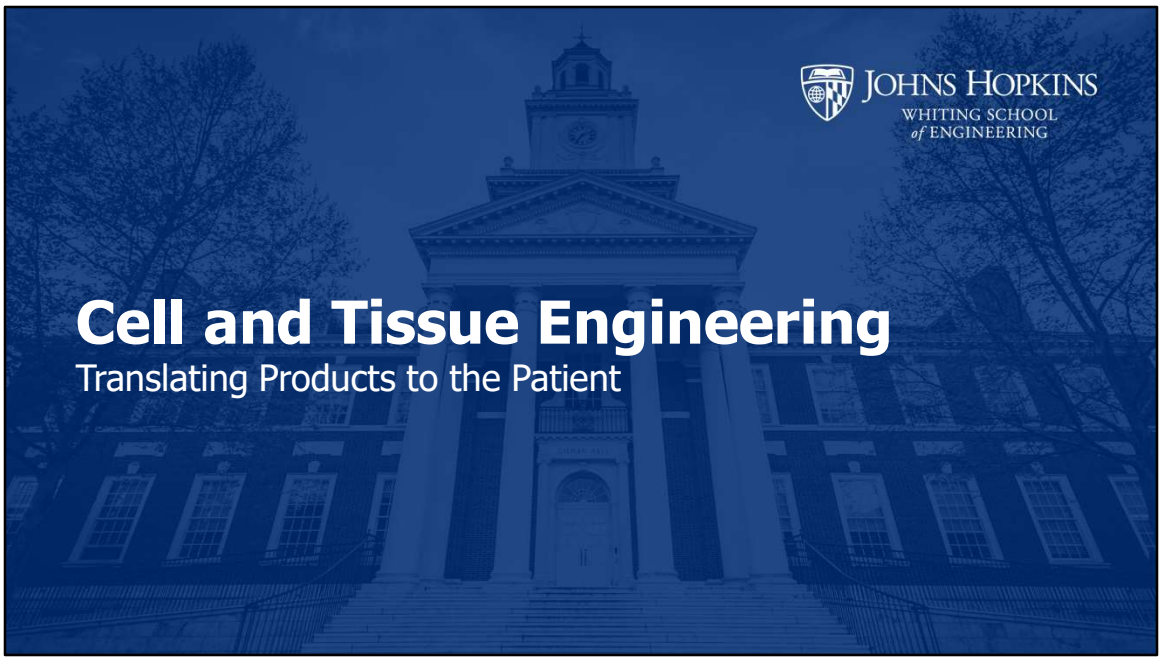
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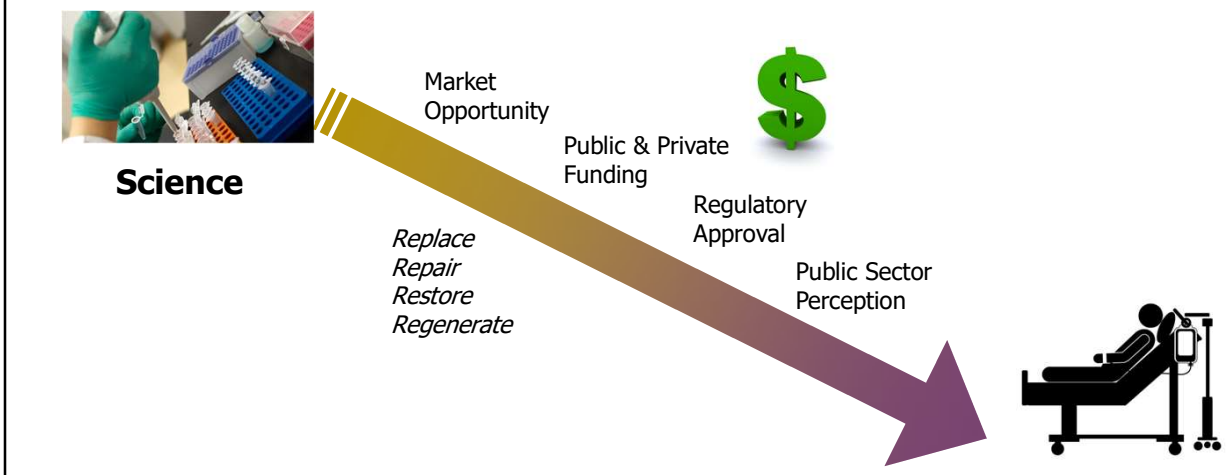
Cell and Tissue Engineering
Translating Products to the Patient

Key Determinants for Translating Science to Products



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Schematic edited from *Topics in Tissue Engineering*

Thus far in the course, it is clear that cell and tissue engineers are working hard to **replace**, **repair**, **restore,** and **regenerate** *human* tissues and organs.

But working out the details in the lab is just he beginning. For a product to make it into clinical use it needs to do *more* than work:

- it must have a **market opportunity**,
- obtain adequate **funding** for development,
- Pass through **regulatory** review, and at the end of the day
- be perceived as **beneficial** to the public and the firm.

The hurdles that come after the science are what we'll be discussing today.

Product Characterization – Cellular

Cellular Components

- Pathogen testing
- Species
- Gender
- Age
- Weight
- Surgical procedure

	Passage 2	
	Excision	Liposuction
CD11a (alphaL integrin)	1.1 ± 1.1	1.1 ± 0.8
CD11b (alphaM integrin)	0.8 ± 0.7	0.5 ± 0.6
CD18 (beta2 integrin)	0.4 ± 0.4	0.5 ± 0.4
CD29(beta1 integrin)	97.0 ± 1.5	96.6 ± 1.4
CD49d (alpha4 integrin)	64.6 ± 24.0	88.4 ± 9.2
CD49e (alpha5 integrin)	97.8 ± 1.2	97.9 ± 1.4
CD51 (alphaV integrin)	97.8 ± 0.8	97.3 ± 3.3
CD61 (beta3 integrin)	29.7 ± 33.2	40.5 ± 21.5
CD49b (alpha2 integrin)	72.6 ± 12.4	88.7 ± 11.1

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Katz et al., 2005; Amos et al 2008

To begin thinking about commercialization of a tissue engineered product, we need to define the **components**.

We know that cellular components can come from **animals**, **immortalized cell lines** or from autologous or allogenic **human sources**.

If it is from an animal source, you need to
- complete **pathogen** testing,
- know the **species**,
- plus a host of properties including the **gender**, **age**, and **weight** of the animal.

All of these characteristics can affect the **quality** of the cells (purity, potency).

You also need to completely define the surgical procedures for **harvesting** and **isolating** the needed cells.

One example of these affects is shown in the table. Here you can see that adipose derived stromal cells – these are an adult stem cell population found in fat tissue – behave differently when harvested via **liposuction** or via **tissue excision**.

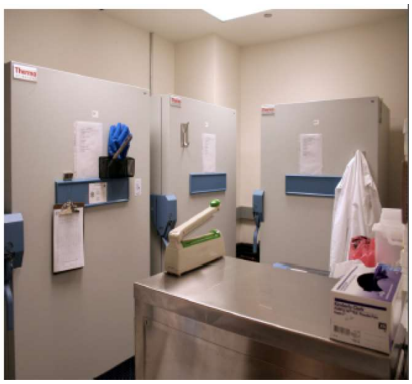
This table shows the **expression of adhesion molecules** on ASCs. Of particular note are the differences in the expression of **alpha 4, beta 3 and alpha 2 integrin**.

Functionally, the different harvest procedure translated to differential responses to **hypoxia** and **therapeutic potential**.

Product Characterization – Non Cellular

Noncellular Components

- Purification techniques
- Production methods
- Degradation rate
- Consumption rate
- Contaminants
- Storage history



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Cincinnati Children's Brooks

Non cellular components include the **biomaterials** – be that natural or synthetic, and supplied biomolecules, factors and proteins.

For these components, instead of characterizing surgical isolation, you may need to characterize other **purification** and **production** methods.

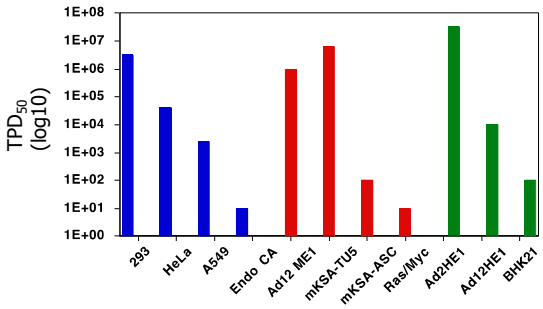
Additionally, for degradable materials, you will need to know the **degradation** and **consumption** rates – which are likely specific to the target tissue and patient population.

Finally, based on the material, you may need to know the **storage history** of the materials. This sometimes means **cold-chain management**. – that is not only **how** they were stored but how they were **transported** between storage sites.

Product Characterization - Safety

Safety

- Toxicity
- Physiologic effects
- Tumorigenicity



TPD_{50} values for cell lines of human, mouse and hamster origin

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FDA.gov

Beyond knowing **what** you have and **how** its been gathered, you also need to know a good deal about **safety–** at a start with regards to toxicity, physiologic effects, and tumorgenicity.

For toxicity, this means knowing if the components in your solution cause **acute** or **chronic** damage to the host. Toxicity effects could be as simple as production of an **apoptotic ligand** by your cells or a degradation of a polymer into **toxic byproducts**.

Physiologic effects are the effects on body systems – for example on **blood pressure** or **heart rate**.

And tumorgenicity, which just as it sounds, assesses the risk of tumor formation. This is a possibility from implanted cells, and also from the host's interaction with the non-cellular components. For example, the cells may form the tumor **directly or transfer oncogenic virus** which can cause cancers to form in the body.

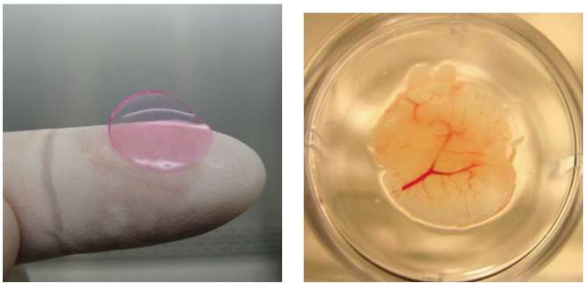
One way to assess tumorgenicity is by using the **TPD50** or tumor producing dose at 50%. This is simply the number of cells required for tumor formation. The fewer the cells required the more aggressive the cell type is for tumor formation.

This graph shows a typical TPD50 curve looks like for cells implanted in nude mice – mice lacking a functional immune response. This study used a number of cell lines, including those HeLa cells we discussed earlier this semester. On the right are cancer cells – A549 is an adenocarcinoma line.

Product Characterization - Efficacy

Efficacy

- Number of cells
- Type of cells
- Fate of cells
- Behaviors: adhesion, migration, production…
- Clinical endpoints: wound closing, protein expression…
- Product parameters: in vivo survival, growth rates…



Human Ectopic Artificial Livers

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http://www.nibib.nih.gov

Efficacy is extremely difficult to characterize with a tissue engineered medical product.

Using cells affords the unique advantage that they **are a thinking machine --** they make decisions and have responsive and adaptive behaviors in the body. However these advantages also make the characterization of their ability difficult.

Efficacy of a treatment can vary with properties of the cell population like **sheer numbers** used, the **fate capacity** of those cells, and their **behavioral** abilities.

With a well characterized cell type, you still need to test the efficacy of the trial with appropriate clinical outcomes.

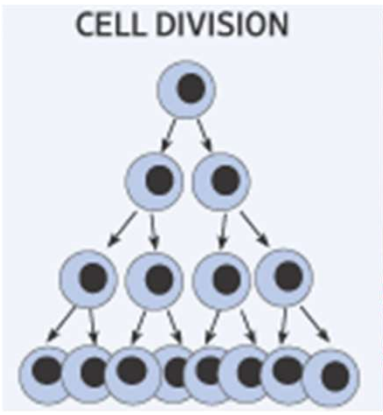
For a skin graft, this might mean measuring not only wound closure but also expression of relevant proteins.

Before reaching clinical trials, you also need to test in vivo with animal experiments. Animal models can be very nuanced, because cellular behavior is known to be host dependent -- therefore developing **humanized animal models** like this ectopic liver

are critical – they help test and define the impact of a tissue engineered product in a non-human model.

Product Preservation

- 1. Freezing
- 2. Dehydration



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Aging Ills Reversed in Mice, Naik, WSJ online

Ok – so say your product is fully characterized – you've really nailed down what's in it, and how it functions in vitro and in vivo.

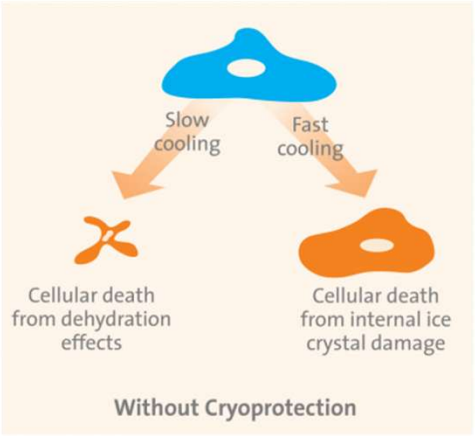
The next issue you need to tackle is **preservation**. How do you preserve the product so that you can have it ready **when** you need it or so that you can **move** it around the country or world to meet the demand.

Note that preservation is not something that only happens when the product is ready. You also need to preserve cells at intermediate phases like **sourcing** and **expansion**. For example you may need to stop cell proliferation at the second generation so that you have the ability to grow these cells later and just some of the cells now.

There are two main ways of preserving tissue engineered materials – the first is **freezing** and the second is **dehydration**.

Product Preservation - Freezing

- Freezing < -130oC



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Let's start by talking about **freezing** or **cryopreservation**.

At low temperatures, molecular motion is arrested, which stops degradation of cellular components. Cryopreservation is ideally completed below negative 130 deg C.

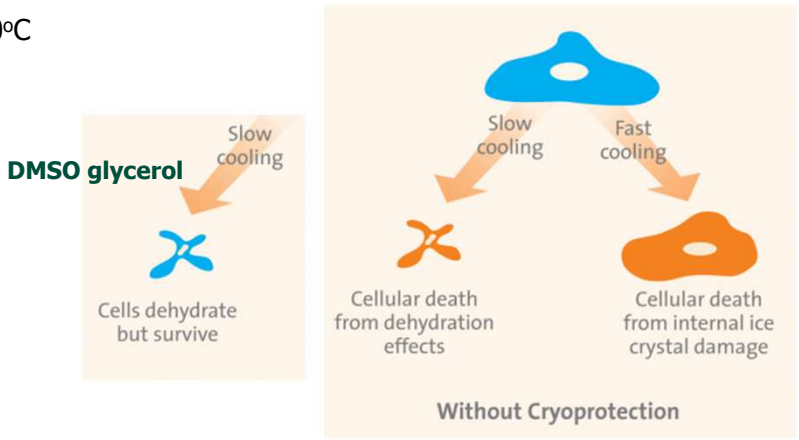
However, the **method** when you freeze your cellular material can impact the function of that material upon thawing.

On the right is an schematic from your reading this week that shows some of the poor outcomes associated with **rapid vs slow** cooling. If you chose to cool rapidly then ice crystals can form within the cell. These crystals can **damage proteins** within the cell leading to differential cell behavior or **low viability** when thawed.

The alternative is **slow cooling** – again ice crystals form spontaneously in the extracellular space – however in slow cooling this will upset the **osmolality**. When pure ice crystals form there is an increase in solute concentration outside the cell. In an attempt to equilibrate, the cell will pump out water – **cycles** of extracellular freezing and water removal from the cells leads to dehydration.

Product Preservation - Cryoprotection

- Freezing < -130°C



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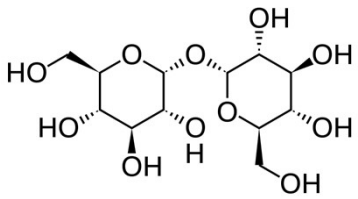
In order to freeze cells without these side effects, **slow cooling** is used with the addition of **cryoprotective agents**.

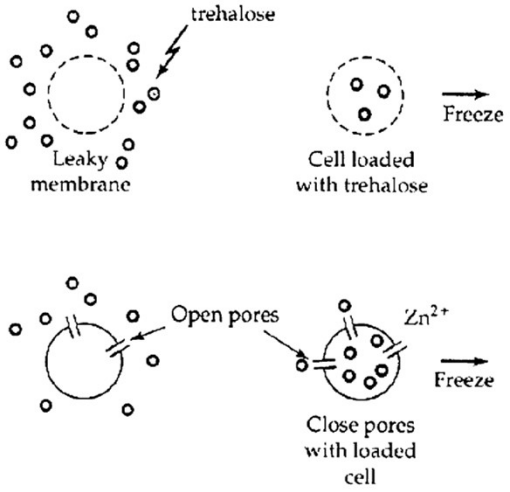
The two most common agents are **glycerol** and **DMSO** or dimethyl sulfoxide – these agents alter the environment immediately surrounding the cell **to prevent excessive dehydration**.

Product Preservation - Dehydration

- Dehydration



Trehalose



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Tissue Engineering, Palsson & Bhatia

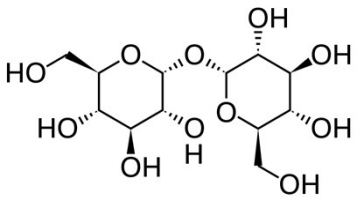
The second preservation method is **dehydration**.

A naturally occurring protein that aids in cell preservation is **trehalose** – shown on the left of the slide. This molecule helps preserve **lipid membranes and proteins** by sheltering them using its **polar side groups**. The interactions that proteins have with Trehalose are similar to the interactions that typically happen with water molecules.

Researchers have found different ways to introduce trehalose:
- through **leaky** or **porous membranes** as shown on the right side of the slide or
- through **forced expression** by the cells themselves.

Product Preservation - Lyophilization

- Dehydration



Trehalose

Lyophilization

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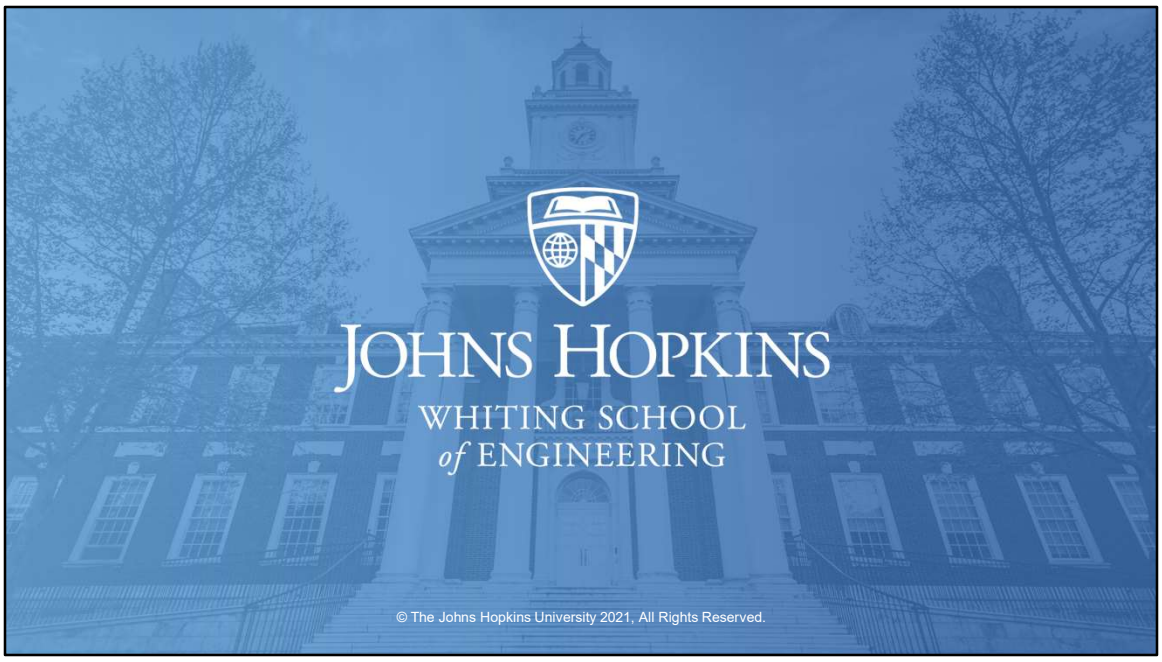
Finally, let's cover lyophilization

In this image you are looking at one method of drying a sample – this scientist is showing you trays of lyophilized or **freeze dried** material. In this method, the material is typically frozen **first** and **then** dehydrated by reducing the pressure. This reduction allows for trapped water to **sublimate**, moving right from a solid to a gas.

Trehalose is not only a cryoprotectant but also a lyo-protectant and is also used as a pretreatment for lyophilization.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