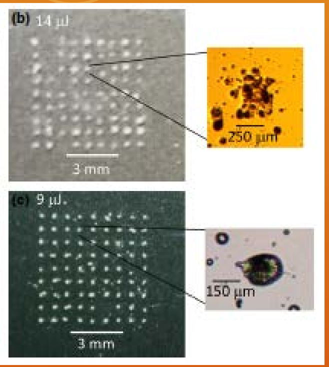
- Aspect Biosystems

http://3dprint.com/16267/aspect-biosystems-3d-bioprint/

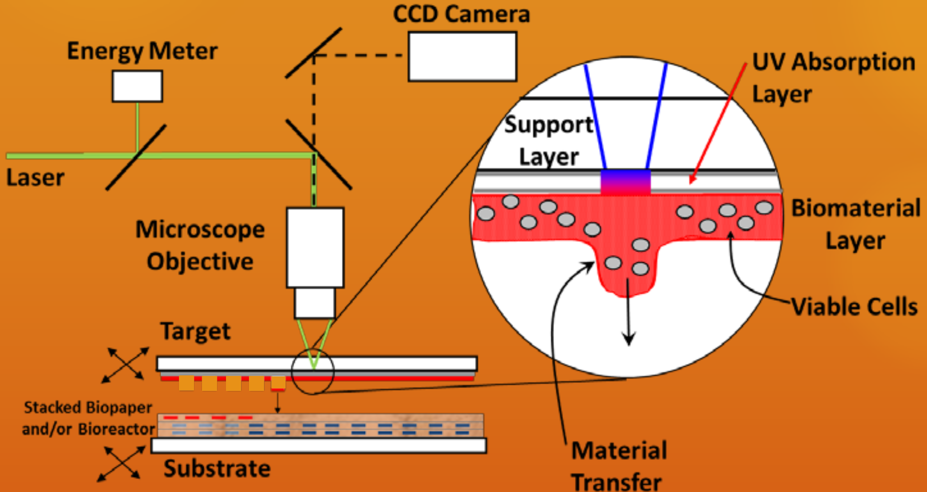
NRL's Bioprinting Tool– BioLP

Biological Laser Printing, or BioLP, is NRL's patented laser direct write tool for creating 2D and 3D patterns of almost any biomaterial including living cells (bacteria, mammalian), soil/sediment, hydrogels and biomolecules

World's First Printed Soil Microarray



Ringeisen, *et al. Methods in Ecology and Evolution* ***6*** *(2), 209-217 (2015)*

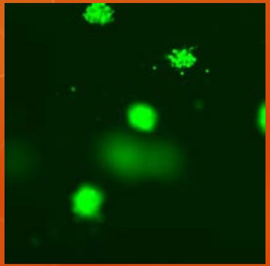


US Pat.#7,294,367; #7,381,440; #7,875,324, #8,669,086

World's First Printed Viable Bacterial Colony

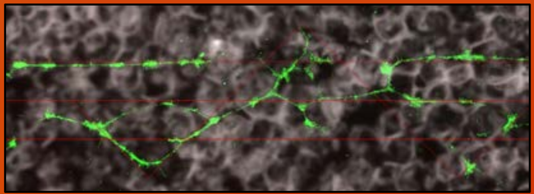
Ringeisen, Jones-Meehan, Spargo, Chrisey. *Biomaterials* **23**, 161-166 (2002)

Printed 3D Mammalian Cell Patterns



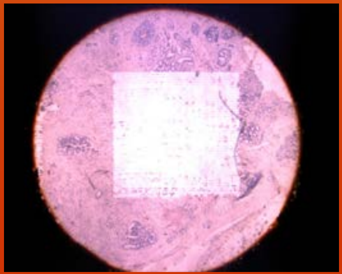
Barron, et. Al. *Biomed Microdevices* **6**, 139-147 (2004)

Printed Microvasculature on Polymer/Gel Biopaper



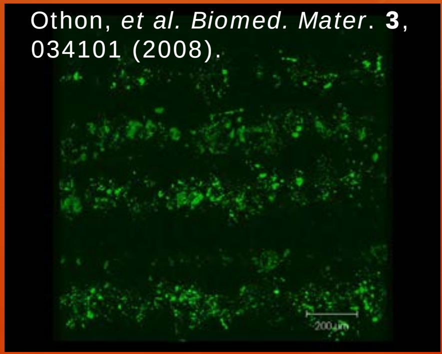
Pirlo, *et al. Biotechnol. Bioeng*. 109, 262-273 (2012)

Licensed for Tissue Microdissection



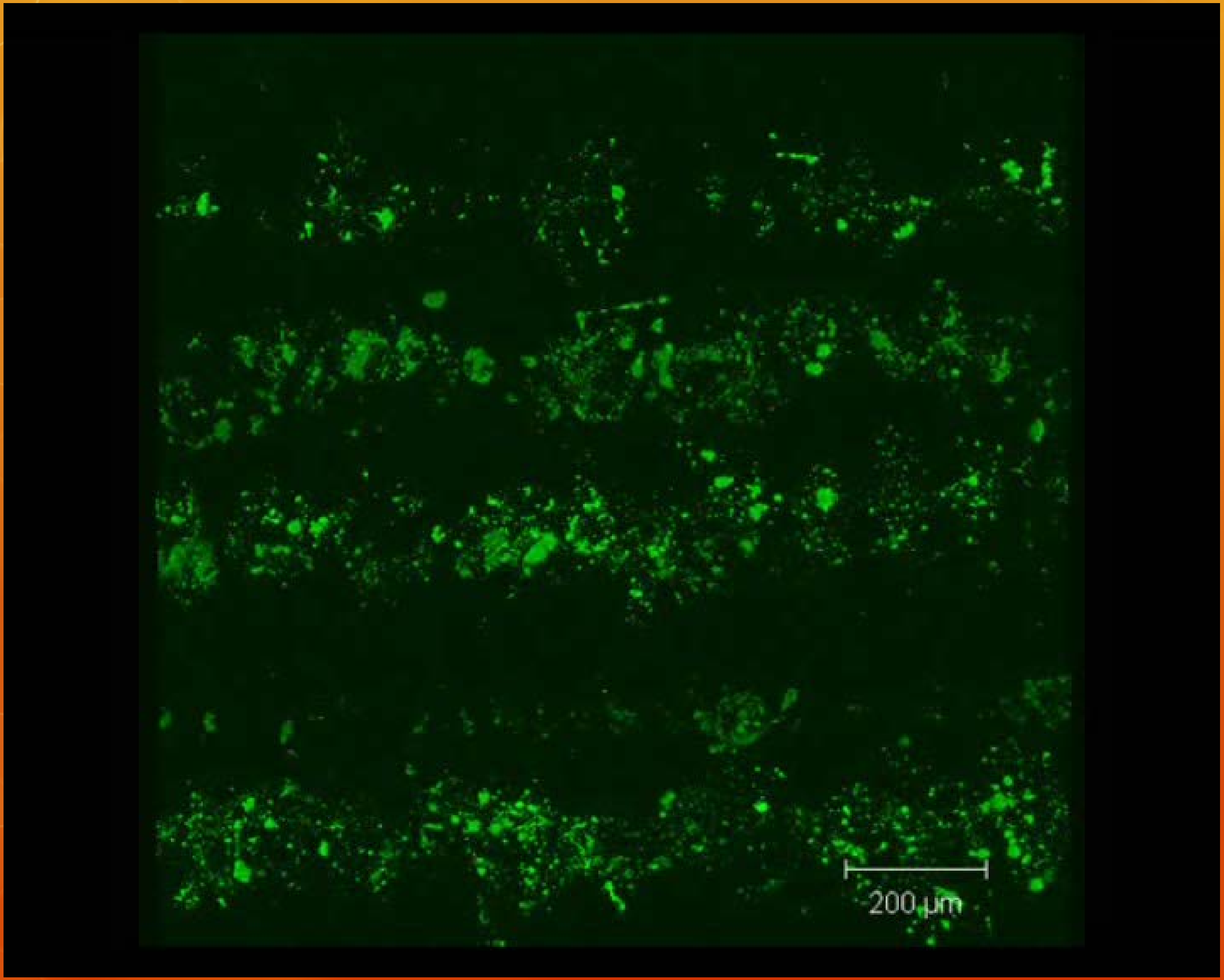
Hood, *et al. Molec. Cell. Proteomics*, **4**, 1741-1753 (2005).

Printed 3D Nerve Conduits



Othon, *et al. Biomed. Mater*. **3**, 034101 (2008).

Harvested Rat Olfactory Ensheathing Cells Printed Into "3D Highways"



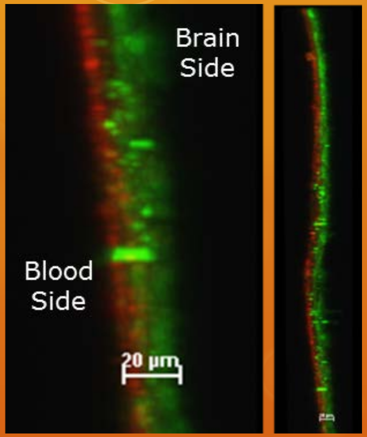
Neurite extension guided by OEC patterns

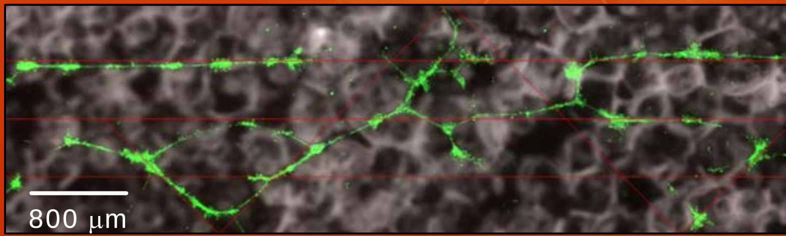
Application where pattern is required for function

Othon, *et al. Biomed. Mater*. **3**, 034101 (2008).

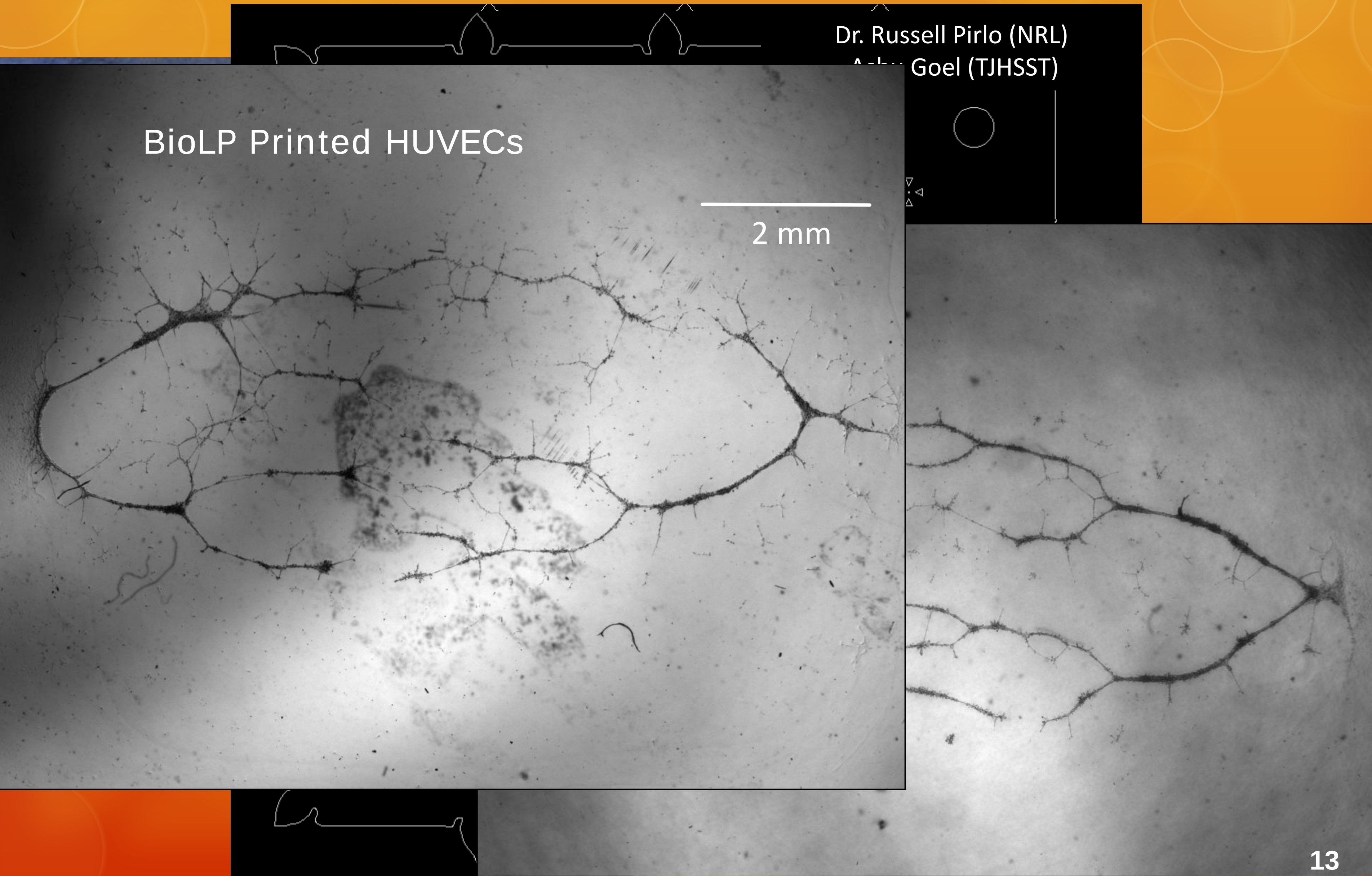
Stacked Biopaper Approach to Bioprinting 3D Tissue Constructs

- Utilize micro- and nano-fabrication tools to create thin layers of biodegradable tissue scaffolds
- Biopapers can be used with or without bioprinting
  - Without Cell Printing: Stand-alone, biodegradable improvements to traditional PET transwell inserts to create/study/use *in vitro* barrier tissues
  - With Cell Printing: Insert biopaper into any bioprinter and it immediately yields a 3D print substrate that can be made of virtually any material with any micro- and nano-scale property
  - Stackable to create 3D structures of any given geometry
  - Allows cell differentiation (post-printing) to occur prior to stacking



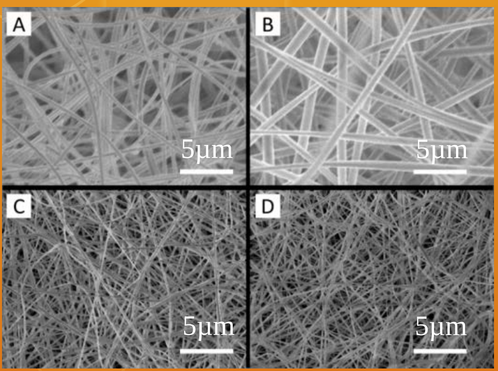


Recent Advances: Cell Printing to Biopaper and Creating Complex Micro-Vascular-Like Designs

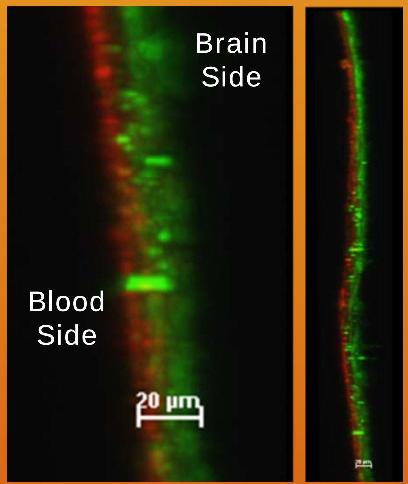


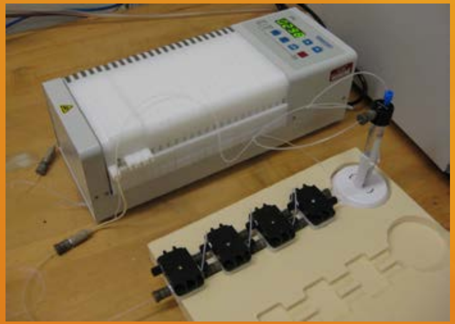
Recent Advances: Biopaper-based Blood-Brain-Barrier (BBB) Tissue Model and Infection Study

NRL Human BBB Tissue Model



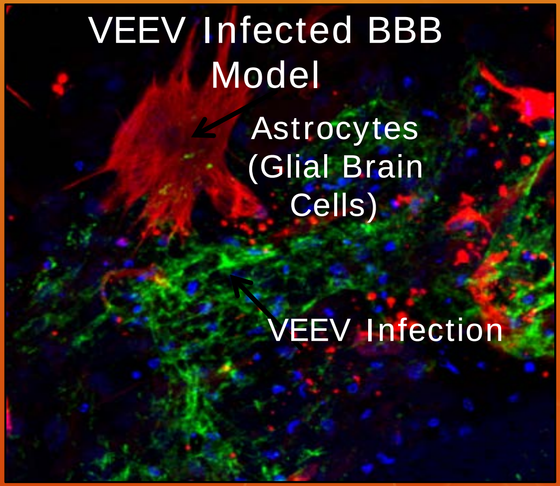
NRL Gelatin Biopaper
U.S. Patent #8,669,086

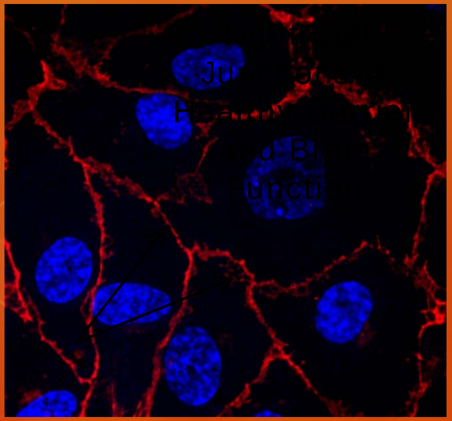


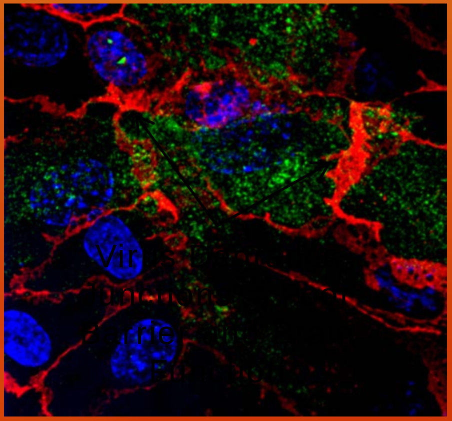


NRL Bioreactors Enable Transport to Ft. Detrick, MD

Venezuelan Equine Encephalitis Virus (VEEV) Infection at USAMRIID







Blood-Brain-Barrier integrity degrades after 24 hours post-VEEV infection

Dr. Russell “Kirk” Pirlo (NRL)
Dr. Lauren Bischel (NRL)
Dr. Connie Schmaljohn (USAMRIID)
Dr. Shannon Taylor (USAMRIID)

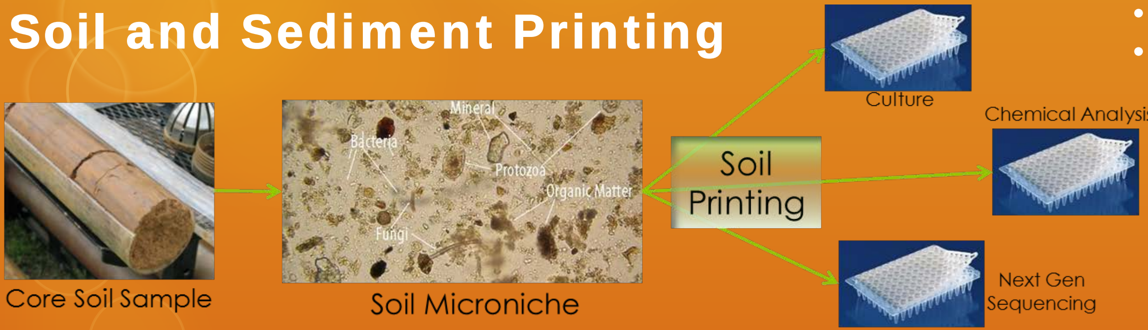
L.L. Bischel, P.N. Coneski, J.G. Lundin, P.K. Wu, C.B. Giller, J. Wynne, B.R. Ringeisen, R.K. Pirlo. *Journal of Biomedical Materials Research - Part A*, *in press* (2016).

DTRA Defense Threat Reduction Agency
USAMRIID United States Army Medical Research Institute of Infectious Diseases

NRL's 3D Bioprinting Center

A collaborative DoD facility that uses a multi-disciplinary approach to advance fundamental science and derive solutions in energy, diagnostics and therapeutics

Soil and Sediment Printing

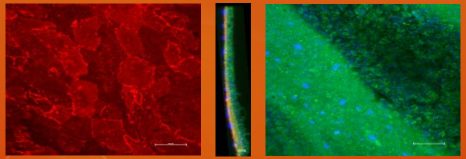


High Throughput Analysis and Culture

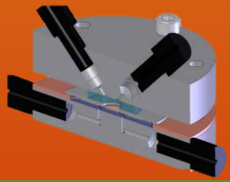
- Culturing the unculturables
- Bioprospecting natural products for energy and medical countermeasure applications

DARPA · Office of Naval Research Science & Technology · George Mason University

In vitro Tissue to Study Bio-Agent Infection



Engineered Air/Lung Tissue Interface



Bioreactor for Aerosol Exposure

- Lung and blood-brain-barrier tissue models
- Studying viral and bacterial bio-agent infections
- Faster and more accurate agent detection, diagnosis and treatment

DTRA Defense Threat Reduction Agency · Harvard · USAMRIID United States Army Medical Research Institute of Infectious Diseases · Biodefense solutions to protect our nation

3D Bioprinting Consortium

- Multi-user facility
- Focal point for inter-DoD collaborations
- Hearing loss, TBI, chem/bio agent defense, radiation exposure, trauma-induced arthritis
- Validation of DoD-funded bioprinting programs

NRL & Navy BUMED

USU · Walter Reed National Military Medical Center · DHA Defense Health Agency

ManTech– Under Secretary of Defense for Acquisition, Technology and Logistics (ATL)

- Manufacturing Innovation Institutes (MII)
  - Currently 6 MIIs ranging across manufacturing disciplines and include additive manufacturing, flexible electronics, advanced fabrics and nano-photonics
  - Requires AT LEAST $75M cost-share from academia and industry
  - 1:1 match of funding from DoD and can be in excess of $100M
- Soliciting input on 2 additional MIIs RIGHT NOW!
  - I am the topic lead for one of these topics called “Bioprinting Across Industrial Sectors”
  - Regenerative medicine, *in vitro* tissue assays, tumor/oncology, energy/biofuel, cosmetics
  - Response time has been extended to April 7th
  - Check FBO.gov or Manufacturing.gov
  - https://www.manufacturing.gov/upcoming-industry-led-workshops-or-forums-addressing-the-dod-manufacturing-innovation-institutes-request-for-information/

Acknowledgements

- Funding was provided by ONR through 6.1 and 6.2 BLK NRL funds and the Defense Threat Reduction Agency (DTRA)
- Thanks to numerous collaborators including:
  - Dr. Russell Kirk Pirlo (NRL Scientist)
  - Dr. Lauren Bischel (NRL PD)
  - Dr. Connie Schmaljohn (USAMRIID)
  - Dr. Michael Lindquist (USAMRIID)
  - Dr. Shannon Taylor (USAMRIID)
  - Prof. Peter K. Wu (Southern Oregon University)
  - Dr. Jason Barron (ex-NRL PD)
  - Dr. Doug Chrisey (Tulane)– inventor of laser direct write technology