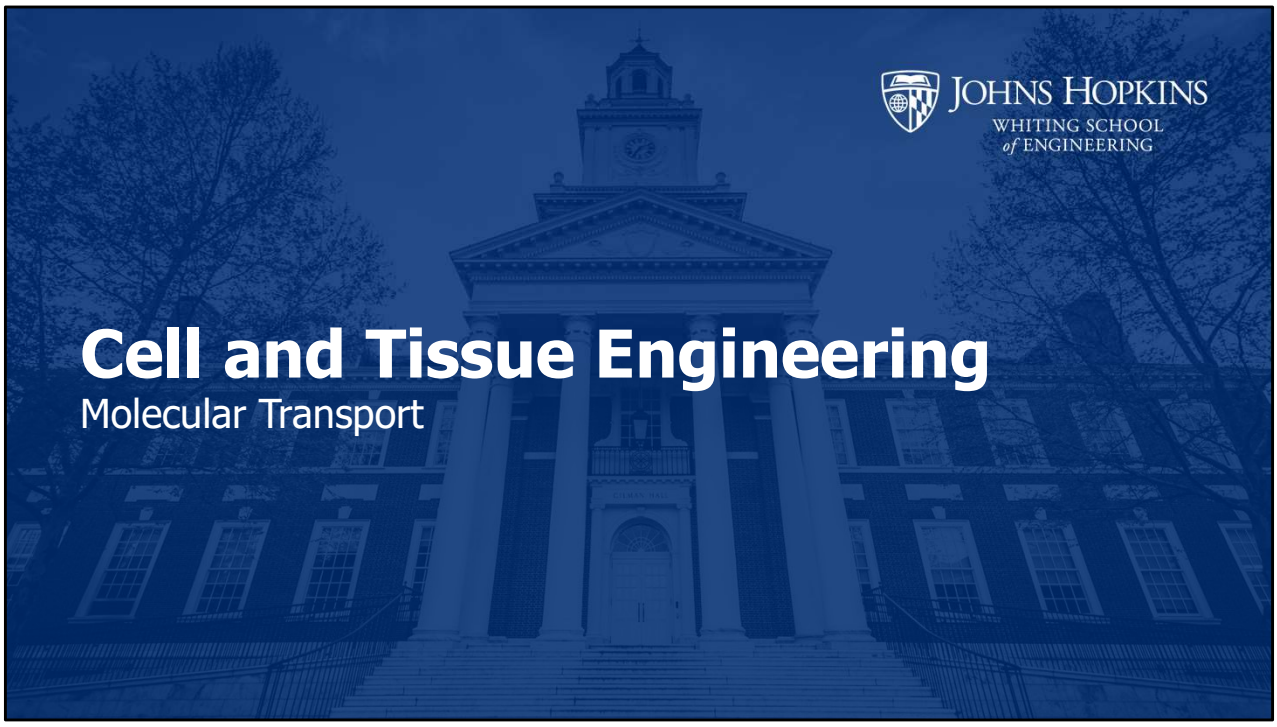
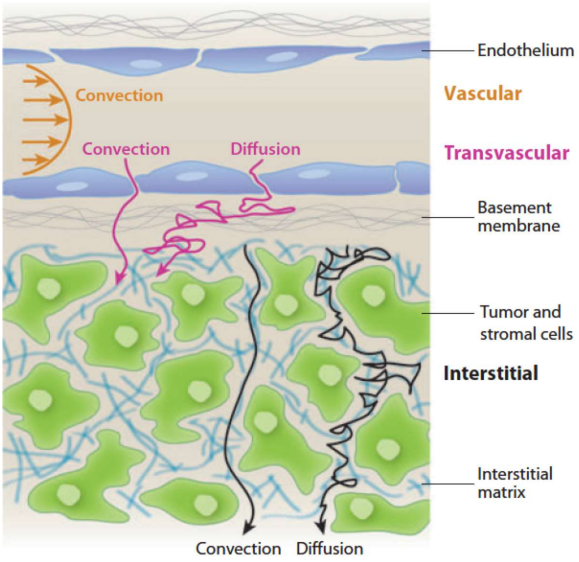


Welcome to cell and tissue engineering. This is Ethan Nyberg. In this video we will discuss molecular transport.

When Do We Need to Consider Transport Barriers?

- **How do we deliver cells and molecules to the body successfully?**
- **How do we deliver molecules and cells in the lab to 3D constructs?**



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Chauhan et al, 2011

In cell and tissue engineering, **delivery** is half the battle. We must consider the following questions:

How do we deliver cells and molecules to the **body** successfully?

Think of engineered cell therapies and molecules produced by engineered cells. Think back to our lecture on recombinant DNA technology.

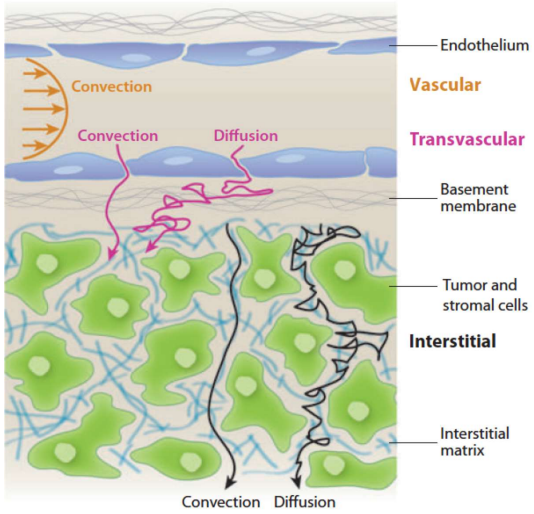
How do we get those **pieces** into the human body?

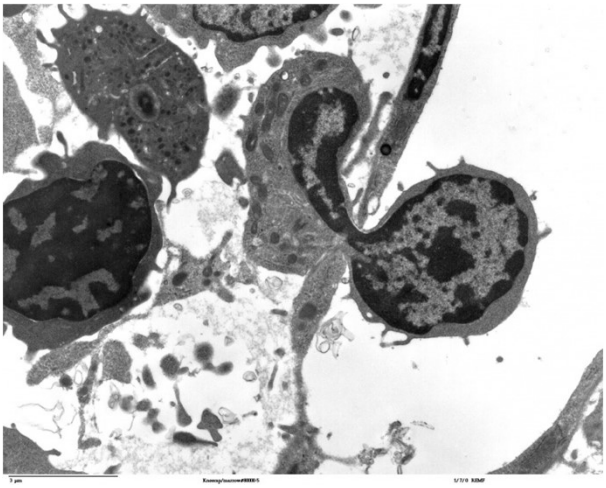
The same issues arise in vitro:
How do we deliver molecules and cells in the lab **to 3D constructs**?
to engineered tissue **substitutes**
to engineered tissue **models**

In this module we'll investigate both **molecular delivery** across the cell membrane, **cell delivery** across the vascular wall, and **cell trafficking** through the circulatory system.

Then in a guest lecture you'll hear about how these same principles are applied to **bioreactor** design.

Molecular Delivery in Fluid Flow





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Chauhan et al, 2011

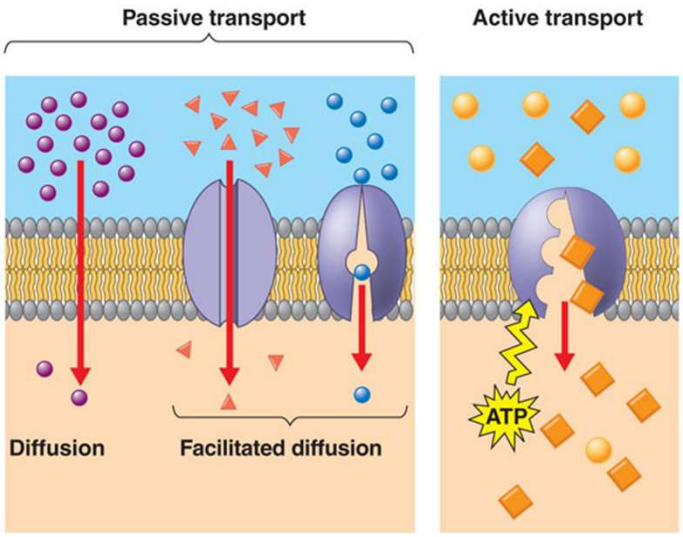
Last module we talked about **forces** acting on cells and tissues – compressive forces acting on bone and shear forces acting on the blood vessel wall.

This module we are going to think more about **fluid flow** in two ways – we are going to discuss how fluid flow **regulates molecular and cellular delivery** from the bloodstream to the tissues in your body.

Here on the left you can see the cartoon of a capillary – with that characteristic Hagen Poiselle flow we saw last module. In the gaps between endothelial cells we see both **convection** and **diffusion trace paths**. We can see similar trace paths through the tissue space.

On the right is another familiar scene – a white blood cell transmigrating through the vessel wall on it way **from the blood steam** to a target **organ**. These images depict things occurring in the **human body** but parallel behaviors happen in tissue constructs.

Molecular Delivery Across a Cell Membrane



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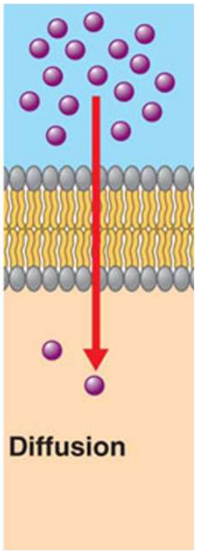
Let's get started with molecular delivery.

When trying to delivery a molecule to a cell the first barrier to consider is the cell membrane. There are several ways to get something passively across a cell membrane – **passively or actively**.

Passive transport mechanisms include both simple diffusion and facilitated diffusion. **Active** transport requires energy to move material across the membrane and doesn't necessarily go down a concentration gradient

Simple Diffusion using Fick's Law



$$\frac{dn}{dt} = P \cdot A \cdot \left(\frac{dC}{dx}\right)$$

$\frac{dn}{dt}$ Rate of diffusion

P Permeability

A Area of membrane

$\left(\frac{dC}{dx}\right)$ Concentration Gradient

$$\left(\frac{dC}{dx}\right) = \frac{C_{out} - C_{in}}{dx}$$

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First up is simple, passive **diffusion**

Diffusion is random motion that moves in a net direction to a region of lower concentration in order to reach equilibrium. This transport efficiency is affected by the **properties** of the cell, the **molecule** trying to pass, and the **solution** on either side of the cell membrane.

We can use a modified expression of Fick's law for small non-polar molecules –

The rate of **diffusion** –dn/dt (that the is number of molecules moving over time)

is equal to the **permeability constant** (depends on the molecules **size** and **lipid solubility**)

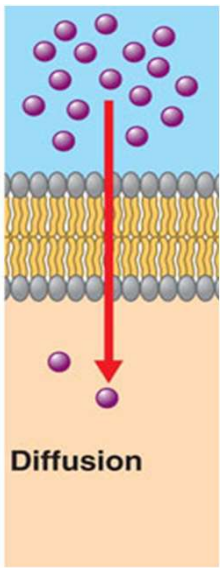
times the **area** of membrane where diffusion is occurring

times the **concentration gradient**.

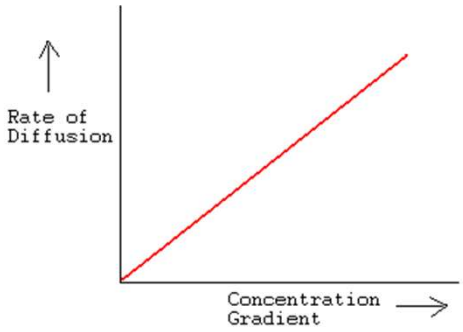
The concentration gradient is simply the change in concentration of the molecule

wishing to cross, divided by the width of the membrane (x)

Simple Diffusion using Fick's Law Cont.



$$\frac{dn}{dt} = P \cdot A \cdot \left(\frac{dC}{dx} \right)$$



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Since P and A are both **constants,** we graph **the rate of diffusion** vs the **concentration gradient**. For simple diffusion we see a linear function.

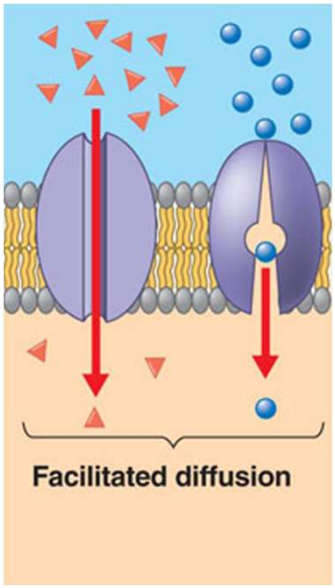
The stronger the concentration gradient is the faster the rate of diffusion is. Think about homeostasis here – the bigger the discrepancy in equilibrium the faster the system will try to remedy that.

Simple diffusion also occurs without a cell membrane – from a gas to a liquid for example.

In tissue engineering we are often concerned with oxygen delivery through the tissue culture media. Fick's law can also be used in this situation to determine the concentration gradient that cells are exposed to.

From earlier modules we know that this gradient can lead to changes in cell behavior and activation– for example **migration** and **phenotype changes**.

Facilitated Diffusion



$$\frac{dn}{dt} = \frac{v_{max}}{1 + \frac{k}{dC/dx}}$$

$\frac{dn}{dt}$ Rate of diffusion

v_{max} Saturation constant

k Constant determining Speed of saturation

$\left(\frac{dC}{dx}\right)$ Concentration Gradient

$$\left(\frac{dC}{dx}\right) = \frac{C_{out} - C_{in}}{dx}$$

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Let's now look at facilitated transport.

As this schematic shows – facilitated diffusion requires carrier proteins – these are proteins that help the molecule pass through the membrane. There are several types – some that look like straight channels and others that rock open to one side of the membrane at a time.

At low concentrations molecules will pass through carrier proteins in a similar way to simple diffusion.

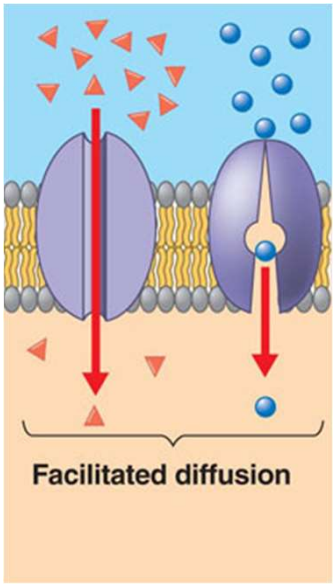
At high solute concentration however all of the carrier proteins can become occupied with diffusing molecules and increasing the solute concentration can no longer increase the rate of diffusion.

This **maximum saturated rate** is called Vmax.

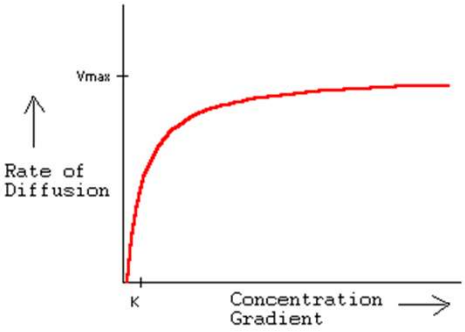
How quickly the carrier proteins become saturated is a **function of constant K** – this is equal to the concentration gradient at which the rate of diffusion is 1/2 vmax.

Both **K** and **Vmax** depend on properties of the diffusing molecule, like permeabilities and surface area.

Facilitated Diffusion Cont.



$$\frac{dn}{dt} = \frac{v_{\max}}{1 + \frac{k}{dC/dx}}$$



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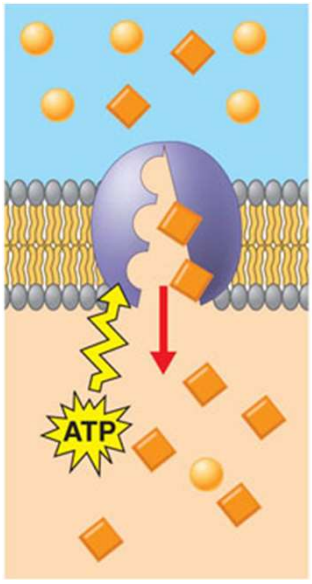
When we graph the rate of diffusion vs. the concentration gradient we see a very different curve.

Facilitated diffusion can increase the rate of diffusion at low solute concentrations but the rate of diffusion levels off with increasing solute concentration.

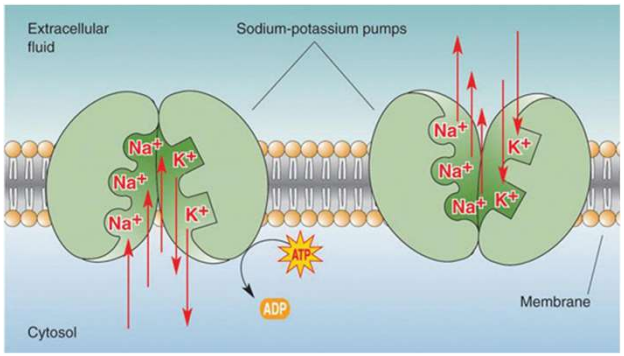
These graphs should remind a bit of what we saw with receptor ligand binding…

Active Transport Requires Energy



Primary Active Transport
Carrier protein hydrolyzes ATP

Secondary Active Transport
Energy from electrochemical gradients



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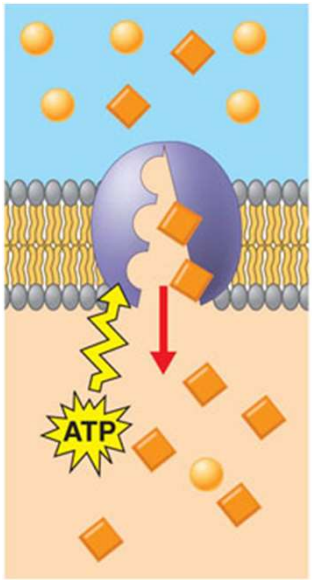
The last form of transport is **active transport.** This transport mechanism is best known for movement of ions and setting up the electrochemical gradients across the cell, however it can also be used to move other solutes.

Common carrier proteins in primary active transport are ATP-ases. The only substances transported by these carriers are positively charged ions – Sodium (Na) Potassium (K) Calcium (Ca) or Protons (H+).

The most important being the Sodium/Potassium pump which moves 3 sodium out of the cell for every 2 potassium in.

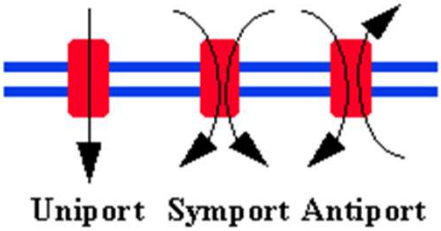
In most cells this pump utilizes 1/3-1/2 of the total energy expenditure of the cell. Without sufficient ATP synthesis cells will die mostly as a result of the inactivity of this transporter and the loss of the necessary electrochemical gradient.

Secondary Active Transport (Co-Transport)



Primary Active Transport
Carrier protein hydrolyzes ATP

Secondary Active Transport
Energy from electrochemical gradients



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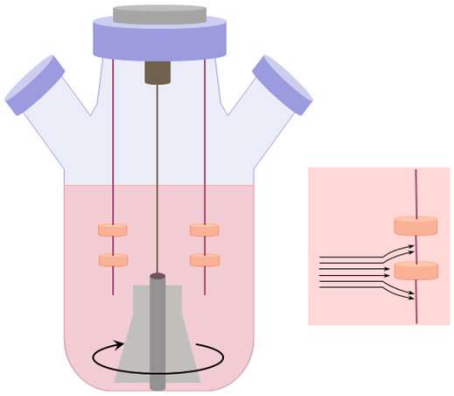
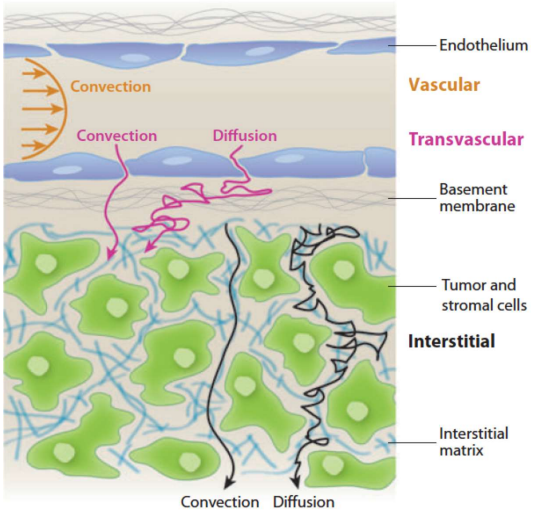
Secondary active transport or co-transport is movement fuelled by energy stored in ion gradients. That is is the energy released when ions move down their electrochemical gradient is used to move other ions up their electrochemical gradient.

This can happen in several ways – **symport** if both molecules are going in the same direction and **antiport** if they are moving in different directions. In antiport you can see the molecule moves down a gradient while the other moves up.

This process is mediated through a series of **conformational** changes in **the carrier protein**.

Molecular Delivery to a Tissue Construct





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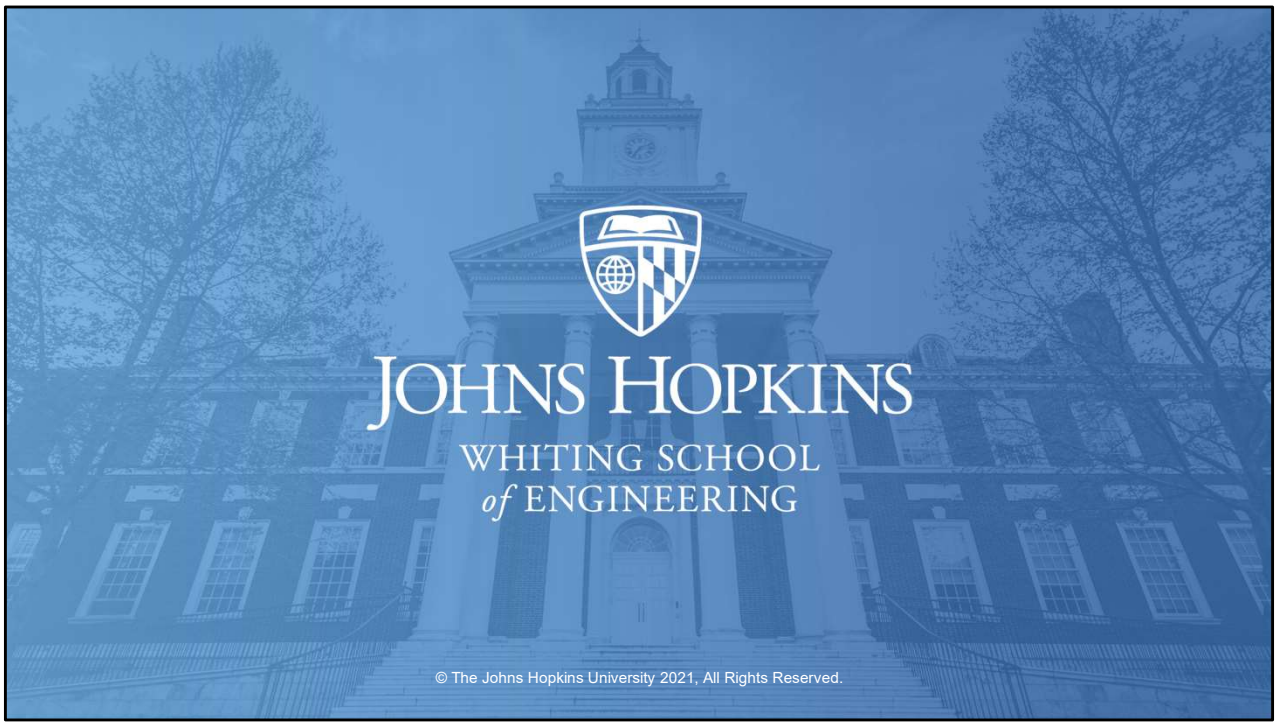
In later videos in this module Dr. Grayson will discuss the use of convection in overcoming mass-transport limitations for the delivery of materials to tissue constructs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