Science AMA Series: I'm Matt Thomson (UC San Francisco), I use colored-light to turn stem cells into neurons. I'm trying to understand how stem cells choose their fate and I hope to one day use this te

Matt$_Thomson^{1}andr/ScienceAMAs^{1}$

[1]Affiliation not available

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MATT_THOMSON R/SCIENCE

ABSTRACT

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What difference do you think this could make to things like lungs exposed to smoke or eyes that don't work. Could it potentially heal them or replace the damaged tissue?

Itsmiles

Yes!....but there is a lot of work to do... This is exactly the long term goal of the field of people working in regenerative medicine. While a lot (a lot) of hard work needs to be done, there is realistic hope that engineered stem cells might provide useful therapies for replacing damaged tissues all over the body in the future. There are a number of basic and safety related challenges that currently stand in our way. What I find really exciting about stem cell biology is that all the technical and health goals and challenges reveal very deep and basic questions about how a single cell can grow into an organisms with many different cell types and tissues organized in space.

In terms of making functional tissues, I am hoping that our work on opticallly controlled differentiation can help solve two big problems in regenerative medicine. Currently, it is very difficult to make a tissue (like brain tissue) that is composed of several cell types organized spatialy. My hope is that optical techniques can be used to guide stem differentiation with spatial precision in order to help cells --like neurons and glia--get to the right location in space. We are currently trying this in the lab with neurons and muscle cells.

Second, there is a fear that --and I see several questions discussing this--if we put stem cells into the body they could become uncontrolled and form cancers. Originally, I became interested in the optical control as a way to "locally" control stem cell differentiation in the body. When you go to the dentist, the dentist can use light to "activate" a filling--(I know this process all too well:). Our idea is that we could make an engineered stem cell that can be optically activated--in a very similar way-- at a local site in the body to differentiate and repair damaged.

How exactly does the blue light drive neural differentiation?

purplepangolin13

Great question! Recently, there has been amazing progress in a field known as optogenetics--broadly

using light to control biological systems. For example, optogenetics is being used in neuroscience to turn neurons on and off (even in awake animals). We are using a set of related methods to turn genes/proteins on and off in stem cells. We take advantage of a light sensitive protein from plants. When this protein is activated by blue light, it can then turn-on the production of any gene. The fundamental work developing this system was done by Yi Yang's group. http://www.nature.com/nmeth/journal/v9/n3/full/nmeth.1892.html?WT.ec_id=NMETH-201203

We have taken this system and placed it in mouse embryonic stem cells--and can now switch on any gene with blue light in those cells. So we are using the light switch-responsiveness of the plant proteins to control the differentiation stem cells.

Research in plant biology has tremendously benefited these developments: by understanding how plants sense light (the proteins they use)--researchers have been able to make other cell types -not in plants--respond to light. This has been an extremely important development and great work has been pioneered in the bay area :

https://en.wikipedia.org/wiki/Light-Oxygen-Voltage-sensing_domain http://pgec.berkeley.edu/pquail

Have you tried these techniques on adult stem cells? How differently do adult and embryonic stem cells act in the experiments you have taken part in?

aminoacetate
Interesting question! We haven't yet tried this technique in adult stem cells, but are actively pursuing this. Adult stem cells could be a great avenue for therapy because they already exist in the adult organisms and can often generate useful cell types--like neural stem cells can generate differentiated neurons. There are fundamental differences between adult and embryonic stem cells in terms of their "potential", and we need to understand these basic differences better in order to manipulate and engineer the cells.

My expertise is in MAPK signaling, and I feel that you should disclose the methods more clearly to this audience. Lay people assume you shine light on these cells and nanog magically does what you tell it to do. But thats not the case, is it. You have genetically altered these cells such that nanog expression is driven by the the light. It's a highly artificial system, and frankly the terminal differentiation of these cells is not guaranteed. Nanog's role in this process is not yet well enough understood for your synthetic biology approaches to be utilized in humans. Not to mention the FDA has a blanket ban on genetically modified cell therapy outside of the cancer space (Chimeric antigen receptor T cells (CAR-T) is a notable exception in the cancer space).

hyperproliferative
This is a great and important point. We have engineered these stem cells (using plant proteins) to be able to "turn-on" genes in response to blue light. We specifically use light to turn-on a gene called Brn2 (Brain2) which is a fundamental controller of neuron specification. Further, we (with the Qi Lab at Stanford) used additional techniques to place an optical tag (GFP) on a stem cell gene called Nanog. This lets us both drive the cells to become neurons and "monitor" the process through the Nanog tag. Understanding the dynamics of terminal neuron differentiation in this system--and how it can be controlled and guaranteed is an important scientific question. In this work, we explored the question of how the cell decides to respond to or ignore our "neural push".

It will definitely take time to verify that this kind of synthetic biology and cell engineering approach is safe for therapy. But there is enormous excitement about this kind of cell engineering approach . One advantage of engineered cells is that safety mechanisms can be introduced. Another area of application is (as you exactly point out) in using engineered T-cells for cancer therapy. At UCSF, as an

example, the Lim lab which is actively pioneering the application of synthetic biology approaches to engineer T-cells for such therapies.

http://limlab.ucsf.edu/papers/pdfs/maf_2013.pdf http://limlab.ucsf.edu/papers/pdfs/wal_2010.pdf

Have you, or any of your colleagues found, or calculated any possible limit or extreme end to what stem cells can do to heal full grown adults? For example, maybe it appears that cancer is curable, but re-growing lost limbs is beyond said scope? There's a general lamens view that stem cells can "do anything." What is known in that regard?

Vidiousp
This is a very interesting fundamental question. Ultimately, we ourselves grow from single stem cells. This means that if we could somehow record the precise conditions faced by each cell in a developing embryo and "replay" these conditions to stem cells in the lab--we should be able to make even complex tissues.

The challenge is that the embryo has enormously sophisticated mechanisms for controlling the signals that stem cells are exposed to in time and space. Currently, we do not have anywhere near the same level of control over the environment for stem cells growing in the lab. The embryo has very intricate machinery for controlling the timing, type, and level of signals experienced by cells. Further, literally hundreds of signals are used in development.

An interesting aspect to your question is that --even without external influence stem cells themselves have the ability to spontaneously grow into organized tissues. People have known this informally for a long time--if you take stem cells and differentiate them in the lab--say to neurons--they start to form structured networks spontaneously. The cells themselves can self-organize fairly complex tissues--so that we might be able to "coax" and hack this process. In fact, our goal with the light control has been to "guide" the innate ability of stem cells to self-organize.

In the movie of our work--you see this happening--the cells start reaching for each other.

https://www.youtube.com/watch?v=CK7NpGQngfg

Amazing recent work has high-lighted the amazing innate capacity that stem cells have:

https://www.newscientist.com/article/dn24114-mini-human-brains-grown-in-lab-for-first-time/

http://www.nature.com/nature/journal/v472/n7341/full/nature09941.html

These examples of self-organized tissue have already been useful for modeling disease.

http://sitn.hms.harvard.edu/flash/2013/cerebral-organoids-a-tool-to-study-human-brain-development/

College sophomore here. My question, in short, is how did you get to where you are today?

I'm very interested in the field of stem cell research and my dream is to one day be able to be in a position similar to yours, actively researching and making new discoveries in the medical field and beyond. I'm currently at a communtiy college finishing up my associate's degree, and plan to get a bachelor's degree in biology from a local university. I'm interested in the path you took to get to where you are.

Thanks so much for your AMA!

lurkeraccount3
This is an extremely exciting time to get involved in stem cell biology and biology more broadly. My main advice is to get involved in research--and also to think about and even tinker with biology on your

own. Even gardening actually provides great experience for the process of biological research-and intuition for biological systems. The other thing that is becoming increasingly important is experience with computers, math, and computer programming. There is just a huge (and increasing) need for researchers who understand the context of biological systems but can also manipulate and analyze quantitative data. You are at a great point in your training to take computer and data analysis courses. These courses will really pay off in the future--even if they can sometimes be challenging in the short term--it is worth it.

Is the technique you used considered to be the use of optogenetics? I see that you don't use that term in your post and I have been a little confused about this technique since learning about it in undergrad.

I see that you used transcription factors Oct4 and Sox2, and the article you linked to mentions the use of CRISPR to fluorescently tag cells. I think this would be the "genetics" part of "optogenetics." You also clearly used optics by using blue light to elicit a response in the cells. Now, what I'm confused about is whether the term optogenetics applies to any technique that combines optics and genetics or if it is more defined technique.

Sorry if this question isn't relevant, I'm just trying to better understand both this technique that I am fascinated, yet confused by, as well as, your study.

khaleesi_dany
Yes --optogenetics is currently applied to a wide range of techniques. In neuroscience there are techniques that allow the opening and closing of ion channels with light to control neural activity. We are using related techniques that allow genes to be turned on and off. The genetics part of optogenetics just means that these capacities can be genetically encoded and programmed into the cells.

We engineer our cells--we alter the genome--and put light responsive proteins into the genome to activate Brn2. We (Yanxia Liu from the Qi lab at Stanford) also used CRISPR to tag the Nanog gene at the same time. So we have two separate genetic modifications in these stem cells.

Light? Fascinating. I've read of other experiments that used the shape of the environment to stimulate stem cell differentiation. How do you control for factors like the shape of the environment or temperature or chemical signals?

ahfoo
Very insightful question. You are exactly right--these stem cells can respond to many different cues--including the signals that they constantly send to one another. In this work, we tried to keep as many things (temperature, chemical signals, growth substrate) as constant and as controlled as possible to focus on one single cue. In the future, we hope to take advantage of these other modes of environmental control along with the optical control.

Since our cells are mouse cells, they need to be kept at a pretty constant temperature to live. It would be much easier for us if they could live at room temp:) The shape or geometry of the environment is a particularly interesting factor for influencing stem cells. In the body, stem cells often occupy structured niches that have well defined geometric and mechanical properties.

https://en.wikipedia.org/wiki/Subventricular_zone

In this work, we kept geometry constant, but we are interested in manipulating the geometry of the stem cell environment to manipulate the decisions that the cells make. We can try to trick them to thinking that they are in an actual animal.

What perfect timing; I just got back from my introductory neuro lecture!

What made you consider the possibility of using light as the stimulant of stem cell differentiation? And how did you decide to use blue light and the specific frequency of blue light to stimulate neural differentiation?

As a side question, what advice do you have for someone beginning their trek into the field of neuroscience? What things can we students be doing to best set ourselves up for the future? (I apologize for how vague my question is. I'm just looking to see if you have any words of wisdom for us newbies.) Thanks!

ProcyonLotorMinoris
what a great time to be learning neuroscience. We used blue light because it allowed us to exploit some existing light responsive proteins from plants to turn-on genes in the stem cells that control neural development.

It is a great time to be starting out in neuroscience. My advice would be to learn and think broadly. I think there will be a great convergence of biology, neuroscience, physics, and engineering. As an example, efforts to engineer machine intelligence are providing an important perspective for understanding natural intelligence as it arises in the brain. I would also advocate taking courses in physics, math, and computer science as these classes will help you learn how to analyze and think about the vast, quantitative data sets that will occupy our future!

My question is in reference to pluripotent stem cells; if we can coax an individual's skin cells to turn into pluripotent stems cells, doesn't that mean that our issues with being able to harvest sufficient amounts of stem cells a thing of the past?

ComradEddie
Yes and no. Now we can get our hands on boat-loads of pluripotent cells--even derived directly from people. This is just an enormous advance. It is hard to emphasize enough how shocking and fundamental the work of people like Yamanaka and Gurdon has been. However, we are still learning how to program these pluripotent cells efficiently into the cell types we need for therapy. There is currently intense interest in this--and I think we can aim to have a comprehensive and efficient programing language for cell fate in the future. Can we learn how to make "all" the cell types in the adult human from a common initial cell state with speed and control? Further, as e_swartz and others point out, there are important issues related to integrating these cells into the body safely.

You mentioned the concept of laser printer human tissue, but recently leaps & bounds have been made in the veils of 3D printing, being able to print highly detailed creations in plastic, & now intricate shapes & designs in foods like chocolate & other candies. Have you considered using 3D printing rather than laser printing? If so, how long would you say before any of this technology is applicable & useful in the field of bio-medicin? I use the prefix bio because there are already groups printing prosthetic limbs for people who can't afford them. But how long would you say before it is possible to print tissue, & from there entire organs?

EDIT:This assuming (here's hoping) you are able to infact 'harness' the power of stem cells.

captainfantastic211
Great point. Yes--there are really exciting efforts around 3D printing cells into tissues.
http://www.nature.com/nbt/journal/v32/n8/full/nbt.2958.html

I am interested in light because it is dynamic-we can easily change the light pattern over time--so we can switch and alter the environment and let the stem cells naturally grow and occupy that

environment. I mentioned in another post that stem cells have an innate ability to self-organize--and the optical system helps us take advantage and sort of guide that innate capacity dynamically.

That said--3D printing efforts are making huge progress. I think it will be possible to 3D print large blocks of cells in the near future. This isnt a field I work in directly--so dont know all the challenges.

One challenge that remains is still this issue of being able to make all the cell types in a controlled and efficient manner. Efforts to solve this problem will help provide good raw materials for 3D printing.

Do you know how the blue light is inducing differentiation? Is it because of blue light exposure (and do other wavelengths of optical light not have the same effects)? Does your specific Nanog timing have an effect on whether the stem cell responds and becomes a neuron or will it change regardless of timing?

This is very interesting. I hope I have access to your articles via my school affiliation.

boringoldcookie
Yes--we are using light responsive proteins that we have engineered into the cells to make them "turn-on" neural control genes when exposed to light. The Nanog timing mechanism appears to be very important--if the timer hasnt "run-out" when we stop exposing the cell to light--the cell actually ignores the signal.

This Nanog timer is a central "gating" mechanism that either blocks or allows progress of the cell through differentiation.