

Assignment 13: Artificial Organs

EN 585.729 Cell and Tissue Engineering

Problems

1. We explored cell and organ printing as strategies to support the fabrication of artificial tissues and organs. Read Chapter 9 in Introduction to Tissue Engineering: Applications and Challenges by Ravi Birla and then complete the following problems:

(A) Discuss the feasibility of using cell and organ printing to support the fabrication of an artificial liver.

Today there are different technics which can be used for cell and organ printing to create an artificial liver (Birla).

- Hepatocytes are the primary functional cell type of the liver; they are responsible for most of the functions of the liver. A variety of strategies using different chemical compounds, can culture stem cells (ESSs, iPSCs) to form endodermal cells, then hepatic progenitor stem cells followed by differentiation to form hepatocytes. Bone marrow MSCs cultured in a 3D PCL nanofiber scaffold have formed mature hepatocytes using chemical conditioning. There are examples of 3D cultures using biocompatible hydrogels (or bio inks) to culture hepatocytes expressing high levels of albumin (Lee et al.) or co-culture (HUVECS + ADSCs; Ma et al.) to form hepatic progenitor cell (HPCs) with enhanced functional and phenotypic maturation. However, 3D culture is more adapted to recapitulate the biological functions of the liver and reproduce the spatial microarchitecture and biochemistry of its ECM components, and the presence of multiple cell types. Spheroid culture technics promoted by custom bioreactor has maintained cell phenotype and function.
- Collagen, fibrin, Matrigel and alginate are biodegradable hydrogels. Specific technologies (e.g., direct injection) can control the properties of alginate to create 3D scaffold with specific porosity, architecture, degradation rate and mechanical stiffness similar to liver tissues (Jeon et al.). Acellular 3D ECM scaffolds (for ex. porous PLLA scaffold) have shown to provide an excellent environment for in vitro growth of primary hepatocytes.
- Strategies like robotic protein printing, photo-responsive culture surface, or PDMS stencils can regulate cell distribution and the placement of different cell types on the culture surface promoting specific cell-matrix interactions.
- By optimizing printing parameter such as gelatin or polymer concentrations, temperature, pressure, printing speed, extrusion rate, and cell density, recent studies have addressed the challenge of angiogenesis by constructing 3D scaffolds with blood vessel-like channel mimicking the liver vascular network (Mohanty et al., Xu et al., Zhang et al.).

- Ex-vivo continuous or multi-step perfusion flow strategies have been key in the localization of liver cells on decellularized scaffolds. And bioreactors with a perfusion system using a peristaltic pump significantly improved functional performance of the culture.

(B) Describe the advantages and disadvantages of using this technology in liver tissue engineering.

Advantages of cell and organ bio-printing

- Alternative to the chronic shortage of donor livers
- Low in cost compared to other strategies
- Biomimetic improved compared to other methods: it could replicate the anatomy, cellular content and the function of the natural organ

Disadvantages include:

- Unwanted cellular interactions
- Time-consuming
- Altered cell-geometry and signaling pathways
- Monitoring solutions are required to assess cell destruction and loss
- Strategies for vascularization need refinement and the development of a mature functional vasculature in a timely manner has yet to be engineered.

There are four bioprinting methods, each of them has its own advantages and disadvantages:

Bioprinting Methods	Inkjet	Micro-extrusion	Laser-assisted	Stereolithography
Advantages	<ul style="list-style-type: none"> • High speed • Availability • Low cost 	<ul style="list-style-type: none"> • Ability to use high viscosity bio-ink • Print high cell density 	<ul style="list-style-type: none"> • High degree of precision and resolution • Ability to use high viscosity bio-ink and print high cell density 	<ul style="list-style-type: none"> • High degree of fabrication accuracy • Low printing time

(Bishop et al.)

Bioprinting Methods	Inkjet	Microextrusion	Laser-assisted	Stereolithography
Disadvantages	<ul style="list-style-type: none"> • Lack of precision in droplet placement and size • Need for low viscosity bio-ink 	<ul style="list-style-type: none"> • Distortion of cell structure 	<ul style="list-style-type: none"> • Time consuming • High cost 	<ul style="list-style-type: none"> • Use of high intensity UV light • Lengthy postprocessing • Lack of compatible materials

(Bishop et al.)

Each bioink types used in cell and organ printing have their advantages and drawbacks:

Bioink Type	Natural	Hydrogels	Synthetic
Advantages	<ul style="list-style-type: none"> • Highly biocompatible 	<ul style="list-style-type: none"> • Hydrophilicity allows for easy exchange of gases and nutrients • Highly biocompatible • Easily modified 	<ul style="list-style-type: none"> • Easily tailored functional groups • Non-immunogenicity
Disadvantages	<ul style="list-style-type: none"> • Limited modification • shear thinning 	<ul style="list-style-type: none"> • Poor cell seeding • Poor mechanical properties 	<ul style="list-style-type: none"> • No cellular attachment sites

(Bishop et al.)

(C) Develop a strategy to implement cell and organ printing to support artificial liver fabrication.

To create an artificial liver, we will follow previous research related to 3D tri-culture model which compared to simple or 2D monolayer has shown phenotypic and functional enhancements [(Bishop et al.), (Mazza et al), (Kryou et al.)].

1. We start by following previous published protocols to differentiate hiPSCs into mature hepatocyte-like cells (HLCs). We maintain in parallel, human umbilical vein endothelial cells (HUVECs) and adipose-derived stem cells (ADCs) in culture media to act as supporting cells for the hepatocytes. We then encapsulate HLCs, HUVECs and ADCs mixing them (in proportion of 3:1:1) into a hydrogel as an encapsulation material (e.g., collagen). Characterization of the cells along the differentiation stages, and measurement of the expression levels of liver-specific genes, are performed to determine the quality of the mature hepatic cells. In addition, albumin level production is assessed following encapsulation.
2. Using a CAD/CAM system, we create a 3D scaffold by stacking 2D patterns from a synthetic biocompatible and photopolymerizable hydrogel with stiffness similar to natural liver tissues. A gel solution with growth factors to support neovascularization

(VEGF) is added to the hydrogel. Photopolymerization of the hydrogel solution create digital masks resulting into patterns resembling the human liver lobules.

3. After fabrication of 3D cell construct, the collagen encapsulating the HLCs, HUVECs and ADCs; is deposited within the lines creating a 3D in vitro hepatic model representing the in vivo liver structure.

Reference:

- Birla, Ravi. *Introduction to Tissue Engineering: Applications and Challenges*. IEEE Press, Wiley, 2013.
- Bishop, Elliot S., et al. "3-D Bioprinting Technologies in Tissue Engineering and Regenerative Medicine: Current and Future Trends." *Genes & Diseases*, vol. 4, no. 4, Dec. 2017, pp. 185–95. *DOI.org (Crossref)*, <https://doi.org/10.1016/j.gendis.2017.10.002>.
- Kryou, Christina, et al. "Bioprinting for Liver Transplantation." *Bioengineering*, vol. 6, no. 4, Oct. 2019, p. 95. *DOI.org (Crossref)*, <https://doi.org/10.3390/bioengineering6040095>.
- Mazza, Giuseppe, et al. "Liver Tissue Engineering: From Implantable Tissue to Whole Organ Engineering." *Hepatology Communications*, vol. 2, no. 2, Feb. 2018, pp. 131–41. *DOI.org (Crossref)*, <https://doi.org/10.1002/hep4.1136>.

Rubric

Question	Component	Total Point Value
1	A	10
	B	10
	C	10
Total		30