Why do we need to bioengineer tissue? Isn’t it true that there are better alternatives out there? Yes, if there are ways to improve the health of the patient through: devices, drugs, minimally-manipulated cells, surgical reconstruction, or transplants. Then we would not need bioengineered tissues. However, often these options are not available, and we must turn to bioengineered tissues.

What are engineered tissues? The basic definition here is biomaterials and cells in various combinations. Examples include biomaterials that recruit cells, biomaterials that house cells, structures such as ECM (biomaterial-free).

In order to design bioengineered tissue, we must first think about the function that we are interested in. This is because often we are not able to replace all of the function of a tissue.

For example, skin replacement lacks hair follicles, sweat glands, immune cells...etc.

So Step one: The engineer must make a deliberate choice to go after the function that is most critical for life due to limitations in technology.
Step two: Pick the raw ingredient and fabrication method.

The **somatic cells** can be from the person (auto-), from another person (allo-), or from another species (xeno-). We can also use **stem cells**, biomaterials, and of course nutrients which are required for putting cells and biomaterials together.

Then after the raw ingredients have been picked, we need to think about how to put these raw ingredients together. This is the process of fabrication through assembly, bioprocessing, and preservation.

Somatic Cells:
Can be derived in various ways, from adult, embryo, or egg. Regardless, they are mechanically dissected and depending on how finely they are processed following dissection they are categorized differently:
Slightly processed: Organ cultures. Grown in environments that mimic that in vivo model.
Finely processed: Explant cultures. Allow cells to grow out of these cultures.
Enzymatically digested: Cell cultures. Cultured in flasks and passage cells to grow and expand the culture.
Stem Cells:
There are a number of ways to obtain stem cells. The most exciting is the fact that we can personalize stem cells. How?

**Nuclear transfer:** Taking the nucleus of a somatic cell and transferring it to an enucleated egg.

**Cell fusion:** Take the somatic cell and fuse it to an embryonic stem cell.

**Induced pluripotency:** Express factors in somatic cells that will reprogram the somatic cells into stem cells.

Biomaterials:
We want to match the degradation of the biomaterial with the synthesis of ECM in the host. The material must last long enough, but if it persists too long, there will be a foreign body immune response.

**Concept originated in experiment with skin:** Looked at wound healing in an animal model. When there’s no scaffolding there will be scars, if the material degrades too fast, scars develop, but further cross linking of material so it degrades slower improved scarring. However, when material is too tough it further causes scarring.
What are these biomaterials now that we know about the importance of their properties?

**Synthetic Scaffolds Examples:**
Polymers: Macromolecules with repeating structures. When degradation occurs due to hydrolysis in the body, the degraded compounds are the same as those already present in the body.

PEG-DA: Can be cross-linked by adding photo-sensitive reagent to the cell, upon interaction with light it will form a cellular hydrogel that has cells embedded in the cross-linked PEG.

**Natural Scaffolds:**
Examples of a complex natural scaffold are whole organs that have been de-cellularized.

Complex natural scaffolds contains all the ECM, growth factors, and other bioactive molecules in the natural material.

The slide shows the de-cellularization and re-cellularization of a heart. We see the perfusion of the detergent and how all the cells are “melting” away, giving us a “ghost heart.”

**Advantage:** Reusing organs with cells from host as opposed to simply transplanting them from a donor.
Hierarchical organization of organ tissues

Cells respond only locally to their microenvironment. For cells to perform their function of interest, the cells must have their microenvironment stimuli.

Tissue engineers must maintain the cellular environment in order to achieve therapeutic outcomes.

How do we assemble these microenvironments?

One way is through 3D fabrication and assembly: Some people call this organ printing. It’s where a 3D drawing is made on a computer, the drawing gets sent to a robot that has a stage that sits in light sensitive material, model is then built layer by layer by shining light in a certain pattern according to the design.

One can imagine going from a 3D medical imaging data of a certain organ and printing a scaffold that will be suitable for replacing the organ.
How do we preserve the tissue to deliver them to the patients?

**Cryopreservation:**
Two different insults from preserving them from this way:

1. De-hydration injury due to slow cooling because water has time to escape.
2. Intra-cellular injury due to ice crystals that form inside when rapid cooling is done.

Different cells must be cryopreserved in different ways.

**Conclusion:**

- Engineered tissue
- Cells from somatic and stem cells
- Natural or synthetic biomaterials
- Hierarchical structures of tissue in organs
- Convergence of cell biology, medicine, and engineering is pushing the field forward
Part 1: Engineering Tissue Replacement

**Keywords**
Tissue engineering, Tissue replacement, hybrid device, scaffold, biomaterial, ECM

**Review Questions**

1. What are some different types of bioengineered tissues described in lecture that demonstrates different combinations of biomaterials + cells?

Examples given in lecture include acellular (biomaterials that recruit cells), hybrid (biomaterials that house cells), and hybrid with cell-derived ECM (biomaterial-free structures).

2. In designing bioengineered tissues, why is it necessary to first pick a function? How is this function chosen?

Because we are not able to replace all of the functions of the tissue, therefore we would make a deliberate choice to go after the function in the engineered tissue that is most critical for life.

3. What determines the type of cultured primary cells? What are they called?

The category is determined based on how cells are processed following mechanical dissection, either slightly processed, finely processed, or enzymatically digested. They are celled organ culture, explant culture, and cell culture.

4. What happens when biomaterials persist too long after implantation?

A) They get incorporated into the host body
B) They cause cell fusion to occur
C) They cause a foreign body response ← Correct answer
D) Nothing different happens
4. **What's an example of a synthetic scaffold that degrades but doesn’t harm the body? How?**

PLGA (or polymers is ok too): It’s a macromolecule with repeating structures of molecules that exist naturally in the system. Therefore when degradation occurs due to hydrolysis in the body, the molecules that have been broken down do not harm the body at low levels.

5. **Why is it important to understand the hierarchical organization of organ tissues?**

Cells respond only locally to their microenvironment. For cells to perform their function of interest, the cells must have their microenvironment stimuli. Tissue engineers must maintain the cellular environment in order to achieve therapeutic outcome.

6. **How does de-hydration damage occur in cells being cryopreserved?**

De-hydration damage injury happens due to slow cooling. The water molecules have time to cross the membrane, and the cell shrinks and a higher concentration of intracellular solute occurs.

**Discussion Questions:**

1. **Describe your favorite bioengineered tissue replacement mentioned in the first 12 minutes of the video. Given the opportunity, what tissue replacement would you engineer and why? What category would it fall in: acellular, hybrid, or hybrid with cell-derived ECM?**

Sangeeta Bhatia describes several examples of bioengineered tissue replacements and categorizes them into different groups: acellular, hybrid and biomaterial free. An acellular example of a bioengineered tissue includes the submucosa of small intestine. This material is first taken from porcine after which cells are removed before implanting into a human. It is an example of an acellular biomaterial because it does not contain cells, but rather a mechanically strong extracellular material that helps recruit cells. The skin is an example of a hybrid biomaterial because it is composed of a collagen-gel with dermal fibroblasts. Another example of a hybrid biomaterial is the bladder balloon in which cells are seeded inside with muscular cells surrounding the outside. Finally, an example of a biomaterial free is the blood vessel in which no scaffolding material from the outside is ultimately used albeit it originally came from a biopsy sample. Tissue replacements that can be interesting topics of contention include, but are not limited to:
~ in vitro meat- basically a combination of muscle cells and protein that promotes tissue growth
~ artificial kidney- same idea as the submucosa of small intestine, whereby they strip away cells to get a strong scaffold and then they seed the material with endothelial and epithelial cells
~ external female genitalia - probably not an idea that has been trialed, however, for women who have undergone female genital mutilation and would like the revert to their original condition then bioengineering a tissue would be very helpful

2. What is the major limitation in culturing primary cells and how do stem cells bypass this?

One of the major limitations in culturing primary cells is that they have a finite amount of cell doublings that they can undergo. This is due to the telomere shortening with age. Stem cells are not susceptible to this therefore strides are being made to utilize stem cells as part of biomaterials. Research into reprogramming cells with transcription factors is a promising avenue because it avoids any ethical issues associated with embryonic stem cells. Also, the reprogramming is done with the patients’ own cells, thus, epigenetic marks persist when cells are programed to a pluripotent state.

3. Why is it crucial to match the degradation of the biomaterial with synthesis of the ECM?

The design process of a biomaterial includes careful consideration of the degradation or persistence of the biomaterial. If the biomaterial degrades too quickly then the function it must perform wont be fulfilled. If the biomaterial persists indefinitely then a foreign body response is elicited. Understanding the characteristics of the endogenous, natural biomaterial is therefore very important. Fine-tuning of the biomaterials or repurposing natural scaffolds can achieve this end. Some important aspects to consider when designing the biomaterial are the rates of degradation and the microenvironments it is exposed to.

4. What are the two types of insults imposed on tissues by cryopreservation? Can you think of other biological substances in which this might also be an issue?

The two insults that were mentioned in the talk were due to either cooling of the tissue rapidly or slowly. If the cells are cooled slowly damage can occur in the form of dehydration and this can cause the cells morphology to shrink. If the cells are cooled too rapidly then formation of intracellular ice-crystals can damage biological machinery. This is cell type dependent and has a lot to do with the 3D form of the tissue. A biological substance in which cryopreservation is an
essential consideration is proteins. During X-ray crystallography, for example, cryocooling can lead to perturbed protein conformation or the favoring of one conformation over the other.

**Explain these concepts:**
Explain the difficulties and considerations for using tissue engineering.

Explain the advantages of tissue engineering.

Explain the function of a synthetic scaffold.
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 affects.

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 blood stream 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 blood stream, 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 100um 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.
Part 2: Micro-scale Liver Tissue Engineering

Keywords
Liver, hepatocytes, microenvironment, tiny technology, drug discovery, *P. falciparum*, malaria

Review Questions

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

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

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

   A) Techno-plating
   B) LED technology
   C) Laser printer technology
   D) Photolithography  <- Correct answer
4. What did scientists learn about hepatocytes from MEMS?

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. What is one improvement to the drug discovery pipeline that Dr. Bhatia discusses?

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. Why would an in vitro human liver system be beneficial for studying malaria?

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.

**Discussion Questions:**

**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?**

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.
A major challenge that has hindered the advancement of cell based therapeutic strategies is due to hepatocytes losing 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.

**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?

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

**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?

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.


**Explain these concepts:**

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

Explain the drug discovery pipeline.

Explain how tissue engineering can be used to help discover drugs or cures for diseases.

**Questions on the paper:**


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

Topographic substrates that contain microscale features were replica molded using poly(dimethylsiloxane) (PDMS) to create a topographic ‘intaglio’ cell-capture substrate 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.

**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?

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.

**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?**

The authors found that organization of stromal compartment alters the iPS-Hep function by patterning heterotypic 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.
What experiments were done with the nude mice in figure 4? What other experiments would you have done?

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:
~ experiments that show that these cells can be grafted, survive and rescue absent liver functions in liver disease model systems
~ 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