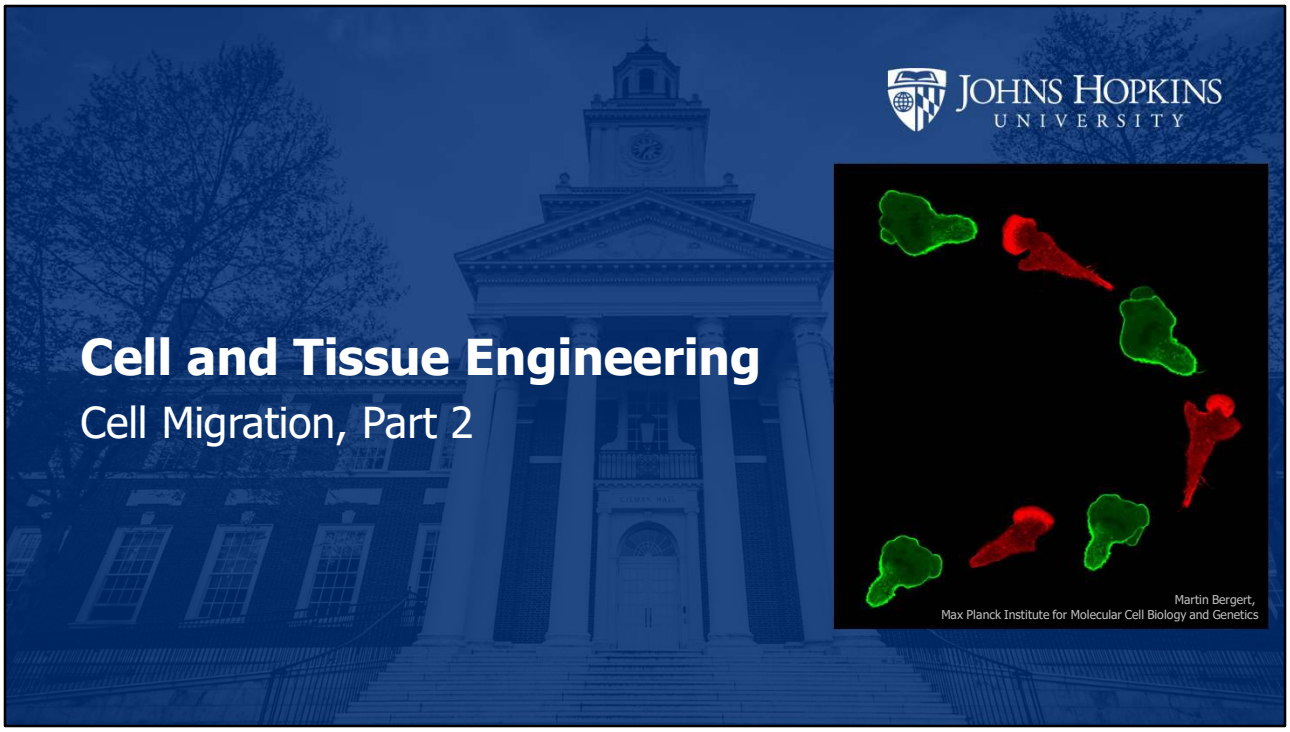
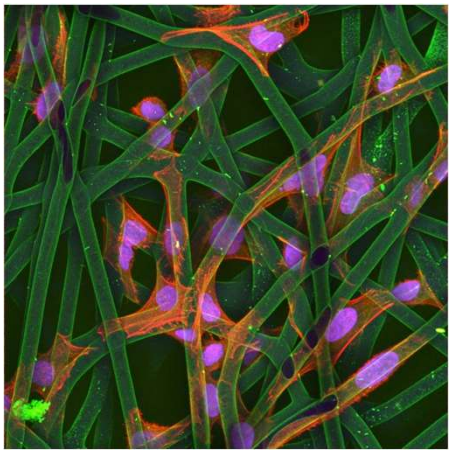
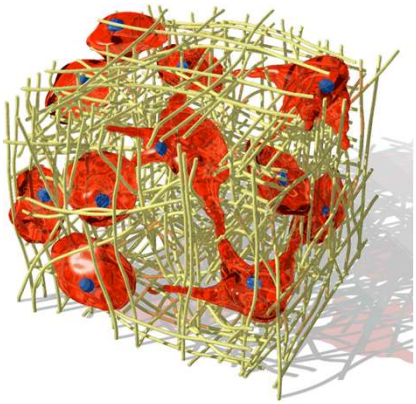


Welcome to cell and tissue engineering. Cell Migration part 2. In this lecture, we will learn how to measure cell migration and discuss the factors that control cell migration speed

How do you measure cell migration?





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Orbiosciences.com; Wodumedia.com

In these pictures, you can see cells seeded into a 3D biomaterial construct.

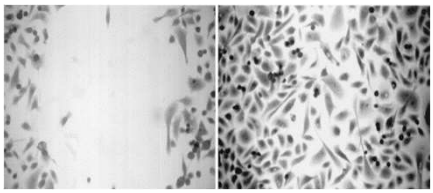
In future modules, we are going to talk about biomaterial design which covers properties like porosity, cell adhesion, and elastic modulus.

When we get there, you'll be armed with tools for measuring both cell adhesion (covered earlier in this module) and tools for measuring cell migration (which we'll go over right now).

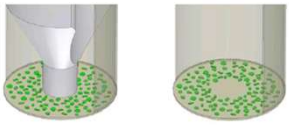
These aren't exhaustive lists – just the most common tools used today.

How do you measure cell migration?

Scratch assay



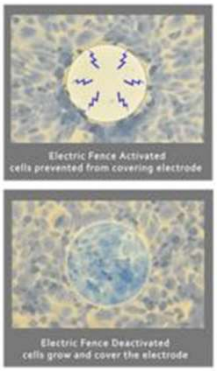
Cell exclusion zone assay



Coat plate, insert stoppers & seed cells

Remove stoppers to reveal detection zone

ECIS



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3

Arunabiomedical.com; Evans et al 1997; Pnwb.com

Keep in mind that some of these tools measure **individual** cell motility and some look at **collective** cell motility.

By far, most assays are commonly done in 2D.

In these examples, cells are **removed** or **excluded** from a particular region of the culture dish and then monitored as they move back into the zone.

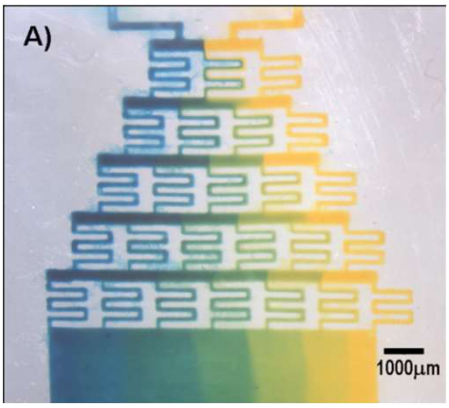
First, lets look at the **scratch assay.** In this assay a tool such as a spatula or a pipette tip is used to scratch a portion of the cell dish, denuding it of cells. Cells then migrate back into the area.

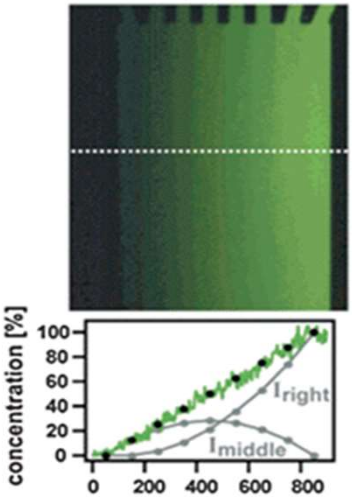
Next are **Exclusion Zone Assays**: If you don't want to scratch cells, you can alternatively block cells from attaching to that region in the first place. You can do this by placing a stopper or other physical limited to prevent cell adhesion to the area of interest. Then you remove the obstacle and allow the cells to migrate.

The third assay is called ECIS – **electric cell substrate impedance sensing**. A pulse of high current prevents cell spreading and attachment, and then when the electric fence is turned off, cells migrate into the open area.

How do you measure cell migration?

Microfluidics





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4
Kim et al 2010
Brandeis.edu

Another more **quantitative** migration assay utilizes microfluidics to generate soluble or bound gradients – that is **chemotactic** or **haptotactic** gradients.

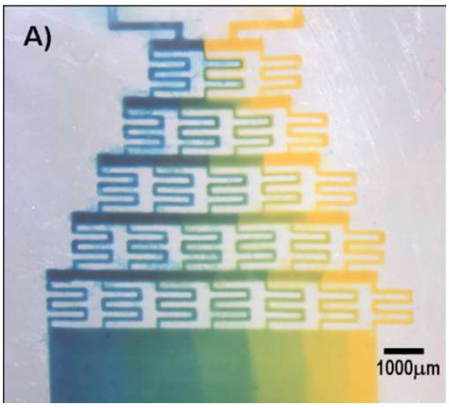
This is called a **Christmas tree chip** – In the most simple configuration you would put a chemokine in one inlet (such as the yellow) and nothing in the other. This results in a linear gradient as seen here along the bottom of the image.

The microchannels within the tree are designed to support only **laminar** flow – therefore mixing occurs through diffusion **only, in the perpendicular** direction to flow.

Because of this the **length** of the channels is maximized, generating the serpentine appearance. This feature allows enough time for mixing to happen.

How do you measure cell migration?

Microfluidics



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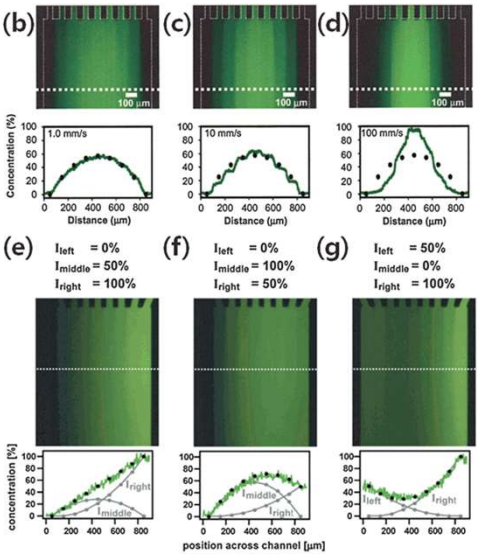


5

Kim et al 2010

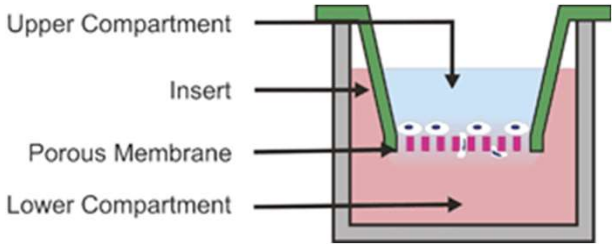
This technology allows for precise control of concentration gradients, and steady gradients (that don't change over time).

On the right you can see come of the more complex gradient configurations for these technologies. These include

- Parabolic
- Parabolic but stepwise
- A steep gradient
- A linear gradient (as on left)
- A shifted gradient
- A hyperbolic gradient

How do you measure cell migration?

Boyden chambers (transwell assay)



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6

Corning.com
Pubs.rsc.edu

A pseudo 3d way to measure migration is a **boyden chamber** (also called **transwell assay**) where cell migrate **through** a porous membrane.

Cells are seeded on to the top of the membrane here in the upper compartment.

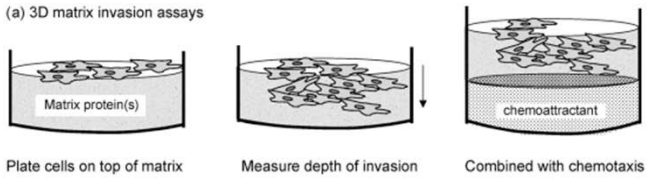
The lower compartment can contain a chemoattractant or some sort of a stimulus such as a coating of ECM protein or material. Alternatively cells can be used as a stimulus for migration seeded on the back side of the membrane.

The cells will migrate through the pores of the membrane as they react to the stimulus, and end up on the bottom side of the membrane.

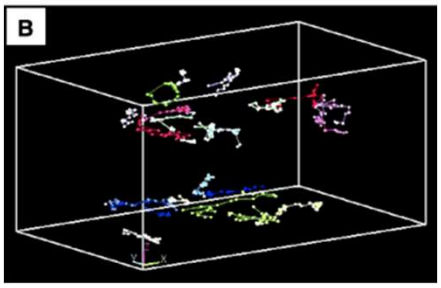
The upper chamber can be removed, and a microscope can be used to observe the cells on the other side of the membrane.

How do you measure cell migration?

Gel invasion assay



Time lapse imaging gives kinetic data



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7

Hazgui et al, 2005
Eccles et al 2005

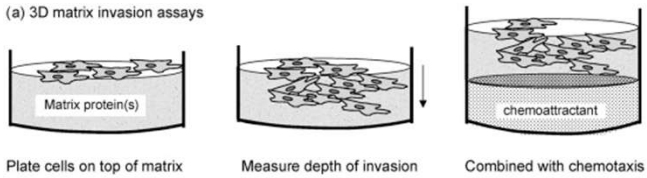
Now we've reached 3D-assays. These have become increasing used in the lab, because 3D- is more representative of the body and the constructs we generate as tissue engineering experts.

Some of the most common 3D assays are **gel invasion assays**. These assays begin with cells placed on the top of a 3D matrix. The cells are then allowed to migrate **in** and the **depth** of penetration is measured.

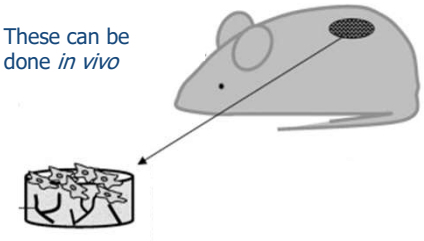
Advanced imaging techniques are used to monitor the individual cells as they move deeper into the 3D volume. Then computer models can give you images like this where each color gives you an individual cell trajectory.

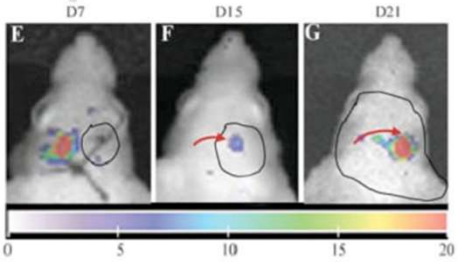
How do you measure cell migration?

Gel invasion assay



These can be done *in vivo*





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8

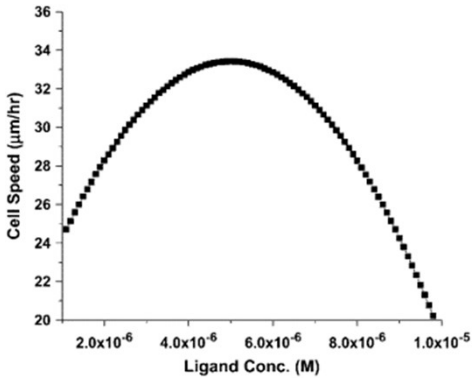
Hazgui et al, 2005
Eccles et al 2005

3D assays can also be run in vivo by placing the gel or construct in an animal and monitoring cell migration in real-time with MRI trackers or fluorescence

Here, you are looking at luciferase expression in neural stem cells. They are migrating towards a growing brain tumor, so your looking at the movement from the left to the right from day 7 to 21.

Migration speed

Migration is **biphasic** with highest speeds at <u>intermediate</u> receptor-ligand binding



Concentration!!

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9

Zaham et al, 2005

Alright so we know the major migration assays now – lets take a look at what we've already learned from performing these assays.

One of the biggest things we learned was that migration speed is **biphasic** with respect to ligand concentration.

Here we are looking at the results of a mathematical model which was used to predict cell migration speed based on parameters for force generation (both traction and contraction), polarity, adhesion and ligand concentration.

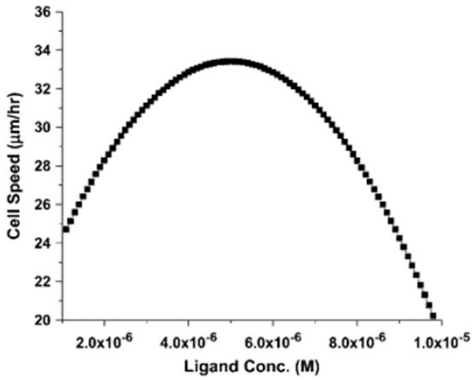
The fastest migration occurring at **intermediate** ligand concentrations. (highlight peak)

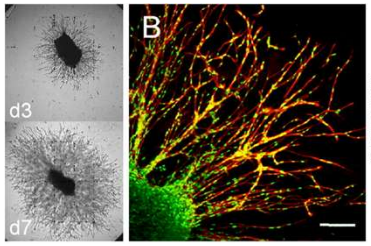
At low concentration few adhesions are made and cells cannot develop necessary traction force to propel themselves forward.

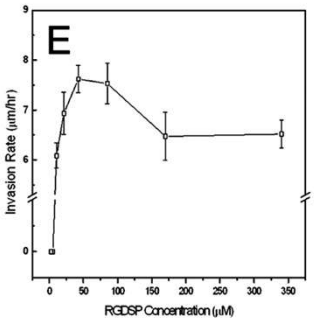
If the concentration is too high however, steric hindrance will inhibit movement.

Migration speed

Migration is **biphasic** with highest speeds at intermediate receptor-ligand binding







Concentration!!

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10

Zaham et al, 2005

If we look at some experimental data you can see the model predictions were accurate in predicting this biphasic response.

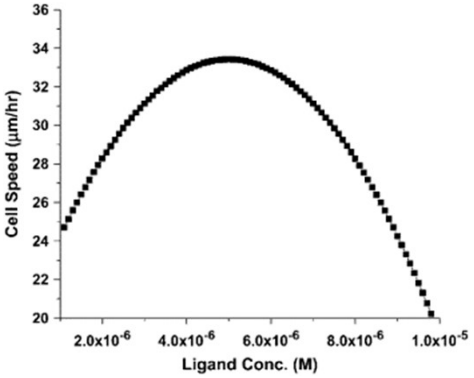
Here you are looking at fibroblasts invading a synthetic hydrogel. The invasion rate first increases with ligand concentration and then decreases, and then plateaus.

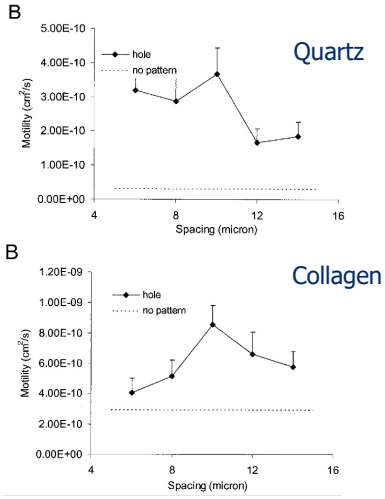
What do you think is happening here??? (pause)

All of the adhesion receptors are full – that is we've saturated ligand binding potential in the plateau.

Migration speed

Migration is **biphasic** with highest speeds at <u>intermediate</u> receptor-ligand binding





Concentration!!

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11

Zaham et al, 2005

This behavior is not specific to fibroblasts however – it is seen with many cell types. Here I'm showing you an example out of the Saltzman lab (the author of your textbook) using neutrophils.

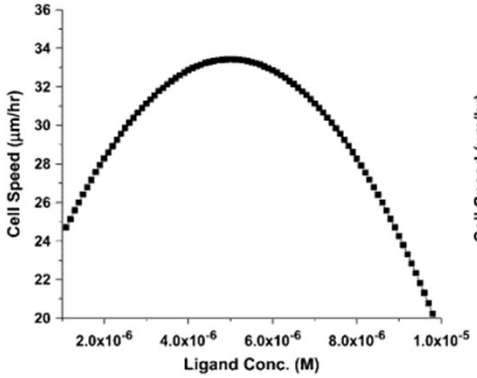
Not only does the cell type not matter but the substrate doesn't matter either. Different substrate matrix composition will require different adhesion receptors.

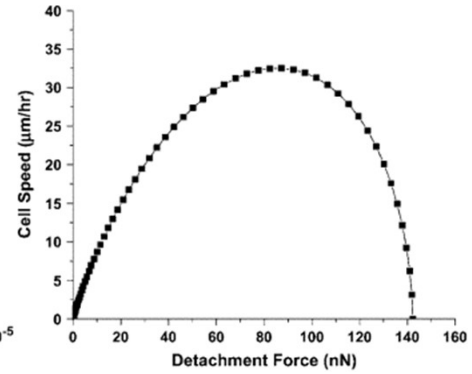
Therefore this tells us that adhesion receptor identity is not a factor in this biphasic response.

Now let's look at the scales (highlight axes). You can see that migration rate is faster on collagen than quartz, and in fact quartz is a **highly adhesive substrate**. (which is the far right of the curve on the left)

Migration speed

Migration is **biphasic** with highest speeds at <u>intermediate</u> receptor-ligand binding





Concentration!!

Strength!!

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12

Zaham et al, 2005

IF we look back at the model again we see that detachment force (which tells us about bond strength) displays a similar curve with migration speed.

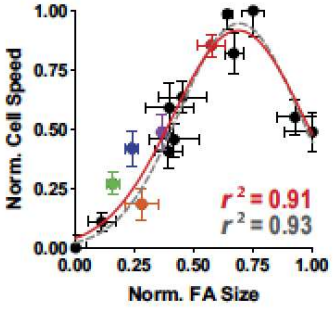
At **low** detachment forces cells break bonds before they can establish necessary propulsion or traction force.

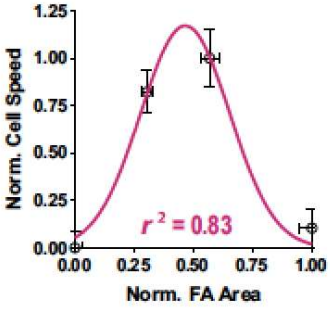
At **high** detachment forces cells can't release their rear adhesions and are left trapped in place

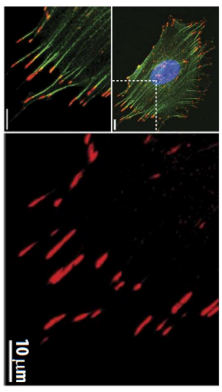
So not only does ligand receptor concentration matter, but also **affinity** and **bond** strength!

Migration speed

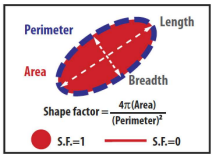
Migration is **biphasic** with highest speeds at intermediate receptor-ligand binding







Focal adhesion size and total adhesion area!!



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13

Kim and Wirtz, 2013

We saw earlier that not only does the strength of **a single bond** matter but you must also consider **the total number of bonds** in an adhesion in determining the detachment force (of the **overall** adhesion).

This research group determined the average focal adhesion area or adhesion size per cell AND total area of focal adhesions per cell, … and looked at how those related to **migration speeds**.

You can see the same bell shaped curve. With the fastest migration occurring at **intermediate** focal adhesion **size** and intermediate total adhesion **area**.

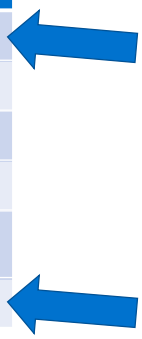
This data all informs the development of biomaterials constructs where you want to **tune** cell migration to your tissue needs.

For example you may want rapid infiltration at the edges of a material for investment and then limited migration at the center in order to maintain your population.

We'll talk more about biomaterial considerations in Mod10.

How fast do cells migrate?

Cell type	Speed um/min
Neutrophil	20
Macrophage	2
Fibroblast	0.5
Endothelial cell	0.4
Smooth muscle tissue	0.5
Neuron on laminin	1-3



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14

So what else have we learned from migration assays??…

This table shows some measured values for migration speeds – you can see that some cells move quite rapidly – namely immune cells, while other move much more slowly.

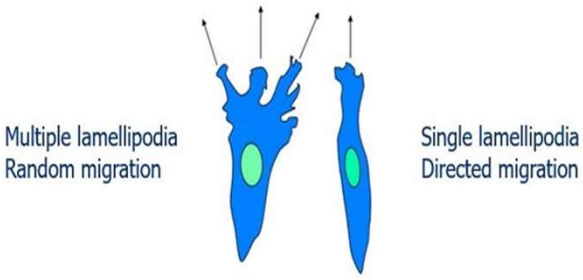
Now the reason for this has a little bit to do with function. Neutrophils need to get to where they are going very quickly and solve the problem. This speed comes because the neutrophils use integrin-independent migration.

Whereas neurons don't really move over time, and this extension time is from their original development which they might want to do quite carefully and slowly so they can be sure to go to the right place.

If you were designing an engineered tissue with centimeter scale dimensions think about how long it would take this construct to fill using migration alone.

How fast do cells migrate?

Cell type	Speed μm/min	Persistence time (min)
Neutrophil	20	1-4
Macrophage	2	30
Fibroblast	0.5	60
Endothelial cell	0.4	300
Smooth muscle tissue	0.5	240-300
Neuron on laminin	1-3	--



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15

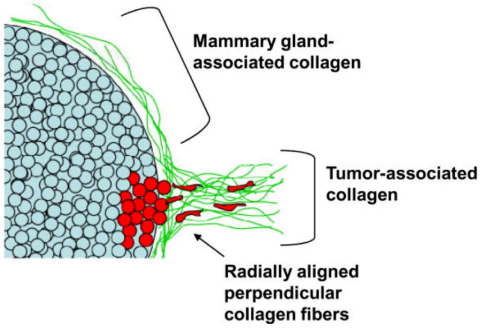
The last column in this table shows **persistence time** – that is how long on average a cell will continue in a given direction.

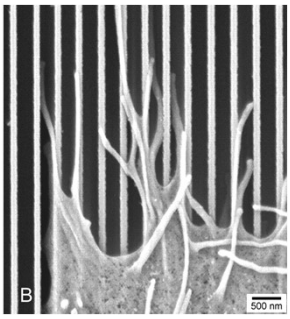
The shorter the persistence time, the more random the migration is.

For directed migration you want **long** persistence times where the cells continue to follow the migratory cue for long times.

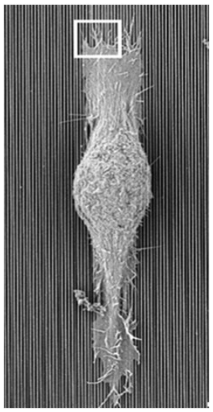
Cells following directed migration cues also display unique morphology with a single lamellipodia.

Directed migration is not always desired





Contact guidance
Surface topology



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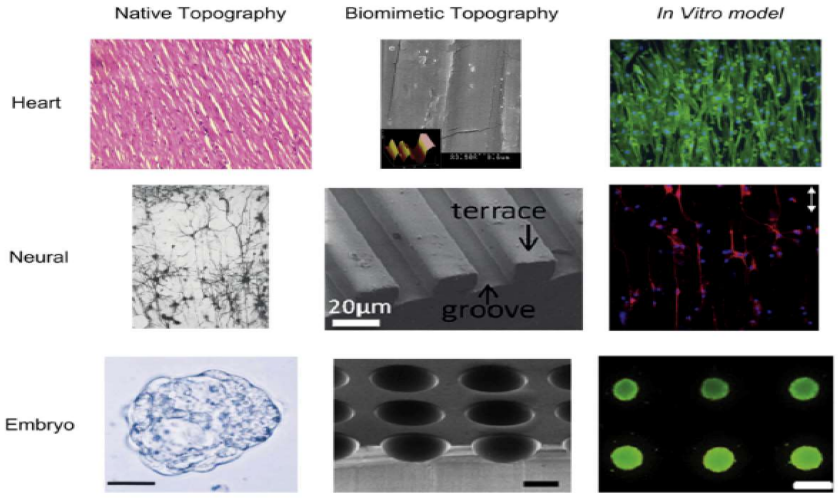
16

Directed migration however is not always what you want. Recall earlier when we discussed contact guidance using surface topographies…

You can see that these grooves that act similarly to ECM fibrils guiding filopodia and orienting the cytoskeleton and cell migration in one direction.

It is also known that highly metastatic cells preferentially migrate along collagen fibers. For example normal breast tissue is surrounded by a dense reticular collagen that is thought corral and restrain cells in the mammary glands, while cancerous tissue shows fibers of collagen extending radially away from the gland supporting motile and metastasizing cells.

Contact guidance for the design of in vitro models and scaffolds



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17

IN the realm of tissue engineering**, contact guidance tools** have been used to create microenvironments **in vitro** that mimic the natural tissue.

This has been done both to create tissue models for study and also design scaffolds for tissue engineered constructs.

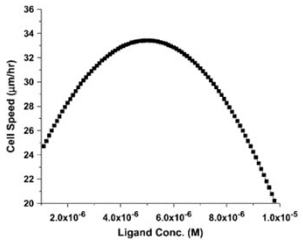
This image from your assigned reading shows some examples. Here you can see a **grooved topography** promoting extension and growth of cardiac cells in a highly aligned fashion.

Using deeper and more narrow grooves for the long-range guidance of axon extension over long distances.

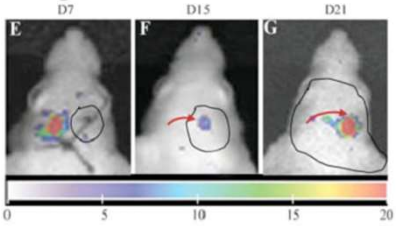
And finally half spheres or cups for embryonic cells similar to what is seen in early embryo development.

Rewind and Review

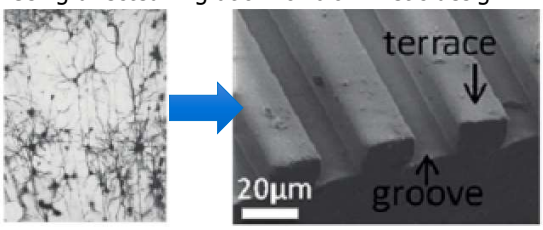
Biphasic relationship
with ligand-receptor binding



Migration assays



Using directed migration for biomimetic design



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18

In this lecture we've covered a lot of ground – let's quickly look back at what we've been through.

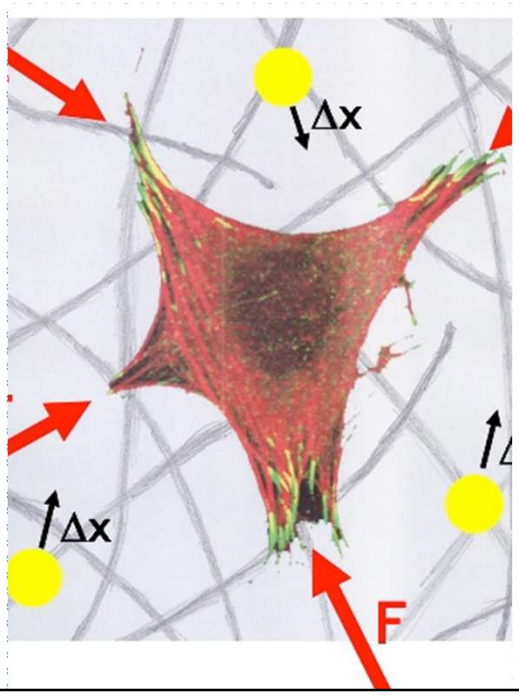
We looked at many different migration assays including both 2D and 3D platforms.

Results of those assays in the field has taught us a number of things about migration, including how cell adhesion parameters work to give us a biphasic relationship between migration speed and ligand concentration.

Lastly we started a discussion on how **contact guidance** has been used for directed migration in the field of tissue engineering.

Looking ahead

- Cell and tissue mechanics
- Cell and tissue engineering



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19

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IN this assigned article this week you'll read more about how substrate topography influences cell substrate interactions --- including adhesion, migration, and mechanics.

In the next module we'll talk specifically about how cells sense forces and ways that we can model these responses.

Our bodies are not static therefore proper maintenance of our tissues hinges on appropriate mechanical cues.

In the next module we'll learn about how those cues are integrally important for controlling cell fate and behavior in context of engineered tissues.


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