

Disruptive: Putting Biofilms to Work

Host Terrence McNally interviews Neel Joshi and Anna Duraj-Thatte. Podcast published on September 12, 2016.

McNally:

Hello. I'm Terrence McNally, and you're listening to Disruptive, the podcast from Harvard's Wyss Institute for Biologically Inspired Engineering.

When I mentioned I was working on a podcast about biofilms, a friend assumed I meant movies about biology. Most of us think little of biofilms. If we do, we're probably imagining slime on stones in a stream, dirty pipes and drains, dental plaque, or worse. It's estimated they're involved in up to 80% of all microbial infections in the body. Scientists, however, are beginning to subvert and take advantage of the very properties that make biofilms so effective as nuisances or threats.

A team at the Wyss have developed a novel protein engineering system called BIND, which stands for Biofilm-Integrated Nanofiber Display. They see biofilms as a new platform for nanomaterials that can help clean up polluted rivers, manufacture pharmaceutical products, fabricate new textiles, and more. I'm going to speak about this with Wyss core faculty member, Neel Joshi, and postdoctoral fellow in bioengineering, Anna Duraj-Thatte.

The mission of the Wyss Institute is to transform healthcare, industry, and the environment by emulating the way nature builds. Our bodies and all living systems accomplish tasks far more sophisticated and dynamic than any entity yet designed by humans. By emulating nature's principles for self-organizing and self-regulating, Wyss researchers develop innovating engineering solutions for healthcare, energy, architecture, robotics, and manufacturing.

[01:34]

Wyss core faculty member, Neel Joshi, is an associate professor of chemical and biological engineering at the Harvard John A. Paulson School of Engineering and Applied Sciences. He earned a BS in Chemistry at Harvey Mudd College and a PhD in Organic Chemistry from the University of California Berkeley.

Joshi is developing new biomaterials constructed from engineered proteins and peptides. The goal of his research is to extract innovative design principles from materials and systems that are the product of natural evolution and embed them in synthetic systems so that we can precisely tune their physical properties to suit our biomedical and biotechnological needs.

[02:13] Welcome, Neel Joshi, to Disruptive.

Joshi:
Thanks for having me on, Terrence.

McNally: [02:17]
Can you share a bit about your personal path, Neel?

Joshi:
Like you mentioned, I did all of my schooling, really, in the field of chemistry. I've always been interested in what stuff is made of, from reading ingredients labels on food to figuring out what types of materials make up everyday objects that we work with all the time. That, I think, fueled my interest in chemistry, but much more at the molecular level early on in my education.

Then, after I graduated from Berkeley, I started a postdoc at Boston University, where I worked on some more materials types of applications. I became more and more interested in how we could put together these molecules, essentially, into larger and larger structures, and build actual materials that had material properties that you and I associate with objects that we see every day.

My postdoctoral work was in the context of tissue engineering, specifically cartilage tissue engineering. Then, when I started my own lab at the Wyss and at Harvard, I wanted to combine my interest in synthetic chemistry, and especially synthetic chemistry involving biomolecules, with my interest in materials to create the new systems that we're going to talk about today.

McNally: [03:30]
We're going to focus on your work with biofilms, but briefly, your current projects that you're involved in employ a range of approaches. You actually are working with a number of the platforms at Wyss. Could you talk just a bit about that?

Joshi:
When I started at the Wyss, my lab was much more interested in synthesizing or recombinantly producing in bacteria interesting molecular components, like molecular Lego blocks, if you will, and putting them together in interesting ways, understanding how they self-assemble to make interesting materials, and mostly in the realm of peptides or proteins. These are molecules made up of amino acids that are really the workhorses of biology.

When I started, I sat next to these people at the Wyss Institute who were doing things like DNA origami, which has really blown the lid off of the field of molecular self-assembly, in terms of its capabilities. They're able to really significantly surpass anything that you could do with peptides, in terms of controlling shape of self-assembled materials.

That led me to reconsider our own path. I don't want to denigrate peptide self-assembly, because that's still a vibrant field and people are doing wonderful things there, but it definitely inspired me to think about the problem in a different way, because the types of shapes and structures that we could assemble from peptides, which was the focus of my research, was never going to reach the same heights in the same way that DNA origami was.

It so happened that we were, my lab, is also neighbors or we interact regularly with many synthetic biologists who are also part of the Wyss Institute, so people like Pam Silver, George Church, et cetera. Looking at the way that they were able to harness biosynthetic systems or living microbes to produce interesting things, we figured why don't we - instead of trying to synthetically make these molecules and remove them from that environment and reconstitute them into interesting materials - why don't we program the living organisms, in this case microbes, to directly make the materials that we're interested in? So that's what really led us to biofilms.

McNally: [05:42]

You referred to it there, but what was it? What is it about biofilms? In other words, when I've talked with some of your peers about why they work with DNA, it was very clear. They said, "A protein does more things, but it's harder to control. DNA, because it adheres so strictly to certain rules and limits, actually allows us to control it more." Out of all the various entities and substances and so on you could be dealing with, what led you to biofilms?

Joshi: [06:11]

One thing is that they are highly abundant. Biofilms are pretty much literally everywhere. You mentioned in your introduction that they invoke images of slime on teeth, or pond scum, or something like that. Really, if you can consider that the vast, vast majority of all bacteria on the earth live as part of some type of community, which is essentially a biofilm.

So they're extremely abundant, and they are robust as materials. They have intricate microstructures in some cases that enable them to perform interesting functions, certainly from the perspective of the microbes that produce the biofilms. They allow them to survive under conditions where they otherwise wouldn't be able to survive.

They also have interesting materials properties. They can be, in some cases, slimy, like some of these examples that we just discussed. In other cases, they can be much more robust and potentially be relevant for the types of materials that we might want for engineering purposes, or even consumer goods, or the types of objects that we interact with regularly in our daily lives.

McNally: [07:13]

Why do cells form into biofilms? Why do they form into communities? What

are the advantages and are there any disadvantages to their forming into communities?

Joshi:

The advantages are that the biofilm enable the cells that make the biofilm to divide the labor of survival up into different lineages, if you will. We generally think of bacteria as single cellular organisms. To anthropomorphize them a little bit, you might think that it was every man for himself in the bacterial community, but in fact they do cooperate quite a bit. This has been known for quite a long time.

One of the ways in which they cooperate to lessen the burden or increase their chances for survival in certain conditions is to form biofilms. What biofilms do is enable them to, for example, adhere to a surface that they might want to adhere to because it's advantageous for them. It's nutrient-rich, or it prevents them from being washed away under some kind of flow, and things like that.

Biofilms also protect the cells, essentially, that produce the biofilms. They can shield the cells from physical stresses, in some cases from environmental stresses, changes in their surrounding environments, in terms of pH, hydration, other environmental conditions, I suppose... Essentially, bacteria are making a home for themselves.

McNally: [08:38]

Yes, and one of the interesting phenomena, I think, is that the cells secrete outside of themselves substances which then create the protective layer...?

Joshi:

That's exactly right. You can think of the biofilm as being composed of two things: one is the cells themselves; and the other thing is the matrix that they produce. This matrix is in the form of polymers that they synthesize and secrete which bind everything together and help to form this slime that we normally think of when we think of biofilms.

McNally: [09:08]

What are the key properties that serve biofilms in nature which make them attractive to you?

Joshi:

I mentioned their abundance, which is nice for us, in that they are easy to access. The other thing is that they are materials that are made by microbes, which are the most engineerable types of organisms that we know of. We specifically work with *E. coli*, which is possibly the best-understood organism on the planet, certainly in terms of its genetics. That affords us a bit of ease and familiarity, in terms of understanding how we can engineer that microbe, from a genetic point of view, to alter the types of biofilms that we can get out of this organism.

McNally: [09:51]

In other words, those things that serve the molecules to form into and the cells to form into communities, are the same things that make them useful for you, the ability to survive, the ability to invade, those sorts of things?

Joshi:

Definitely. In many cases, the robustness of the material, that matrix that I mentioned, that's composed of various biopolymers, the cells themselves want that material that they're making to be robust for the reasons that I mentioned. It protects them from all of these environmental conditions. It allows them to adhere to surfaces. In many cases, those are the properties that we also want as materials engineers for various functions. If you want to design a surface coating, for example, obviously adhesion to an underlying surface is going to be very important. Then, just being able to withstand many different conditions is also important in various contexts, from an engineering point of view, for materials.

McNally: [10:42]

What is self-assembly, for those who that might be new to, and how does it work in biofilms, and how does it serve you?

Joshi:

Self-assembly is essentially a property that allows molecular components, in this case that are synthesized by the cells themselves, to come together to form larger structures. You can think of it as an individual polymer, if you were able to look at it in isolation, would look something like a tangled spaghetti noodle. It wouldn't have a lot of inherent structure on its own. It would be in some random orientation. But, because of the properties of the molecule that makes up that chain, it's able to interact with its neighboring molecules and other entities in its environment, like the cell itself, to form materials that have more structure.

For example, the system that we work with forms fibers. Instead of being in some random orientation, these units come together in order to form essentially a line, a stack, which is then a chain in itself. It's forming this hierarchical structure. There's, on one level, the structure of the polymer chain itself, in this case a protein. That protein folds into a particular shape, and then that you can consider as a Lego block of sorts that comes together with its neighboring proteins in order to form larger structures. Then those structures come together in turn to form still larger structures, et cetera. This is basically the way that biology builds structure, in general. We are built that way. All materials in biology are built this way, from the bottom up.

McNally: [12:08]

The concept of a microbial factory has been around for a while. What is it that your team is doing that's new and different?

Joshi:

We do see a parallel with the trajectory of the concept of the microbial

factory. When microbes were first discovered, it was in the context of their role as a health threat. The germ theory of disease that started with Pasteur and Koch, ~~and stuff like that~~, really led to the understanding that these microscopic organisms are causing disease, and that is what fueled a bunch of research into how they operate. It was the genesis of fields like microbiology and the discovery of DNA. All of that came out of this, in part, out of this germ theory of disease, and the concept that these things are bad and we need to understand how they work. Because of that intense research into microbiology and similar fields, we can now harness these microbes, or certain microbes, anyway, as factories, like you mentioned.

I would say, up until very recently, the concept of using these microbes as factories has focused on programming them to produce soluble molecules of interest. So, for example, a therapeutic like insulin, for example, that's produced recombinantly would be a protein, in this case, that you can have a solution of. You can have it in a vial, and that can be used for some useful purpose.

Alternatively, biofuels have been a large focus in the field of thinking about microbes as factories. These are essentially small molecules that can be useful in the context of fuels.

But what we would like to do is figure out how we can make materials which, in addition to producing the molecules of interest, necessitate organizing through self-assembly those molecules into these higher-order structures, which is something that we think is a frontier for the concept of the microbial factory.

McNally: [13:56]

By the engineering that you do, you program what they will do, and that's what you mean by that second step. They don't just produce building blocks, but they actually have a "plan," in quotes, if you will, of what they're going to do with those building blocks.

Joshi:

Exactly. I shouldn't take too much credit here, because we are essentially relying on what nature has already developed, to a large degree, in the form of these proteins that already have this self-assembly property built into them.

E. coli, for example, for the system that we use, has already figured out how to use these proteins, form them into fibrils, use those fibrils to adhere to surfaces, et cetera. We are essentially making minor tweaks to that structure to imbue these materials with new properties.

McNally: [14:39]

What are some of the novel properties or functions that you've endowed biofilms with through your programming?

Joshi:

When E. coli forms biofilms, the biofilms that E. coli forms usually have two major structural components, in terms of those polymers that I was talking about earlier. One of the types of polymers that forms the biofilm is cellulose. This is a biopolymer that we're all familiar with. It forms, to a large extent, the structure of plants. It turns out that E. coli also uses cellulose as a polymer in a structural context. That's one component of the biofilm.

Then, the other major structural component of the biofilm, is curli fibers. These are protein-based fibers that are formed through a process of self-assembly. That's the system that we exploit when we make the synthetic materials that we are engineering.

The way that this curli system works is there's a particular protein called CsgA that is secreted by the cell. It exists in this random conformation, a random shape, after it's secreted by the cell. It's just floating around outside the cell until it encounters another protein on the E. coli surface, which induces it to form a specific fold. That fold you can consider as the Lego block that can be put together with a neighboring Lego block to start building a structure.

Then, that starts a growing fiber from that's anchored to the cell surface. As more and more of these CsgA proteins add on, the fiber grows out from the cell surface and eventually becomes long enough to encapsulate all of the cells. If you look at the cells under an electron micrograph, for example, the cells will appear as these oval-shaped cells, which is what E. coli looks like, and they're embedded in this fibrous mesh. They almost look in some cases like eggs sitting in nests, or something like that. This entire fibrous mesh is essentially made of a single protein in the case of these E. coli strains that we have. We see that as an engineering opportunity.

McNally:

That, by the way, Neel, that is what nature does.

Joshi:

That's right.

McNally:

...and then you insert your cells into that system.

Joshi: [16:47]

Exactly, by altering the structure of that protein monomer, the protein building block that undergoes that self-assembly process. The way that we alter that structure is to append extra domains onto that protein. These extra domains are intended to imbue that material with some new function of interest.

Through a process of genetic engineering, the domains can be appended to

that CsgA carrier protein. The extra domain then just goes along for the ride through the whole secretion and self-assembly process. Then that extra domain is displayed all over the surface of the biofilm. That fibrous mesh that I mentioned earlier would be coated in this extra domain.

The way that we choose that extra domain is based on an understanding of some particular new function that we want to introduce into the biofilm. For example, things that we've done are to take a domain that has known surface binding activity.

There's a particular peptide that we show in one of our publications that is known to bind to steel surfaces. This is a domain that was isolated from a completely separate organism. It turns out that that 17-amino acid sequence in isolation is able to bind to steel surfaces quite strongly. We can take that sequence and now append it to our CsgA protein. It gets displayed all over the surface, and it will imbue the E. coli biofilm that we form with the ability to bind to steel strongly. Whereas, the unengineered system can easily be washed off of steel.

We have the ability, for example, to control which surfaces our biofilms will stick to, which is important in the context of certain engineering applications. Biofilms are advantageous, in that they are easily replenishable. You can consider them as living materials that you can program. The downside of that is that they can, for example, clog plumbing, which would be not desirable in many applications. So the ability to control which surfaces our biofilms adhere to could be advantageous in that context.

McNally: [18:53]

How do you know, how do you find, how do you select both the thing, the peptide, or whatever molecule has the property you want, and then how do you guess, or is it trial and error, that it will properly fuse and work with the molecule you're already working with?

Joshi:

It turns out that there's actually a huge, huge library of thousands, maybe tens of thousands of such sequences that are pretty well-characterized in the literature. That saves us the work of having to develop them ourselves, which is nice.

One of the advantages of our system, as we've characterized it so far, is that it seems to be compatible with quite a wide range of different sequences and structures of these appended domains that I'm talking about. It's quite versatile, in that regard. In terms of figuring out which sequences will give you which desirable function, there are many techniques that are available to engineer such sequences.

Phage display is one of them. It's a directed-evolution type of process that is able to identify sequences with desired properties, for example, the ability to bind to certain surfaces. Overall, the point is that there is a huge body of

literature out there that essentially provides us a sandbox of different domains that we can choose from.

So far, a large fraction of them seem to be compatible with our system, in that they can be displayed on the biofilm. Then, to some extent, it is a trial-and-error process to understand whether or not those are functioning properly after they're displayed.

McNally:

In other words, it had a function in this context. Will, when we display it with ours, will it have that same function? To what extent? Et cetera. That's the thing you're then looking at...?

Joshi:

Exactly.

McNally: [20:35]

I saw a quote that you said. You said, "Until recently, there was not enough cooperation between synthetic biologists and biomaterials researchers to exploit the synthetic potential of biofilms this way, and we're trying to bridge that gap." Can you talk a bit about that?

Joshi:

I think these types of interfaces between fields are coming up all the time. It just so happens that I think these two particular fields, there's an interesting interface between them at the moment.

As I mentioned with the concept of microbial factories, the initial focus was on the production of soluble products. So far, there hasn't been as much focus on producing materials themselves, but that idea is actually taking off and has quite a bit of research in its early stages right now. We see it as exciting, in that regard.

[21:22]

When I talk about things like this, it sometimes seems like these ideas are very, I don't know, out there, science fiction-y, but I like to bring up a few examples of actual companies that are marketing products now. These are relatively new startups that, I think, fall under the umbrella of what I'm talking about.

There's, for example... This is more of a boutique application, but there are fashion designers out there, one in particular, who've shown that you can grow cellulose mats and form them into clothes. It so happens that some bacteria produce large cellulose mats when they're grown in culture. What this particular fashion designer does is takes... I think she's derived it from a kombucha culture. I don't know if you're familiar with...

McNally:

Sure.

Joshi:
...the fermented tea, kombucha.

McNally:
I'm in Los Angeles. Of course I am.

Joshi:
Right. Exactly. This is accessible to anybody.

She grows these microbes in her bathtub. They form a bathtub-shaped mat, which is like a several-inch thick mat of pure cellulose, essentially. She takes that mat and then dries it out in the sun. It forms a kind of cellulose leather, if you will, that she then shapes into garments.

Her name is Suzanne Lee. She's got a TED Talk. You can check it out, and everything. When she's giving her talk, she is wearing one of the garments that she's made. It's kind of like an almost see-through or translucent-looking material. It's very unusual. She mentions that even though it looks very cool it would actually make a poor functional garment because it absorbs water very readily. If you walk out on a humid day or if it's raining, it will immediately swell up and obviously become a very poor garment.

At the end of her talk, she makes a plea for engineers who can figure out how to make versions of this cellulose that are more appropriate for things like garments. We see that as a call to arms for engineering microbes that can do similar things as this naturally-occurring microbe that produces the cellulose, but make materials that are more suitable for engineering purposes.

McNally:
Interesting. I must say one thing. I actually, years ago, did ferment kombucha. What you end up with is what looks like a large pancake.

Joshi:
Exactly. That's the cellulose.

McNally:
Or a moo shu. It looks even more like a moo shu. That's what you're talking about.

Joshi:
Exactly.

McNally:
What that is that I'm talking about is a very sturdy and robust biofilm.
Correct?

Joshi:

Yes. That's exactly right. In addition to that example, there's a couple of other examples of people who are taking this concept into an industry that is potentially actually commercializable and will be available to the general public.

[24:08]

Another example is a company called Ecovative. There's also one other company whose name I forget that is doing something very similar. It turns out that there's a fungus called mycelium, which survives by breaking down cellulosic plant matter. The fungus survives by eating the cellulose in plants, but, while it does that, in order to form a community again, it secretes its own polymers. What it does is essentially form a biofilm of its own, except instead of the more slimy type of biofilm that we normally think of, this one is much more mechanically robust. It's like a foam, almost.

What these companies have figured out how to do is, they will take plant matter, inoculate it with this particular fungus, the fungus will eat the cellulose in that plant matter, and in the process synthesize all of these biopolymers to stick everything together in a matrix. What they get at the end is a solid block that is composed of whatever residual plant matter is not digestible by the fungus and then the material that the fungus itself has made to bind everything together. They get a solid block. That block is completely biodegradable. You can take that block and throw it in your garden and compost it. What they're doing now is making packaging materials out of this material.

The Styrofoam corner piece that you get when you buy a new TV or computer, or something like that, instead of having that made out of Styrofoam, which is a petroleum-derived material, you can make this out of this fungus, essentially, and plant biomatter. That packaging material can then be thrown in the garden and can compost itself. That's another example of harnessing the biosynthetic power of biology in order to make materials.

McNally: [25:52]

What are amyloid proteins? Amyloids usually get a bad rap, because they play a role in health challenges such as Alzheimer's. Correct?

Joshi:
Yes.

McNally:

...but in this case, they work for you. Tell us how they come into play.

Joshi:

It's funny, actually, that biofilms themselves have a bad rap, in some regard, and amyloids within those also have their own bad rap, but it turns out that we're trying to reform the image of both of those things simultaneously.

The initial understanding of amyloids were that they were a protein misfolding problem. They were proteins that had some aberrant folding that led to the formation of fibrils. This is another self-assembly process. In its original context, it was understood as being bad because it contributed to the formation of these plaques that are characteristic of diseases like Alzheimer's, et cetera.

But it turns out that that particular protein fold that's characteristic of amyloids is an extremely common type of protein fold. Some time after the amyloids were discovered in these pathological conditions, another class of amyloids were discovered called functional amyloids.

These are, instead of proteins that have adopted some fold that they shouldn't be in, these functional amyloids are classified by the fact that the organisms have purposefully formed these amyloid fibers because they provide some benefit to the organism.

In the case of the system that we're working with, the curli fibers, the individual proteins that form the fibers are amyloid proteins. The fibers themselves are formed through the polymerization of those amyloids to form large amyloid fibers. Except, in the context of *E. coli*, the organism is doing this on purpose because those fibers contribute to some fitness benefit. In this case, for example, it helps them adhere to surfaces by forming these fibers. It also provides structural support to the biofilm structure itself.

McNally:

One thing I've read is that amyloid proteins are enormously tough and strong.

Joshi:

That's right. This is both an advantage and a disadvantage for us, because when we want to work with these proteins and engineer them, we're able to form materials that are quite robust because of the robust nature of the proteins themselves. At the same time, it's difficult to analyze the composition of those materials, because it's difficult to break down those proteins into their constituent components to analyze them, to digest them, et cetera, which is a key tool that is usually available to people who are working with such systems.

McNally:

That presents a challenge, but the payoff is super tough natural materials performing functions you desire.

Joshi:

To give you a benchmark, usually when people think of proteins, they are somewhat sensitive. You can think of drugs, for example that are antibodies, which are proteins. These are relatively sensitive entities. They have to often

be refrigerated. They have to be kept under sterile conditions. Things like that. Amyloid proteins are much more robust. Our amyloid proteins, we can boil them in detergent and they still won't break apart into their constituent components.

McNally: [29:00]

Very good. What is your team doing with enzymes?

Joshi:

It turns out that enzymes are used quite frequently in many different fields. Pharmaceutical companies routinely use enzymes to help them achieve certain chemical transformations. Enzymes are essentially nature's catalysts. They're good at doing certain types of chemical transformations which are necessary in some cases, for example, in the synthesis of drug intermediates, and things like that. There's a need, to some extent, to understand how to use these enzymes in a process where you're synthesizing a certain compound.

What we're able to do is modify our very robust biofilm-based material with enzymes on its surface using the technique that I described to you before. It turns out that that enables the enzymes to become more stable than they otherwise would be. It also adheres them to the surface so that they can be recovered from a reaction mixture and then reused, which brings down the cost of the manufacturing.

Another advantage is that it's completely biologically-produced. Our system doesn't require any purification of those enzymes or reconstitution, in the context of some bioreactor. We have a system that is a single organism that could potentially create the material that binds the enzymes together and the enzymes themselves, and then incorporate that into a manufacturing context to facilitate the production of certain chemicals.

McNally: [30:27]

You've talked about proteins, peptides, enzymes. What are these things? What defines these things, so that people have a notion of what we're talking about.

Joshi:

As you mentioned, you had talked to other people at the Wyss who are working with DNA, which is one type of biopolymer. Proteins are another type of biopolymer. They really form the workhorse of biological systems. They, in some cases, play a structural role, like the curli proteins that we are talking about here, in their ability to self-assemble into larger and larger structures. In other cases, they are able to perform chemical catalysis. Those types of proteins would be enzymes. What characterizes proteins is really just a linear string of amino acids. This is a type of biopolymer that all living things use to perform various functions.

McNally:

So an enzyme is a type of protein. What defines it as an enzyme?

Joshi:

The thing that defines an enzyme is its ability to perform catalysis, which is just facilitating a particular chemical reaction.

McNally: [31:39]

You're also doing some work in the biomedical space, work with the gut, for instance. Can you talk a bit about that?

Joshi:

When we initially characterized our system, we were able to show some of these functions that I mentioned before, where we could control the adhesion to certain surfaces. We could, in other cases, immobilize things onto this biofilm. We could really start to think about programming how the microbes and the biofilm that they were forming was interacting with its surroundings.

We thought that we could also do something similar inside the body. It turns out that our guts are full of microbes, as we are more and more aware of. Things like the Human Microbiome Project have also fueled a bunch of research into understanding what these interactions between the microbes that live in our gut look like when they interact with our body.

All of those microbes live, again, as part of some community, which you could define as a biofilm. We envisioned our BIND system being used inside the body to essentially reprogram the way that certain microbes were interacting with our bodies and use them for some therapeutic purpose.

McNally:

How has your work progressed?

Joshi:

Just like we appended extra domains onto our curli fiber proteins in order to control adhesion to, for example, steel surfaces, we thought that we could append other domains that had known tissue-binding capabilities and use something like that to, for example, control the residence time of a particular engineered probiotic bacterium inside the gut.

Right now, most probiotics don't really stick around for very long inside the gut. There are some that stick around for longer than others, but it would be nice to start to think about engineering probiotics as essentially drug delivery vehicles and being able to control how long they live in the gut, where they live in the gut, and things like that. We have thought about using our BIND system in a similar context.

McNally: [33:47]

How are you going about that work? What have been your steps, your obstacles, your overcoming of obstacles, and so on, along that path?

Joshi:

It turns out that translating this technology from the lab into something that's suitable to put into a living organism, for example, a mouse or a human, for that matter, has many challenges. The first is reconstituting the system in a probiotic or commensal microbe. This is a microbe that has the capability to traverse your gastric system and then colonize your intestine for some extended period of time. The types of organisms that are able to do that are not exactly the same as the types of organisms that we typically use in the lab.

You can think of the organisms that are able to survive in the gut as animals that are able to exist in the wild, and the lab strains of E. coli that we typically use and that we've developed our system with are more like domesticated animals. You can imagine what would happen if you took a domesticated animal and just released it into the wild without anything to protect it. That's essentially what would happen if we took our system as it stands and fed it to a mouse or a human, for that matter. The cells would just immediately die, essentially, because they aren't able to cope with the stringent conditions that exist inside the body.

McNally: [35:13]

That's the problem. How do you overcome that?

Joshi:

It turns out that there are various probiotic strains of E. coli, which many people don't know about. They're just more familiar with pathogenic strains of E. coli, which you hear much more often about.

It turns out that there are certain strains of E. coli that actually have quite a long track record of safe use in humans as probiotics. You or I could go down to the drug store right now in the States and get a pill that has probiotics in it just over-the-counter. These strains of bacteria that you can get in that manner are typically things like Lactobacillus and similar types of strains that have known safety standards.

It turns out that there are E. coli strains that also have similar safety standards that you can use as a starting point for the types of engineering that we want to do.

We have reconstituted our system now that I described in the context of the lab E. coli. We've reconstituted that BIND system now in a probiotic strain of E. coli that's now more appropriate for deployment inside the gut. That comes with its own set of challenges, in terms of the genetic engineering. We're just at the stage now where we are taking this engineered microbe and actually determining its ability to colonize the gut, just like its unengineered precursor, in mouse studies.

McNally: [36:37]

We now turn to Anna Duraj-Thatte, one of the researchers leading the work on adapting biofilms for therapeutics in the human gut.

Anna, who grew up in Poland, explains that her current work at the Wyss brings together two long held passions.

Duraj-Thatte:

I think if I go back in the past, I always remember that I always was interested in biology and biological sciences. I always liked animals, I always loved going to zoos and I loved watching many programs about biological sciences even when I was a kid.

I loved engineering. When I was even in high school I used to love to ... I liked to combine these two things, that's why I decided to go to get a bachelor in engineering, biological sciences, and biotechnology in Poland.

There I had first exposure to engineering microbes and then I decided to go to Georgia Tech to get my PhD degree and then I was working on the protein engineering. I wanted to go more into the bioengineering field. That's why when I met Neel, I decided to join his lab at the Wyss.

McNally: [37:51]

But she wasn't immediately involved with biofilms.

Duraj-Thatte:

When I joined Neel's group I worked on a totally different project. It was on protein switches, but I was the only person who was working on that project at that time in his lab. Other members of his group were working on the biofilms on engineering.

You know, if you are between people who are so excited and vibrant, talking about engineering of biofilms, after some time you are really getting interested in that too. I was only looking at engineering biofilms in a slightly different perspective than other peers in my group because I'm a biochemist, so I was thinking mostly about biofilms as how we can use them, for example, for drug delivery.

At that time I started discussing with Neel, and we decided to change slightly part which everyone was moving in the lab for engineering of biofilms, and decided to use a BIND system for the drug delivery in the gut.

McNally: [38:55]

The public it seems to me has begun to pay more attention over the last few years to the gut and to the microbiome in the gut. What have we learned over the last few years that have caused us to pay more attention there?

Duraj-Thatte:

We learned that we know very little about the microbiome in the gut. In the last few years there have been done some of those amount of the research

done on the microbiome in the gut, but still we don't understand a lot. We know also that there is a high increase of many disorders related to the gut like inflammatory bowel diseases like Chron's or ulcerative colitis. Even diabetes 2 is linked to the microbiome.

That's why in the last three years there is a high interest to engineer a microbiome for not only therapeutics but also for learning exactly specific communities in the microbiome and how does it correlate to the specific disorders.

McNally: [39:55]

Neel and I had talked earlier about the bad rap that biofilms and amyloids get. Add E. coli. Some strains are potent pathogens, but some are probiotics – beneficial in the ecosystem of our gut. Neel and Anna's team are engineering novel probiotics by engineering a strain of E. coli – Nissle 1917, named for Alfred Nissle who isolated it during the first world war.

Duraj-Thatte:

This is probiotic known from last 100 years. It's the most well studied probiotic. Because our technology BIND system works in the E. coli, we decided to engineer E. coli Nissle probiotic for delivery specific therapeutics.

McNally:

And how would they do that? The extracellular matrix which the cells of the biofilm secrete is made up primarily of curli fibers. Discovered in the late 1980s, curli fibers are involved in the biofilm's formation and its adhesion to surfaces. The Wyss team fuses the curli fibers with therapeutics.

Duraj-Thatte:

Because we are able to engineer these biofilms for a specific thing. In the gut, for example, because we are able to engineer curli fibers which are part of the biofilm, we are able to add a few specific domains which are able to bind to the epithelium or the mucus layers in the gut and increase colonization of the bacteria. We are using the biological nature of the biofilms and also engineering part that we can engineer the matrix for a specific purpose in the gut.

McNally: [41:31]

What is it that your lab is doing that other labs haven't done?

Duraj-Thatte:

In our case, our bacteria is designed in a way that it adheres to the surface of the epithelium in the gut, and because of specific delivery sites it's able to deliver to a specific place ...therapeutics. In a specific place we have a higher concentration of therapeutics in the localized sites of inflammation in the gut.

McNally:

Even though they take it orally and it goes into the system down the throat just like any other, you are able to target where it lands and adheres?

Duraj-Thatte:

Our bacteria is engineered that way that on our biofilm curli fibers we have displayed human anti-inflammatory cytokines which we know that they bind very well to the mucus. We are able to deliver them to a specific closer inflammation site compared to other engineered bacteria.

McNally: [42:30]

Neel Joshi expands a bit on how they're working with our own biology.

Joshi:

These are small molecules that are proteins, really, that are secreted already by our own intestines in order to try to dampen inflammatory responses. We are taking those domains and now appending them to our biofilm to see if we can similarly further dampen inflammatory responses, especially in cases where inflammation has run amok, as is the case in many chronic inflammatory diseases.

McNally:

We return to the story of the biofilm development process -

McNally: [43:02]

How and why did you begin to work with the gut-on-a-chip out of another platform at Wyss?

Joshi:

That is one of the unique, I would say, advantages of working at a place like the Wyss, because people are developing things like gut-on-a-chip which can really facilitate this type of research.

It turns out that when we are trying to make this transition from the microbial strains that we engineer in the lab to figuring out how they interact with a living organism, there aren't really very many intermediary steps that are appropriate. You could imagine that's a big leap, to take this microbe that we've been engineering in the lab and understand what it does in the context of a mouse, for example.

In other cases, there are certain in vitro systems that you could think about using. For example, one thing that we have done and that many people do is to culture cells that are derived from your intestine. We can take human cancer-derived cells that are from the intestine, so these are colon cancer-derived cells, and grow them in a dish and look at the interaction of our engineered microbes with those cells.

That's a baby step in the right direction, but it turns out that the problem is that you can't really co-culture bacterial and mammalian cells very easily at all, because the bacteria are much more adept at using nutrients. They will

immediately overgrow in the course of about an hour and kill the underlying mammalian cells, which makes the characterization of this interaction very difficult.

What the gut-on-a-chip does is it recapitulates many of the physiologically relevant factors that are present in the gut. For example, instead of just having these static cells in a dish, the gut-on-a-chip incorporates an element of flow. Also, it mimics the process of peristalsis. This is the movement of the walls of your intestine that is critical for your intestines to function. It turns out that those two things in particular are quite important for preventing the overgrowth of bacteria. This gut-on-a-chip now is somewhat unique in its ability to facilitate these host-microbe interaction studies.

McNally: [45:12]

You've got the mammalian cells and the bacterial cells. When they're in the gut-on-a-chip, those physical actions allow the mammalian cells to survive longer against the bacteria?

Joshi:

You can think of it that way, or you can think of it as they just wash out bacteria that isn't really adhered, and so the bacteria don't overgrow as quickly as they would in the context of a static dish.

McNally:

The closer it gets to the actual conditions of an organism, that's what changes the way the two kinds of cells interact.

Joshi:

Exactly. So, for example, we have taken our engineered microbes and put them on the gut-on-a-chip or in the gut-on-a-chip. We can co-culture the mammalian cells with the bacterial cells for two days, and even sometimes beyond.

This is way beyond what you could do in a static dish before you would achieve overgrowth, essentially. For example, interactions that take a longer time to develop. The formation of biofilms themselves, for example, usually takes many hours to days, possibly. Those types of interactions can't very easily be studied in the static dishes, but the gut-on-a-chip really facilitates the study of those types of processes.

McNally: [46:22]

Anna Duraj Thatte offers an assessment of where their work stands now and what their next steps might be.

Duraj-Thatte:

We were able to *in vitro* show that our system works, that our engineered bacteria are able to treat inflammation. Right now we are moving to testing our system in mice to try to find out how our engineered bacteria is reliable in an *in vivo* system.

McNally:

If the current work you're doing is successful, what will you have learned?

Duraj-Thatte:

If my current work will be successful for me, it means that in the *in vivo* system we were able to treat inflammation inside the gut of mice. What we learn, first of all, is that we develop new methods of the delivery of therapeutics inside the gut, a localized delivery. All other systems which exist right now, as I mentioned, they deliver inside the lumen therapeutics. We are able to deliver to specific site of the inflammation or any site of the problem in the gut because, of course, the system doesn't need to be used only for treating inflammation but also for many other things.

Duraj-Thatte:

I think we would like to see some kind of testing in the humans for the next step. If our engineered probiotic is able to colonize the human gut and deliver therapeutics in specific sites of inflammation, that could be really great.

McNally: [47:53]

Where do you see your work evolving over the next five or ten years?

Duraj-Thatte:

I think I would like to stay in the field of engineering of the microbiome in the gut. I think it's such a dynamic and innovative area right now. A lot is going on around even in Boston if you look at other groups. I really think that I would like to work more on trying to engineer delivery of therapeutics inside the gut and engineer the microbiome in the gut.

McNally:

And she sees herself continuing that work at the Wyss.

Duraj-Thatte:

It's really a great environment for working because it's such a vibrant and dynamic environment. We have so ... It's not small, but in a relatively small place, we have such multidisciplinary people. If we have a problem with our project, we can discuss with folks from a totally different background than we are and try to find an answer from a different angle. This is an excellent environment for collaboration with different groups... not even in the same platform, but between the platforms.

McNally: [49:03]

Neel Joshi is likewise excited about the prospects for their work with biofilms.

Joshi:

We're particularly excited about some of the biomedical applications of this BIND technology. We really see it as a biomaterial that's resident inside the

gut that can be genetically programmed. This is something that could persist in the gut for weeks, months, perhaps even longer. The functions of that material that are living in your gut can be programmed genetically. They can potentially respond to their environment, so we can create different versions of the biofilm depending on whether that biofilm is experiencing certain types of disease or other environmental factors.

It can really be a very dynamic type of material. You can almost think of it as a prosthetic or a device that would live inside the gut and respond to its environment. That, I would say, is a grand vision for where this could go, in terms of the biomedical applications.

McNally: [49:55]

Could you talk just a bit about what it's like to work at the Wyss?

Joshi:

Working at the Wyss has been a unique opportunity for me. I've already described to you a little bit how just the people that I was around really had a dramatic influence on the type of research that we were pursuing. In fact, our entry or our interest in engineering biofilms came from essentially a mixture of our interest in protein engineering and the really amazing stuff that is happening in synthetic biology that we were privy to as part of the Wyss community.

In addition to that, the somewhat unique focus of the Wyss, in terms of producing technologies that are going to be useful in the real world in the context of commercialization or some other type of real-world deployment has shaped the way that we think about our own research.

Instead of creating things that are just really cool from a scientific or engineering point of view, we are constantly surrounded by people who are actually developing technologies that are making it out into the real world or designed to make it out into the real world. That also influences our own thinking about our own projects to think similarly or along the same lines.

McNally: [51:02]

Can you provide an example of how that influences your work?

Joshi:

This is something that I didn't talk to you about, but we have some previous work where we've made certain synthetic peptides that have very interesting self-assembly properties, from a fundamental science point of view. We eventually moved away from that research because, even though those particular peptides that we were studying formed these very interesting nanotubes that have interesting material properties, we didn't see those as having that much of a future, in terms of their real-world applicability.

By contrast, some of the biofilm stuff that we are doing we see as being much closer to being a technology that could be used in the real world,

either in the context of the biomedical applications that I just mentioned or in the context of industry.

The enzyme catalysis that we discussed is something that already happens in industry, in terms of the use of enzymes and figuring out new ways to integrate those enzymes with manufacturing processes is, I think, very close to a real-world application. We're already collaborating with certain industrial partners to implement these systems in contexts that would be relevant from a manufacturing perspective.

[52:12]

Another application that we think could be quite interesting for the BIND technology is for the creation of scalable materials. You can think of plastics that you encounter every day in your life, which are essentially derived from petroleum. These are resources that we are extracting from the earth, reconstituting into various plastics, and then they make the chairs that we sit on and the consumer goods that we use all the time. It's a non-renewable source.

In a grand vision, you could imagine making those things through a process of fermentation, essentially, through a system similar to BIND, where you could program what types of material properties you wanted genetically, and then have microbes produce materials of interest, and then harvest those materials from these microbial cultures, and reconstitute them into actual products.

McNally: [52:59]

Thank you very much, Neel. It's been a pleasure.

Joshi:

Thanks, Terrence.

McNally:

You've been listening to DISRUPTIVE: Putting Biofilms to Work.

I'm Terrence McNally, and my guests have been Neel Joshi and Anna Duraj-Thatte. You can learn more about their innovative work with biofilms as well as an exciting range of other projects at the Wyss website. That's wyss.harvard.edu, W-Y-S-S-dot-Harvard-dot-E-D-U, where you'll find articles, videos, animations, and additional podcasts.

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My thanks to Seth Kroll and Mary Tolikas of the Wyss Institute, and to JC Swiatek in Production, and to you, our listeners. I look forward to being with you again soon.