Teaching Tools were prepared by Karina Perlaza and PoAn Brian Yang.

Contents

1. Keywords and Terms
2. Lecture Notes
3. Review Questions
4. Answers to Review Questions
5. Discussion Questions
6. Answers to Review Questions
7. Explain or Teach These Concepts to a Friend
8. Questions for Discussion Paper
9. Answers to Questions for Discussion Paper

1. Keywords and Terms

Liver, hepatocytes, microenvironment, tiny technology, drug discovery, P.falciparum, malaria
Tissue engineering of the liver:

- The liver has over 100 billion cells and 500 different functions in the body
- 300 million people worldwide have liver disease
- Scar tissue occurs in liver damage
- Scar tissues (cirrhosis) increase cancer risks

The best solution is to replace the liver through whole organ transplantation. However, there are not enough donor organs available.
Because of how vital the liver is and the lack of donor organs, groups have thought about supporting liver function through cell-based therapies.

Hepatocytes are liver cells that perform most of the 500 functions of the liver.

**Extracorporeal Device**: Devices outside of the body that could house hepatocytes. Blood would run out of the body and into the machine to be processed.

**Implantable Constructs**: Constructs that can be implanted and support the function of the liver as it regenerates.
Problem: Hepatocytes in devices do not perform the 500 or so functions of the liver.

Cause: The microenvironment of the liver is disrupted in these devices.

Microenvironment:

- Repeating unit called acinus
- Hepatocytes align into structures called hepatic plates
- Along each hepatic plate is a blood vessel
**Problem:** The structure is small, at about 10-100 um length scale.

**Solution:** In computers, we’ve seen how we’ve been able to go from a single transistor to 100 million transistors in the same area due to the technology called photolithography.

This is done by shining light on photo-sensitive material and making a pattern through this process. We can take this same technology and use it to make microenvironments for the liver.
When cells were randomly distributed versus when they are plated in a certain pattern, scientists have seen a striking difference in the functions that they perform.

**MEMS:** Microelectromechanical systems that is done by etching techniques. The example shows two interlocking combs with a hepatocyte on one side and supporting population on the other side.

What they’ve learned from MEMS is that the cells need to touch each other for about a day, and after the first day, soluble factors are produced and can support hepatocytes.
Long chain polymer with reactive end groups + light sensitive chemical + cells = 3D hydrogel for hepatocytes after light is shined on the mixture.

One can change the pattern of light that one shines and create different structures.

When looking at albumen secretion (a liver function), a graduate student has shown that the more structure, cell to cell interaction (endothelial cell with hepatocytes), and ECM interaction, the more functional the hepatocytes are.
Drug discovery pipeline:

15 years and approximately 1 billion dollars.

**Problem:** After in vitro and animal screens, in phase I of the clinical trials, about 1/3 of the time the drug has toxicity to the human liver and thus will not pass the trial.

**Solution:** Bridge the gap by making an in vitro engineered human liver to test drug delivery and potential side effects.
How does it work?

Once a drug is developed, there won’t be too much of that compound, therefore a high throughput method is required.

**Multiwell device:**

At the bottom of each well are micro-patterned hepatocytes and fibroblast cells. This is done by:

1. Having stencil at the bottom of each well
2. Pour collagen into well
3. Plate human hepatocytes onto ECM
4. Co-culture with supporting cells
Another use is to look at disease models: Malaria - Does not infect animal models, must be studied in human.

1. A motile infective form of the plasmodium to the host
2. The plasmodium travels through the blood vessels to liver cells
3. It reproduces asexually in hepatocytes.
4. Bursts the hepatocytes and goes into the bloodstream and infects the red blood cells.

If we can kill the plasmodium while it’s still in the liver and before it’s in the bloodstream, we can prevent symptoms and also prevent spread of disease.
Tiny technology is very powerful for studying microenvironments in tissues. Though the example shown is in livers, it’s broadly true for all tissue types because the scales of these repeating units are in the same micro-scale.

There are now tiny technologies from 100 um scale all the way down to nm scale that are ripe to be borrowed.

**Conclusion:** We can use this technology to mimic microenvironments to construct, interrogate, and interact with the in vitro tissue models such as the liver.

### 3. Review Questions

1. According to the introduction, what’s important about the liver? Why are people interested in studying it?

2. What is an extracorporeal device and how does it help a patient?

3. What solution from computer technology did scientists borrow to overcome the micro scale structures required for hepatocyte functions?
4. Answers to Review Questions

1. The liver provides more than 500 different functions for the body. People are interested in the liver because liver disease is so prevalent and yet organ transplantation, the best solution, is often not available.

2. It's a device outside of the body that would house hepatocytes. Blood would run out of the body and into the machine and be processed. It helps the patient by allowing the liver to recover and regenerate, and replaces the function of the liver when necessary.

3. D. Photolithography is the correct answer.

4. Fibroblast and hepatocytes need to touch each other for about a day, and after the first day, soluble factors are produced and can support hepatocyte functions.

5. After in vitro and animal screens, phase 1 clinical trials show about 1/3 of the drugs have toxicity to the human liver. The improvement that can be made is to engineer an in vitro human liver to test drug delivery and identify side effects.

6. The parasite does not infect liver of animal models such as rat and mouse, and since the number of parasites in the liver is limited and during the liver phase malaria is without disease symptoms, it would be beneficial to discover ways to kill the parasite while it's still in the liver.
5. Discussion Questions

1. Relevance: How does the therapeutic approach for liver diseases contrast with other organ systems? What is the microenvironment of hepatocytes? What is a major challenge that has hindered the advancement of cell based therapeutic strategies?

2. Construct: Describe two examples of light-based microfabrication techniques used in hepatic tissue engineering. What features do these techniques control for and why is this important? What would you design using one of these techniques?

3. Interrogate: What are some applications for the arrays of liver tissue in drug development? Can you envision a biological question that may be addressed via these micro-patterned co-cultures, be it with liver arrays or other organ arrays?

6. Answers to Discussion Questions

1. Due to the scale and complexity of the liver structure, developing therapeutic strategies is challenging. Currently, there are no therapeutic approaches that collectively augment the collection of affected functions. This approach contrasts with other organ systems like the heart because the deteriorating tissues can be treated with medications that improves the contractility without the need of immediate transplantation. In liver failure, an organ transplant has been the only permanently effective therapy to date.

As mentioned in the talk, the microenvironment of hepatocytes is complex with the liver performing about 500 functions.

All hepatocytes are lined with blood vessels, which enables them to process blood very efficiently, and to produce secretory proteins, such as albumin, for the blood stream. liver-specific functions and the ability to replicate in an in vitro setting. The microenvironment requires signals from soluble factors, extracellular matrix components and heterotypic cell-cell interaction that cannot be recapitulated when culturing these cells in-vitro. Thus, the recreation of an in-vivo setting is important to study the biology of hepatocytes and furthermore to one day use them for therapeutic solutions.

2. Sangeeta Bhatia describes many light-based microfabrication techniques in her second talk. One of them is the micropatterned co-culture. These experiments lead to the observation that having hepatocytes either with homotypic or heterotypic cells
increases the mortality of these cells. Another example is that of microelectromechanical systems (MEMS) which was used to create transient interactions of heterotypic cells and discovered that the interaction need only persist transiently for the cell to retain its function. It does not require persistent contact. Another example of light-based microfabrication is of a 3D stereolithography printer. The robot moves the stage to cross-link the light-reactive polymer in regions dictated by the design made through the CAD software.

The follow-up design question raised interesting examples:

- heart scaffold
- liver
- heart valves

3. In vitro liver models facilitates the studies of the behavior of pathogens that target hepatocytes, including hepatitis C virus and malaria. Also, high throughput screening of drugs can assess the pharmacokinetics, metabolism and toxicity of new drugs. This can save a lot of money since it can screen against drugs that can potentially be toxic or rejected by the liver.

The second part of this question can result in a discussion about modeling of the full liver stage of *P. falciparum*. Since this was a topic of discussion in Sangeeta Bhatia’s talk, it can be insightful to review the *P. falciparum* life cycle. This can be used to discuss any potential experiments that might prove this system a successful recapitulation of the liver stage infection of *P. falciparum*. A paper in the journal Cell Host & Microbe, March et al., addresses this question. http://www.ncbi.nlm.nih.gov/pubmed/23870318

7. Explain or Teach These Concepts to a Friend

1. Explain the overall function of the liver in the human body.

2. Explain the drug discovery pipeline.

3. Explain how tissue engineering can be used to help discover drugs or cures for diseases.
8. Questions for Discussion Paper

Discussion Paper:


1. What are the steps for the InVERT molding technique? Briefly describe figure 1a (or 2b) and redraw the steps on the board.

2. What experiments were done to further characterize the new InVERT technique and why were they important when trying to understand the relevance or versatility of this new technique? In the first part of the iBiology lecture, Sangeeta Bhatia talks about designing the right biomaterial. What aspects of the InVert technique allow for biomaterial design?

3. Describe how interpenetrating, juxtaposed and paracrine conformations were patterned (figure 3). How do the organization of compartments (figure 3 and 4 a,b) relate to the importance of cell organization discussed in the first part of Sangeeta Bhatia’s talk?

4. What experiments were done with the nude mice in figure 4? What other experiments would you have done?

9. Answers to Questions for Discussion Paper

1. Topographic substrates that contain microscale features were replica molded using poly(dimethylsiloxane) (PDMS) to create a topographic ‘intaglio’ cell-capture substrated with recessed voids. The cells were then added in the solution then isolated in the intaglio cell-capture substrates with centrifugation and then they were embedded in a 3D hydrogel. The hydrogels that contained the patterned cells were removed from the intaglio substrate and inverted which expose the ‘relief’. The microscale features from the first cell population project from the hydrogel. Then, a prepolymer solution with the second cell population is loaded on top of the inverted hydrogel and centrifuged into the relief. Polymerization was triggered to encapsulate
the cells thus yielding a 3D hydrogel containing micropatterned cellular compartments.

2. The researchers wanted to test the scalability, referring to the size of the tissue, as well as the patterning fidelity, referring to percentage of microstructures that were successfully patterned in a macroscopic hydrogel. They showed the versatility of this new molding technique by utilizing topographic patterning substrates from other sources. In addition, they found that one of the main advantages of this technique is the ability to separate the cell capturing and encapsulation into two distinct steps. This allows for cell encapsulation with different natural and synthetic materials with diverse properties. For example, the encapsulation can be made with fibrin, agarose and polyethylene glycol all of which have unique features. An interesting but not surprising finding due to the knowledge accrued from the first lecture was that the material used dictated the pattern maintenance. Another supporting evidence on the versatility of this technique is the ability to use ‘sensitive’ cell types. Since the molding is based on cell sedimentation, the process is robust regardless of the cell-specific properties that impact cell patterning in other systems.

3. The authors found that organization of stromal compartment alters the iPS-Hep function by patterning heterotopyc cells in different forms. They were patterned in a compartmentally distinct lattice (‘paracrine’), adjacent to iPS-Hep aggregates that had already been compacted (‘juxtaposed’), and directly into the wells with the iPS-Heps (‘interpenetrating’). They found that the interpenetrating method resulted in greater albumin production, a proxy for hepatic function.

In addition to looking at the compartmentalization and how it affects the iPS-Hep function, they also looked at whether the number of hepatocytes per homotypic aggregate would affect liver functionality. They found that 100 hepatocytes per homotypic aggregate was ideal. They also found that a ratio of 1:2 hepatocyte: fibroblast yielded the highest functionality. Together, the results provide strong evidence that tissue architecture optimization modulates the hepatic function in vitro.

4. The experiment consisted of implanting hepatocytes expressing luciferase under the control of an albumin promoter and patterned in tissues that contained heteroaggregates of hepatocytes and fibroblasts. When they explanted the tissues they saw that the patterned aggregates were generally retained. In addition, they found red blood cells in some of the explants suggesting that there were de novo vessels derived from the host. The second part of the question can yield various responses. For example:
a. experiments that show that these cells can be grafted, survive and rescue absent liver functions in liver disease model systems
b. hepatocytes are in proximity to 4 different cell types (part II of Sangeeta Bhatia’s talk) thus it would interesting to recapitulate all 4 cell types in a pattern that’s closest to physiological conditions