3D bioprinting for medical and biotechnological applications

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tu-dresden.de/med/tfo + www.biofabrikation.de
Bioprinting

- Integration of cells during scaffold fabrication
- Spatially controlled positioning of one or more cell types
- Combination of cell encapsulation and additive manufacturing
  - Complex constructs as **tissue models**
  - Basic technology for **tissue & organ printing**
  - Probably new applications in **biotechnology**

Scheme: Utrecht Univ., Hydrozones bioprinting
Common AM technologies

Common methods for 3D bioprinting

- **Inkjet printing**
  - thermal
  - piezoelectric

- **Robotic dispensing**
  - pneumatic
  - piston
  - screw

Single cells or cell aggregate: ✓ high resolution
Cells suspended in hydrogels: ✓ high volume

3D bioprinting with cell aggregates

Smooth muscle cells

Ovary cells (CHO)

Fibroblasts

C. Norotte et al., *Biomaterials* 2009, 30, 5910
Design template for tubular structures

Pink: supporting agarose rods
Orange: spheroids

C. Norotte et al., *Biomaterials* **2009**, *30*, 5910
3D bioprinting with cell aggregates

Building a double-layered vascular wall

Pink: supporting agarose rods
Green: smooth muscle cell aggregates
Red: fibroblast aggregates

C. Norotte et al., *Biomaterials* 2009, 30, 5910
3D bioprinting with cell aggregates

V. Mironov et al., Biomaterials 2009, 30, 2164

C. Norotte et al., Biomaterials 2009, 30, 5910
Functional tissue models: “Organ-on-a-chip”

Human Cells
- Primary, or iPS
- Normal or diseased

NovoGel® Bio-Ink
- Cell mixture
- Proprietary media
- Spatial control

NovoGen Bioprinter® Platform
- Biocompatible
- Multimodal
- Spatial control

Human Tissues
- Reproducible
- Scalable
- 100% cellular (scaffold-free)

Ex Vive™ Liver

J. C. Irelan et al., Eurotox 2016 (Poster)
Methods for 3D bioprinting

Inkjet printing
- thermal
- piezoelectric

Robotic dispensing
- pneumatic
- piston
- screw

Single cells or cell aggregate
- high resolution

Cells suspended in hydrogels
- high volume

Extrusion-based bioprinting

Printing → Stabilization (crosslinking)
Extrusion-based bioprinting: challenges

- New strategies
- Easy 3D fabrication
- Cell culture
- Polymer concentration, crosslink density, stiffness

Main strategies

External stabilization

Technical solutions

Internal stabilization

Core/shell bioprinting

D. Kilian et al., *MRS Bull.* 2017 (accepted)
Suitable bioink for extrusion-based bioprinting

3% alginate

3% alginate/9% methylcellulose (alg/MC)

Crosslinking after plotting: incubation in 100 mM CaCl$_2$ for 10 min

Adjusting the viscosity

Strong increase of viscosity by addition of methylcellulose to 3% alginate
→ Allows plotting of 3D scaffolds in air

Bioprinting of hMSC suspended in Alg/MC

hMSC = human mesenchymal stem cells

MTT staining of viable cells

Nile red staining of lipid vacuoles

30 Layer scaffold with embedded hMSC, 1 d after printing

Adipogenic differentiation of embedded hMSC, 21 d after printing

Example for tissue printing: skin

4 components:
(a) hFB, (b) Plasma, (c) CaCl$_2$ und (d) hKC

N. Cubo et al., *Biofabrication* 2017, 9, 015006
Example for tissue printing: skin

After 17 d of differentiation at the air-liquid interface

N. Cubo et al., *Biofabrication* 2017, 9, 015006
Challenges for printing of complex tissues

- For clinically relevant tissues/organs: enormous quantities of cells are needed (e.g. for liver: ca. 100 billion = $10^{12}$ hepatocytes)
- High complexity: various cell types, defined arrangement
- Fabrication of volumetric constructs versus precise arrangement of small groups of cells into functional units
- Biomaterials: suitable for processing by AM, enable fabrication of constructs of sufficient stability, ensure cell survival and function
- Hierarchically structured vascular system, microsurgically connectable to host vasculature (e.g. liver: 3 x blood, 1 x bile = 4 independent tubular systems!)
Main problem for clinical application: vascularisation

Liver

http://hepatitis-c.de/leber2.htm

Coculture hMSC+HUVEC

red: CD31, blue: cell nuclei

M. Quade et al.
Direct printing of tubular structures

A. R. Akkineni et al., Biofabrication 2016, 8, 045001
Direct printing of tubular structures

3D printed scaffold of hollow strands

Direct printing of tubular structures

3D printed scaffold of hollow strands

MTT staining of viable cells

Cell nuclei stained in blue
A. R. Akkineni et al., *Biofabrication* **2016**, *8*, 045001
3D printed model of the renal proximal tubule

PTEC = Proximal tubule epithelial cells

3D printed model of the renal proximal tubule

Bioprinting offers opportunities for:

- Immobilisation of cells in macro-porous constructs
  → Possibility for continuous perfusion culture
- Combination of more than one cell type and local positioning with good spatial control
  → Setup of multicellular systems for cascade reactions, e.g. for drug production
Microalga: *C. reinhardtii*

Alg/Aga/MC –
3D bioprinting of plant cells

Embedding of *Ocimum basilicum* cells

green: live, red: dead cells

Collaboration with Dr.-Ing. Juliane Steingröwer (Bioprocess Engineering, TUD)

J. Seidel *et al.*, *Biofabrication* 2017 (in revision)
Summary

3D bioprinting develops very fast in the moment:
✓ New printing technologies
✓ New printing materials ("bioinks")
✓ Stem cell technology (pluripotency, differentiation)
✓ Advanced cell culture systems for expansion
✓ Coculture models: towards functional units

Simple tissues (cartilage, skin) and very small units can already be manufactured (e.g. as test systems)

Main problems: upscaling, vascularisation, suitable cell sources

Novel applications in biotechnology
Thank you for your attention!

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→ www.biofabrikation.de