This Week in Microbiology

With Vincent Racaniello, Michael Schmidt, Michele Swanson

Episode 167: I have one word for you: Flink

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Vincent: You are listening to TWIM, This Week in Microbiology, episode 167, recorded on December 14 2017. I am Vincent Racaniello and you are listening to the podcast that explores unseen life on Earth. Joining me today from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello!

Vincent: We got snow here, Michele.

Michele: Oooh, so do we.

Vincent: (laughs)

Michele: A lot of it, I think we got maybe 5 inches yesterday. The schools are closed today.

Vincent: Wow.

Michele: It's a winter wonderland.

Vincent: We had three inches on Saturday, it melted, and then last night it went another one or two inches and it seems to be melting, but it is very cold here.

Michele: It is December.

Vincent: Of course, in certain parts of the country it is not snowing. And also joining us from Charleston, South Carolina, Michael Schmidt.

Michael: Well, it did snow in the upstate of South Carolina this past weekend and so we are fortunate here on the coast, it is just a cold, damp rain, and today it is a little bit sunny, I had to look out the window. It is a little bit sunny but it is December, but I think snow is a better alternative than the poor souls out West dealing with the fires. I mean, those poor souls in Los Angeles and Santa Barbara and Ventura County, it just has to be freaking everyone out.

Vincent: Yep, fires are tough. They can take your house.

Michele: Especially if the wind is blowing at 60 mph. It is just unpredictable.

Vincent: We will be without Elio for the next two episodes because he is out East on vacation with his family.

Michele: Nice.

Vincent: So enjoy that, Elio. And in the meanwhile we will continue to deliver to you the finest microbiology that a free podcast can deliver.

(laughter)

Michael: At least we are going to try.

Vincent: Since we are all card-carrying microbiologists, we will probably be alright.

Michael: Yes.

Vincent: We have a snippet and a paper, and for the second episode in a row we are going to talk about influenza virus. Michele picked this paper.

Michele: I did. It is titled "Pandemic H1N1 influenza A viruses suppress immunogenic RIPK3-driven dendritic cell death" and it is by scientists from Mt. Sinai Medical School in NYC and the Fox Chase Cancer Center in Philadelphia. And the authors are Hartmann, Albrecht, Zaslavsky, Nudelmman, Pincas, Marjanovic, Shotsaert, Martinez-Romero, Fenutria, Ingram, Ramos, Fernandez-Sesma, Balachandran, Garcia-Sastra, and Sealfon. I'm sorry I'm butchering these names.

Vincent: Wow, that was a mouthful.

Michele: Yeah. I'm sorry, pronunciation is not my strong suit.

Vincent: By the way, Mt. Sinai Department of Microbiology, that is where I got my PhD many years ago. Do you have an affinity for flu or for RipK3, Michele?

Michele: Well, it is flu season, and I thought this was a hopeful paper, actually. So H1N1, our listeners might have heard of this, it is the type of flu virus that caused the famous pandemic in 1918 that is estimated to have killed 50 million people world wide. Another version came through in 2009. You might remember the swine flu caused a lot of sickness and death. So what are these names, what do they come from? The H and the N both refer to surface proteins on the viral shell. The H is hemagglutinin, the N is neuraminidase, and we have to name the viruses because flu is a quick change artist. It has genetic mechanisms to alter at a very high rate its surface structures so that the virus that we see this winter will be mostly replaced by another virus variant next year. And that is why we have to be diligent and go out and get our flu vaccines. Have you two been vaccinated this year?

Vincent: Yes I have.

Michael: I have, too.

Michele: Alright. And how did they do with predicting the vaccine for this year's viruses? Vincent, do you know?

Vincent: So the vaccine has an H3N2 and an H1N1 and one or two influenza B strains, the H1N1 is a good match but H3N2 is a problem because as we discussed last time although the strain was matched, you grow it in eggs, which is what you do for most of the vaccine, you lose the mutation that you need for a good match and so it is not a good match. However, you still have less serious disease, the other isolates will protect you. So it is good to get it. I was listening to a tech podcast a couple weeks ago and they were talking about this mismatch and

they were lamenting that it wouldn't be worth getting the vaccine so I wrote in to them and I said it is still worth getting because you will have a less serious disease and these other two strains are still well matched. And you know what they did? They made fun of me and said I was a big pharma shill just wanting to sell vaccines.

Michele: Oh, you're kidding.

Michael: Oh my.

Michele: That's awful.

Vincent: I was stunned, here I was trying to just inform them, you don't have to get the flu vaccine but you should make a decision based on good information, and they made fun of me. And the thing is, I don't even work for big pharma (laughs)

Michele: We work for free. No. Plus, isn't it money in the bank even if it is not a perfect match? We now have that information in our memory cells and so next year we will have protection if that other one arises?

Vincent: In a way, yeah. It depends. I mean, the memory for flu vaccine isn't great, but even if there is a mismatch and you get infected you will have less serious disease.

Michele: Right, right, it is not a black and white phenomenon.

Vincent: Exactly.

Michele: So the question arises, how is it that seasonal flu will kill people, especially the very young, the very old, people that are otherwise ill, but by and large people can recover from seasonal flu. And yet, we have these pandemic versions of the H1N1 that wiped out millions of people. So this group wants to understand at a mechanistic level what is the difference between the seasonal flu and the dreaded pandemic H1N1s.

So the system that they used is white blood cells called dendritic cells from human blood. They do all their studies with human cells because the components that recognize and fight infection are somewhat different between mice and humans. So that is one strength of this paper. And what they know from the literature is that we have in our bodies surveillance systems that are able to detect and quickly respond to foreign molecules made by microbes, including viral RNA.

So in this case we know that if your cells in the lung are infected with flu virus the cells can detect the viral RNA through a surveillance pathway, and that infected dendritic cell in that case will recognize that it is infected and then purposefully undergo a programmed cell death. By doing so they deny the virus a chance to replicate because of course viruses depend on host machinery to make copies of themselves, and they also send out signals. So they don't just die quietly, but they scream out to the neighboring white blood cells "I'm infected" and they capture key information about who is infected as if they had taken a snapshot and have shown it to the rest of the immune system. So this is called antigen presentation.

So when the infected dendritic cell dies, undergoes this suicidal programmed death, it releases signals and it provides captured antigen to neighboring uninfected dendritic cells which then take that information and trigger CD8 T cells to begin to multiply and then the T cells are responsible for moving around the body, finding infected cells and killing them. So that is why most of us will be miserable if we get the flu because we didn't get our shots. But we will recover.

Michael: You'll recover more quickly.

Michele: We'll recover more quickly, yeah. You will still perhaps wish you could die because you are so sick, but (laughter) But you wont. So how is it that the pandemic flu virus is different, is the question they ask. So in earlier work they had identified a signal transduction pathway that is important for this and one of the key components is called, in the title, it is the RIPK3 so this is a receptor interacting protein kinase, but I am going to call it the Rest in Peace kinase.

Vincent: (laughs)

Michael: So you remember what it is doing.

Michele: Yeah, but it is a part of this coordinated plan to die and at the same time capture the antigen and activate my neighboring white blood cells to fight the infection. So what they do in the first figure, figure 1, is they have these dendritic cells and they infect them with either a strain of the seasonal flu or the pandemic flu, and then they measure cell death. And they do that in a couple ways. You can look at the morphology of the cell when they undergo this programmed death, their nuclei go all fragmented, so you can measure that. They also measured how many fragmented DNA molecules were inside the dendritic cells, and they could also measure how much components from the cytoplasm were released as these cells burst.

And what we see very clearly is that the seasonal flu viruses do trigger this programmed cell death whereas the pandemic cell viruses trigger less of it. So only about 10% of the infected cells exposed to the pandemic virus die whereas about 60% of the seasonal virus infected cells are undergoing this programmed death to alert their neighbors. So they know they have now an experimental system and they can use it to start to identify the mechanism. How are the cells recognizing and responding so quickly to this seasonal flu virus but not the pandemic virus?

So in the next figure that I will talk about, figure 2, they learned by using genetic tricks and drugs to block particular pathways. They were able to identify that RIPK3 was one of the key molecules in this sensing pathway that coordinated the programmed cell death. They also learned that the virus needed to replicate in order to cause this death. So then in figure 4 they did a really cool experiment where first they infected the dendritic cells with either the pandemic flu or the seasonal flu and then they intentionally exposed it to synthetic RNA to give it a load of viral RNA. And by doing that they learned that if the cell already had pandemic flu virus in it it was now resistant to a stimulus of synthetic RNA. So in that experiment then they could deduce that the pandemic flu could inhibit the signaling triggered intentionally by a synthetic RNA molecule. So now they can start to think about mechanisms.

Somehow it is inhibiting this recognition and cell death pathway. So then I will jump to figure 6B which I found really elegant. The whole figure, what they do is take advantage of the fact that flu viruses are segmented. So they have 8 different chromosomes, if you will, and it is possible experimentally then to make viruses that are chimeras, that have 7 segment from the pandemic virus and only one segment from the seasonal virus and vice versa. So they made a whole series of these chimeras and then could ask which chromosomal segment did the suppression of cell death track with, what did it map to? And by doing that they were able to determine that it is the hemagglutinin molecule, one of the surface protein molecules, the H in the H1N1, that was contributing and accounting for the ability to block programmed cell death.

To explore that further, they did a really intelligent experiment which is they took advantage of many different H1N1 viruses that have been circulating in the human population over decades. So they looked at 8 different viruses from different time periods, so they would have slightly different versions of the H protein on their surface. They sequence them and ask how similar are each of these H proteins to the pandemic potent H

protein that can inhibit cell death? And they then directly measured cell death inhibition by each of those variants. And in doing so they could come up with genetic data from human populations that was consistent with their model that in fact it is this H hemagglutinin protein on the surface that correlates with blocking cell death. Is that clear? How am I doing so far?

Vincent: Very clear. Beautiful.

Michele: I thought it was a really great experiment. Vincent, how many different types of flu genomes are out there for experimentalists to….

Vincent: Thousands.

Michele: Really?

Vincent: Thousands and thousands, because they do a lot, WHA coordinates a global surveillance and they have tons of sequences in the data bank, and you can just get those and synthesize the segments and put together your own virus.

Michele: Wow.

Vincent: Very easy to do, if you take 8 plasmids, one for each segment, and you put them in the cell you can get a virus out. So you could make any combination of all these sequences that you want.

Michele: Wow. So it is like nature had done the experiment.

Michael: This is actually then illustrating the hallmark of this virus, namely the shift in drift. So as in the pandemic variant you have not yet undergone the sin of all RNA viruses, namely the drift in it genome, because the RNA viruses, especially flu, has no proofreading activity. And so as with each round of replication there is some drift in this hemagglutination antigen and at the same time you have the ability of the influenza virus to pick up segments from other viruses or as the antigenic shift and so the thing that troubled me is what is unique about his hemagglutinin antigen in the pandemic strain? How does the dendritic cell effectively know that this is the pandemic strain? And that is their elegant experiment with the mixing and matching.

Michele: They then did another experiment that is going to help them think about the mechanism, which was they asked if they could just purify the hemagglutinin protein and add that to the dendritic cells, would that be sufficient to inhibit this cell death pathway? And based on that and some other really careful experiments, they were able to deduce that only when, well they are predicting that in fact the infected cell has to be newly synthesizing the hemagglutinin RNA or protein in order for it to be competent to block this surveillance pathway. So it is not going to be as simple as just adding HA protein to a cell and have it processed.

So they are starting to think about what is actually happening during viral replication to have this occur. But that gets maybe into a little more nuance. I do want to describe one other experiment that I thought was really beautiful. They did verify that a dendritic cell that was infected with the seasonal virus was not only able to commit this programmed cell death but those infected cells did in fact present antigen to other dendritic cells which could then stimulate proliferation of the appropriate killer T cells that would then go do the job of hunting down and killing infected host cells. So they verify that T cell proliferation was also occurring for the seasonal virus but not for the pandemic virus.

So again, to this group's credit, they wanted to take advantage of whatever data was out there and ask whether this was likely to be happening in an intact human with their whole immune system. And so they took

advantage of a published data set, this was work that was published in PLOS Pathogens in 2015 by another group who had enrolled 1,600 healthy adults and then followed those adults during the flu season and they took blood samples and profiled their white blood cell gene expression while they were healthy and then when a subset of those people in that trial or experiment came down with the flu, they were able to take another blood sample early in the infection and then a few days after their flu infection and again capture the RNA profile of the white blood cells.

So that data is publicly available and what this group did was use computational methods to extract from that data set information about what genes were expressed in the healthy people before they were infected and then after they were infected, either with the seasonal virus or the pandemic virus. Specifically what they did was pull out molecular signatures of the T cell population because their earlier experiments had shown that the seasonal virus does trigger lots of T cells to expand whereas the pandemic virus does not.

So in figure 7E they showed data from 9 different patients in each of those groups. Statistically they do find that there are fewer T cells in the blood of a human who was infected with the H1N1 of the pandemic type, so lower T cells than what they measured in the control or the comparable group that had a seasonal flu. So they admit that this was only 9 patients and it was somewhat indirect but nevertheless it is consistent with their model that they developed from these experiments I described and many others that I don't have time to describe that in fact the pandemic viruses have got a special hemagglutinin on their surface that when the virus is replicating and making these new HA molecules that in some way they are interfering with the natural surveillance system of our white blood cells that typically can recognize the viral RNA and trigger programmed cell death to alert their neighbors of the problem. But the pandemic strain is able to somehow short circuit that pathway. So as a result, the immune system is not alerted and the virus gets an upper hand and presumably that is why you get a higher viral load and more illness and more death.

Michael: So what should we be vaccinating people with to prevent a pandemic from occurring? Should we be looking at this HA molecule in greater detail to try to decipher what piece is unique or should we be focusing on the neuraminidase associated with the pandemic and make that more antigenically robust in order to effect sterilizing immunity for a vaccine candidate? Which way do you think we should go? I don't know if there is an answer.

Vincent: The main neutralizing antibodies are against the HA, not the NA, so the focus is on the HA. I think it will be interesting to know if they can identify what motif in the HA is responsible for the effect. Then you could do the surveillance and say are there any circulating strains that look like that and which we have to keep an eye on.

Michael: Yeah.

Vincent: If there is some property of HA that makes it lethal like this, in the mechanism suggested by this paper, then we should be able to track it. But I think in terms of a vaccine the real thing is to make a universal flu vaccine which will neutralize any strain out there and people are working on that.

Michele: Yeah, and they are avoiding the surface parts of the HA and the NA molecule and instead going for the stalk, the deeper embedded part of the molecule that doesn't change as much from season to season. So that is the strategy that is underway now. I think there are human trials underway.

Vincent: There are, yeah. I guess in the next 10 years we will have something licensed for that. Even if it is not once in your life, if it is once every ten years, that would help. But if that kind of vaccine could protect against a brand new pandemic strain then problem solved. And we could take all that flu research money and put it towards other stuff.

Michele: Yeah. I am also wondering if knowledge of how this HA is interfering with this particular pro inflammatory cell death, if there might be some clinical scenarios where you would want to mimic that with maybe a small drug.

Vincent: Yeah, good idea.

Michele: To avoid overamplification of the immune system which can be toxic also.

Vincent: I think that is a good idea, using a virus to probe these systems will give you some mechanistic insight that you can translate, yeah. That is a good idea.

Michele: And drug development targets.

Vincent: Yes.

Michael: Or even for transplants to prevent rejection.

Michele: Could be. Right. Now, fortunately for us, we do have many different redundant pathways for detecting different invaders and so they will converge on maybe a few molecules that are the best drug target, but we might need a cocktail drug to block all of these cell death pathways.

Vincent: This is a really cool paper and it is in an open access journal so listeners can go see it if they want.

Michele: They also did a great job of using color coding and schematics to make it easier to follow all their different constructs and chimeric viruses, so I appreciated that. It will be a good teaching paper for those of you who are looking for a neat way to teach some basic flu virology.

Vincent: Alright. Thank you, Michele, that was very nice. Cool paper, interesting stuff.

Michele: Let's talk about 3D printing.

Vincent: Okay, so I came across a paper in Science Advances. It is called "3D printing of bacteria into functional complex materials." So I saw the title and I said we immediately have to do this which could be dangerous, it may be that I don't understand any of the paper, but it is not the kind of paper that I would be fully comfortable with but I think we can get the gist of it to you.

Michele: It is really a fascinating paper.

Vincent: The ideas are great. The authors are Schaffner, Ruhs, Coulter, Kilcher, and Studart, and they are from ETH Zurich and University College, Dublin. So as Elio would say, what is going on here?

Michele: (laughs)

Vincent: The goal of this paper is to try and use the diversity of bacteria which can live almost anywhere, they have huge diverse metabolic activities, more so than any other organisms. They form biofilms, and biofilms can adapt to their environment to match the conditions, they can degrade lots of compounds, they can form all sorts of chemicals. To use this, in fact to use our ability to program all of these properties, to create 3D materials that contain bacteria that can do things that are useful. They have 2 examples in this paper of useful

things. One is to degrade a compound and the other is to make what could be a medical device made out of bacterial cellulose.

Michael: The other thing you need to point out is what this really is is a machine. It is a very small machine designed to do a specific task. As Vincent said, task 1 that they demonstrate is to degrade a toxic waste product, and the product they are going after is phenol. And the other is to make a scaffold that they can grow skin on. So it is absolutely fascinating. The other thing we always have to remember is biofilms need not be one single organism. And this is where the machine really gets pretty cool because you can actually have organism 1 doing one particular task and organism 2 doing a second task and when you think about the 3D printing you can actually vary where the organisms are laid down in 3D. So you can actually design your biofilm. It is going to be fun.

Michele: And use different colors of ink, too, 2 or 3 different colors so you can put different species of bacteria at different places.

Michael: So you know where they are.

Vincent: Yeah, exactly. So the idea here is to immobilize the bacteria in a hydrogel and try to manipulate it in a way to have beneficial properties. In addition to the two examples they say we could make fuel cells, they say we could make biosensors this way by embedding bacteria and programming them to do certain things. So what they do in this paper is to create some proof of principle for this. They make a 3D printing platform. Now, if you think of a 3D printer, it lays down layers of, if you want to print, say, a virus particle, it would take the 3D coordinates and then a machine would lay down thin layers of a polymer plastic or a clay like material that would then harden to form the final object. What is going to happen here is they are going to embed bacteria into a matrix that will have strength and flexibility. They call this functional living ink, or FLINK.

Michele: I love that.

Vincent: A bio compatible immobilization medium that exhibits the viscoelastic properties required for 3D printing through multimaterial direct ink writing. So that is complicated but basically they have to make this matrix strong enough and printable, it has to be squeezed through a nozzle and shaped into different forms and have different kinds of properties. And that is what they do in this paper.

Michele: It has to have enough solidity that it will hold its form but it also has to keep the bacteria alive.

Vincent: That's right.

Michele: That's where the tension is in this design project, between those two goals.

Vincent: Right. You want to put them in an ink that can be extruded and what they do is they make filaments, plus you have to keep them alive, and they have to metabolize because you want to use that metabolic activity to produce something or to degrade something. And so what they have settled on is a hydrogel composed of hyaluronic acid, kappa-carrageenan, and fumed silica. They have learned that this is compatible with bacterial growth and metabolism, it can be printed into scaffolds, and they go through a little bit of illustration to show that. In the first one is biodegradation by Pseudomonas putida immobilized in a 3D printed lattice, and the second is making cellulose by A. xylinum. What does the A stand for? Michael must remember.

Michael: Uh.

Vincent: Acetobacter.

Michael: Acetobacter. It's an acetogen, yeah.

Vincent: It makes cellulose. So basically they are going to put these bacteria in the inks and then they are going to extrude these filaments into different shapes and see how they work. So they go through quite a bit of experimentation to find the right concentrations so the bacteria survive. They have to make sure these filaments don't dry out, they have to be strong enough that they don't shear, they have to flow properly out of the ink nozzle. These are all process based issues that I don't usually think about, like shear forces on the printing process and that sort of thing.

They basically come up with in the end with a good composition of this FLINK. They show that you can actually incorporate bacteria into very specific regions of a printed structure. They have a picture here with a shirt a grid and some other things, and you can see that the bacteria can be placed in very specific places. I'm not sure how that works, actually. I believe them but I don't know how you would make sure that the bacteria are in a particular place, right?

Michael: Just like you have a four color printing process, you can actually have the ink because they are using, my guess is a variation of the standard inkjet printer that we all know and love. Many of us have it in our office. So you would put the bacteria in the black well or the yellow well or the cyan well and it would just squirt it on when it is calling for black or blue or yellow.

Vincent: Just like printing, yeah.

Michael: It's just like printing, and the trick, I think is the matrix that they are using. It is nothing more than a grid pattern and their inherent design of this grid pattern is that the bacteria will actually be able to grow out of the ink and actually fill the space. So that is what the cool part is, that it is both solid and not. So the microbes can actually get across or outside of the matrix that they are doing very similar to the way biofilms actually continue to expand and slough and do all the wonders that biofilms do.

Vincent: In fact, they even mention that they loaded the tanks of printing ink with different bacteria, so it is exactly as Michael said. You can imagine that wherever a certain color has to be it would mix the inks together and you would get a mixture of the different bacterias so they could be placed very specifically in this structure just by injecting them.

Michele: So Vincent, imagine what this is going to do to your agar art competition in the future.

Michael: Oh, God.

Vincent: Oh yes, for sure!

Michael: This is going to--

Michele: Each bacteria could be a GFP or RFP.

Vincent: Sure, all different colors, yeah. And whoever is going to be able to afford this, right (laughs) Even 3D printing is not cheap at the moment, you know. I just went to a company online and ordered some 3D viruses printed and have them here on the desk in front of me. They are about 2 inches in diameter and they were 30 dollars each. I could go broke on viruses.

Michael: You just have to go down to the dental school and meet your friends in the dental school. Many of the dentists are using 3D printers to print appliances that actually, night guards and other things. So they may have a 3D printer in their lab, and if you get the coordinates of the virus you could make your own. The materials aren't that expensive, what you are paying for is the hardware.

Vincent: That's right.

Michael: And the know-how.

Michele: That's a beautiful set up for the second part of the paper, the cellulose producers.

Michael: That's right.

Vincent: They have a lot of experiments where they make sure that the bacteria can live in these things. They do viability studies, they do some crosslinking, they look at viscosity and so forth. They do make a grid which if you think of a screen with very large openings they can print these bacteria in that kind of an orientation and they look at the swelling of the gel in water versus medium and then they do an experiment where they make one of these grids and put Pseudomonas putida in it and ask can it degrade phenol?

So just think of a screen but much thicker and with bigger openings and your bacteria are embedded in this and you can place that and they place this in a flask with phenol in it and they show that the concentration decreases. And what they can do that is really cool, they can take out the grid, wash it, and put it back in again and it will go through a second incubation period and remove even more phenol and convert it into biomass. So they show that this actually works. A lot of bacteria are released from this grid as Michael mentioned before, so it must be a combination of those plus the bacteria in the grid that are doing the degradation. So that is interesting but Michael, tell me why is it better than just if you have a phenol spill, can you inoculate your bacteria into the spill, why is this better?

Michael: Well, if you think about it, a lot of time what you want to do is go into aquifers and what you will do is pump out the material and then you can drive air across it and you can vaporize it. That is the way a lot of these aquifers are cleaned is they sink a well and they drive air across it and they vaporize it and let the volatile organic just diffuse into the atmosphere. So what you can imagine is you can blow the phenol laced air into this reactor technology and the microbes are suspended in this very hydrophilic gel and you are providing them a carbon source and if they are bathed in the other essential materials that they need to grow and they were using a minimal medium where the defined chemical composition is known, and the only carbon source that the microbe has access to is the phenol.

You can actually design it so it can degrade in real time, so rather than trying to figure out how to get the phenol out of the water or separate the water or concentrate the phenol, you can set up a surface to volume ratio so the air is literally feeding the microbes, and when the air comes out the air is devoid of the phenol because you have that incredible driving force of the microbe wanting to eat or needing to eat the carbon in energy source for it. This actually has so many applications in the environmental world. You can imagine this on plants that are trying to deal with the OC loads carbon sequestration or all sorts of things. You could imagine going after that.

Michele: And the ability to make these grid-like patterns as you pointed out increases the surface area to volume ratio. So it increased the efficiency in a smaller space.

Vincent: For example, if you had an oil spill in the ocean, you could make a grid and put it right in the oil, right?

Michael: Right, right.

Vincent: And when it is done you pull it out and wash it and reuse it maybe.

Michael: And if you think about it, with the spill that we had in the Gulf a few years ago, they were effectively just inefficiently dumping fertilizer into the major parts of the oil and the microbes were already there and it was not as efficient. Could you imagine if you had the right mix of oil degrading organisms suspended in these grids, you could effectively create a wall as the oil would move with the current. It would degrade in real time simply as it would pass through this. Because of the 3D geometry you are getting all that catalyst that is effectively going to degrade it or transform it so that it is not as sticky or as heavy and you can have various organisms at different levels that may be chewing at one aspect of the oil, one particular carbon fraction versus another.

So by demonstrating that this works for something as straightforward as phenol, but you can well imagine that the next experiments are to build these complex geometries with organisms that will have different predilections for one carbon source over the other. It is whether your kids like peas or broccoli. And oil is composed of both peas and broccoli, and you could actually sort them out based on the organisms you dynamically load into it.

Michele: So to borrow a phrase from the movie The Graduate, I have got one word for you. FLINK.

Vincent: Flink!

Michael: Flink!

Vincent: That's right.

Michele: Flink! It's the way of the future.

Vincent: I've got one word. Flink.

Michael: That could be the title.

Vincent: That's right, I like that. So the other thing they do is they take A. xylinum which can make cellulose when exposed to oxygen. So they make a matrix containing that bacterium and then they put it on a face mold, so they have a mold of that person's face, and they layer this on top of it and the bacteria make cellulose, they can then wash out the ink constituent, and they leave behind a network of fibers. They call it nanofibrillated bacterial cellulose. So they say this could be skin. This could be used as human skin.

Michele: I thought that was just amazing. Wow.

Michael: You could almost imagine that if you could create this matrix of bacterial cellulose that you could then deposit kidney cells. Could you make a real time dialysis unit where you would just pass the blood where you would allow the waste products to go through this filter, to effectively take out, to do effectively what a kidney does and just return it. It is a variation of peritoneal dialysis but with a real machine.

Vincent: I mean, what we should point out is you can remove the bacteria so they can mold this face and then remove the bacterium and then if they have to they can put it on your face if you had lost all your skin. But you can get rid of all the bacteria which is important.

Michele: They also point out that this is not far fetched. They have cited a couple of reports where bacterial cellulose is already being used in the medical industry for tissue engineering. So you can read more if you pull this paper off the world wide web.

Vincent: I can imagine all kinds of films and coatings, implants, to avoid organ rejection, and they also talk about incorporating oxygen producing bacteria in these gels. And that oxygen is needed for the A. xylinum production of cellulose. So you can have really big things made, right, the experimental one they made here is rather thin because it needed to be permeated with oxygen but if you include oxygen producing cyanobacteria you could make it much thicker.

Michele: Wow.

Michael: And you add a light so you don't even have to feed it. It will just photosynthesize.

Vincent: That's a human face. A cute little picture there of a human face with some bacteria making a cellulose film on top of it. Skin replacement, organ transplantation, any 3D shape you can imagine, you can make. So that is pretty cool.

Michele: It is. I'm a little puzzled by how you would control, like what is the optimal amount of bacterial replication you would want?

Vincent: I guess that is the sort of thing you have to figure out, right?

Michele: Right. And it probably differs depending on your application and which bacterial strains you are using. It is a living, dynamic system.

Michael: And the carbon source that you feed it.

Vincent: That's right. We envision that long term medical applications will benefit from the presented multi material 3D printing process by locally deploying bacteria where needed. This is so cool. Unprecedented functionalities, adding a new dimension to 3D printing. That is very cool. So if you lose your skin on a part of your body they could synthesize a new skin out of cellulose and put it back on, for example, and as Michael suggested maybe whole organs one day. So this is pretty impressive, I would say. The potential of this is really amazing.

Michele: They also point out that the incredible diversity in the microbial world, since the microbes live everywhere on the planet, in so many different environments, that the metabolic toolchest that engineers would have to apply to this technology is essentially unlimited. I am sure there are engineering issues that are going to need to be solved but the potential is amazing. Flink, one word, flink.

Vincent: Flink! What did you say, the future is Flink?

Michael: I have one word for you, Mrs. Robertson.

Michele: Was it Mrs. Robertson that gave the--?

Michael: No, no, it was a guy standing at the pool who was telling Dustin Hoffman that.

Vincent: Yeah, that's it. All you have to do is Google "I have one word for you."

Michele: Flink.

Vincent: Plastics.

Michael: And it's not plastics. It's Flink.

Vincent: That's funny, Flink, yeah. In fact if you search you get the clip from The Graduate, the one clip where he's saying it. That's so funny. A young Dustin Hoffman. Anyway, I thought that was an impressive paper, even though most of it was not microbiology, but it was still very cool. There is a nice picture, I don't think this is an open access article--

Michael: No, it is.

Vincent: Is it?

Michael: It is. It is distributed under a Creative Commons attribution non commercial license.

Vincent: Nice, very good, I can even use the image, then.

Michael: Yes!

Vincent: Wonderful, I love when they do that. Thank you very much. Well, there you go, 3D printing of bacteria into functional, complex materials. Just ask for one under the Christmas tree (laughs) or the Hanukkah candle.

Michael: Or dreidel.

Vincent: Isn't that cool?

Michael: You know, I developed back in the early 90s a vapor phase bio reactor that would take these VOCs and it was based on membrane oxygenators in which we would blow the air across the oxygenator and the biofilm was effectively growing on these hollow fiber polypropylene tubes that had little tiny holes in them so that gas would effectively come and the bacteria were on the outside of the tube, if you will, and they were bathed in media, and the problem depending on which medium that we used is that you could only have so much catalyst at the surface and this solves the problem of putting catalyst in 3D. Because that was always our limiting capability, of how much catalyst we could have per surface area and the cost of the membrane was very expensive.

But this then makes the whole device get much smaller and we could feed that membrane oxygenator as much VOC as we could pump through it. And it would be all gone in real time. It was incredible to see how well it worked but the engineering limitation we had is we had no way to cheaply make more of the catalyst available short of buying more membrane oxygenators, which were very expensive. So this solves the problem and because you can use, you can make as many color inks as you would like, which means different bacteria, you can go after very complex waste streams that can be degraded in one single pass which is really code for what a kidney or a liver is really doing in the human body. We are constantly feeding ourselves complex waste streams that our liver and kidneys deal with in real time just by passing blood across that cell matrix.

Vincent: Neat stuff. Okay, we don't have any emails, so I think, yes Michele?

Michele: I have a little current events as today is December 14, when we are recording this and so on Capitol Hill our elected officials are trying to reconcile the House and Senate tax package and one of the issues that we

are concerned about in academia is whether or not graduate student stipends are going to be taxable. Currently you are not taxed on the tuition waivers that many graduate students receive. Currently the word is that that will not be included in the final bill, so we are hopeful because that will really drive up the costs of graduate education.

Vincent: Yeah, if they have to vote on it that should go out, but I am hoping it will not pass because it is a lousy bill either way.

Michele: Yeah. That's a bigger question.

Vincent: But I don't know what is going to happen, that's politics. We are microbiologists. So we don't have any emails, but I am going to have to give away a book next time to start getting some emails, and so our next, this episode will be released on the 21st of December, we will record another one on the 28th. So then we will do a book giveaway. That will be released on the 4th of January, so early next year you can start trying to win another book. Try your hand. This is TWIM 167, you can find it at asm.org/twim, you can find it at Apple podcasts, and of course if you have a phone or a tablet and you listen to podcasts on an app, it is very easy to subscribe. Please do that so you get every episode. It also helps us if you subscribe, that helps our numbers.

If you like what we do, consider supporting us financially. Go to microbe.tv/contribute. Some of you may know that Patreon had a little issue in the past week or so, they were going to charge patrons a certain amount of money, and that caused us to lose quite a few patrons who were contributing to us. So they have rescinded that move and so I hope those of you who left will come back. If everyone just gave us a buck a month, that's 12 bucks a year, that's like 3 coffees, it would help us so much. So consider doing that for the holidays, give us a holiday gift. We would be very grateful. And of course send your questions and comments to TWIM@microbe.tv. Michele Swanson is at the University of Michigan, thank you Michele.

Michele: Thank you!

Vincent: Very nice explanation of a virus today on TWIM.

Michele: Well, I like to keep the melody clear, not get bogged down in so many of the details.

Vincent: And also you benefit because you do teach and it helps you with that, right?

Michele: Right. And I am just winding up teaching a first year seminar called Current Topics in Microbiology, and I can't wait to incorporate this one on 3D printing of bacteria in next year's rendition of that class.

Vincent: That'll be great, they'll love it. That's good. Michael Schmidt is at the Medical University of South Carolina, thank you, Michael.

Michael: Thanks Michele and Vincent.

Vincent: And I am Vincent Racaniello, you can find me at virology.ws. I want to thank the American Society for Microbiology for their support of TWIM, Ray Ortega for his technical help, I want to thank Ronald Jenkees who wrote the music that you hear on TWIM, he is at ronaldjenkees.com. I want to thank all of the listeners, especially those who support us financially, but everyone for listening and being a part of the team, and I hope everyone has a great holiday and a very good New Year. Thanks for listening everyone, we will see you next time on This Week in Microbiology.

(music)

Transcribed by Sarah Morgan.