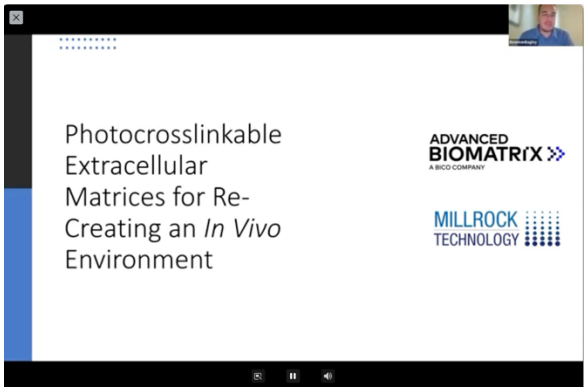
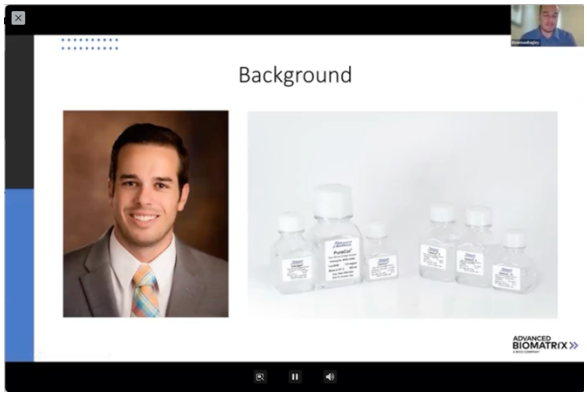
Photocrosslinkable Extracellular Matrices for Re-Creating an In Vivo Environment (webinar transcription)
Bowman Bagley, CEO – Advanced BioMatrix



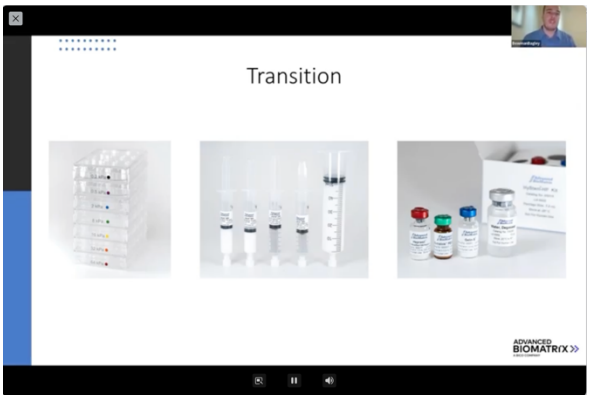
All right, everybody. Thank you very much for joining us this morning for this webinar. I am very excited and honored to be presenting in conjunction with Millrock Technology. My name is Bowman Bagley, representing Advanced BioMatrix. The title of this webinar is very long, and potentially boring sounding, “Photocrosslinkable Extracellular Matrices for Recreating an In Vivo Environment.” But, as I was trying to think about what I really wanted to talk about, this is the title, and essentially the statement, that kept coming back to my mind, as really this is our goal with these materials. And so, I'm excited to talk about them today. If you have any questions, put them in the chat and then, at the end of the presentation, I'll open up the chat and I'll go through those one at a time and do my best to address those and answer those. And at the very end of the presentation, I have all my contact information, so you can reach out with additional questions or if you want to request additional information.



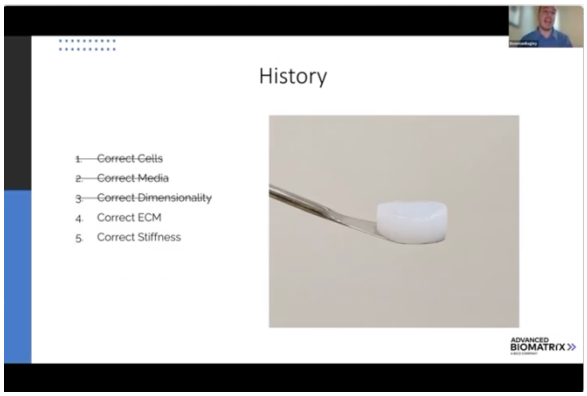
So that's me. Bowman Bagley with Advanced BioMatrix. My background is, I studied neuroscience and business, and really with the intention of wanting to help understand the research, the science, help create new materials. And then, also, focus on, how we actually translate those from the lab into the hands of additional researchers, to really progress science even faster. I think a lot of materials get created and then get stuck. And so, I think, for me, it's a lot of fun getting it out of the lab and into more hands, so it can be a more functional and used material. I've been with Advanced BioMatrix for eight years now, and I've been having a lot of fun. I don't want to do a huge plug into the company, but I think telling a little bit about where the company started and where it's going, ties a lot into why we bring these types of materials and why we're seeing a huge spike in customers using these types of modified materials, and maybe the reasoning behind that.

So the very first product that we started with was a pure Type 1 collagen, called PureCol. It's 3 mg/ml, enzyme extracted atelocollagen. And over the years, we started getting more requests of, "Can you have

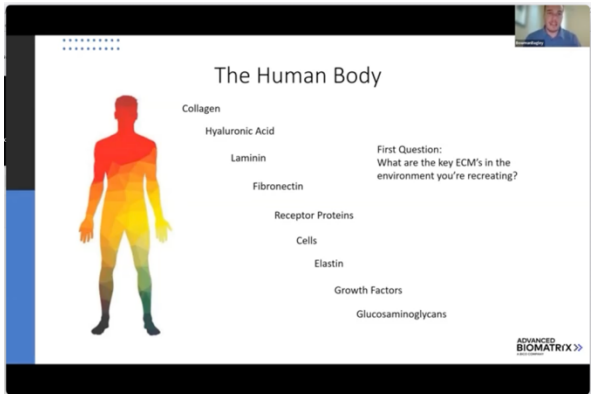
a higher concentration? Can we have an acid extracted telocollagen, instead of an enzyme extracted atelocollagen?" And so, we've built out our collagen portfolio, different species, powders and solutions, really addressing that market. But then, over the years, we also started getting requests of," Hey, our plastic dishes are too rigid. Can you have some soft substrates?" And we created the CytoSoft line. We got requests for "Hey, collagen typically is really, really soft. Hydrogels, "Can you create something that can be bioprinted with an extrusion printer?" So we created these bio inks. And we got requests for different types of ECMs, fibronectin, vitronectin, and then more recently, a big focus on modified proteins and sugars.



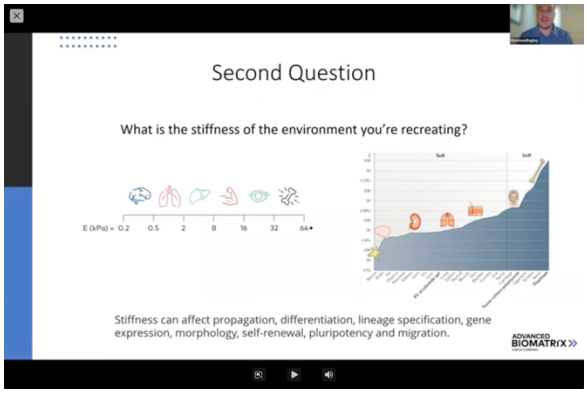
And really with each new product, our goal has been how can we help the researcher get one step closer to really recreating an in vivo-like environment in your research? Now, I hate history slides because everybody on this webinar, I'm sure, knows a lot more about the history of science than I do. So, I really simplified it to just kind of show back in the day it was focusing on, "Okay, we got to just study these cells." And then we realized, "Okay, media can really change how these cells differentiate or grow." And then we start looking at, "Okay, maybe two dimensionality is not as relevant as three dimensionality." And then from there, this is what I want to talk about. We're now really strongly focusing on, "Well, let's make sure that we have both the right protein or sugar, the right environment, and then also the right stiffness of that surrounding material, that hydrogel, that tissue needs to have the same stiffness and really represent the native environment."



So now we're in this phase, focusing on four and five. So, the first question, and I'll get back to that in a second, but... We're focusing on the extracellular matrix. These proteins and sugars, they're critical for cellular adhesion, communication, migration, differentiation. Countless other functions. And the most abundant protein in the body, as you all know, is collagen. The most abundant glycosaminoglycan is hyaluronic acid. And so, I put those at the top, because they're the two most prominent and most widely used laminins right there, fibronectin, and there's all sorts of other receptor proteins and cells and growth factors. Our body is composed of so many beautiful proteins and sugars, and everything seamlessly working together. And so, when we're talking to customers and researchers, the very first question we ask them when we're trying to help them advance their research is, "What are the key ECMs in the environment, or the tissue, that you're trying to create?"



If they want to make a skin model or they're trying to bioprint a piece of heart tissue, or a liver, or lung models, what are the ECMs of that environment? So let's first solve that problem. Answer that question.

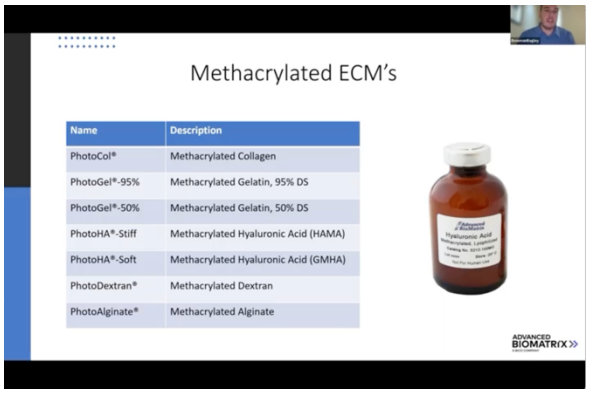


And then once we understand the ECMs, we asked the second question, what is the stiffness of that environment that you're recreating? Years ago, I had made this graphic on the left with my wife. She did some graphic design work for us, and we looked at some publications, and pieced this together. But I ended up finding a publication, the picture on the right, which is a little, it's more scientific. But it really shows this broad range of tissues and the stiffnesses. If you're studying the brain, you're going to be in a very, very soft environment, whereas bone is going to be significantly off the chart. Way stronger.

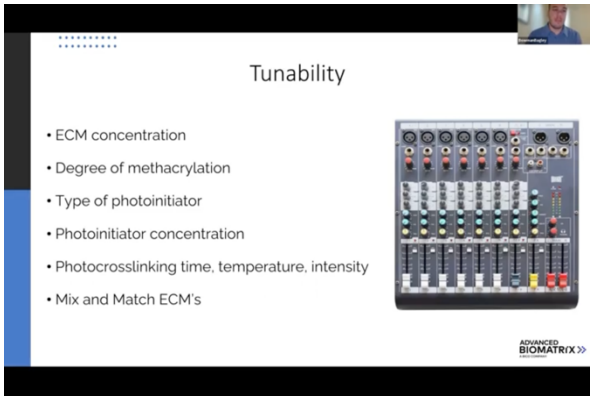
And then everything in between. So, a lot of researchers will have the perfect cells, the right media, it'll be a 3D environment. They'll have the right proteins, but then they're growing neurons in an environment that's as strong as cornea or bone or tendon. And so, stiffness can affect almost as much as the protein, propagation, differentiation. I guess the list is right there at the bottom. But gene expression, morphology, there's been hundreds or thousands of publications now, showing how subtle changes in the stiffness of that environment can drastically change the viability, or health, or just even morphology, of your cells. And so, we're focusing on, how do we tailor both the extracellular matrix environment and then the actual kind of macro stiffness of that environment?

Initially the first answer that we had was, "Okay, our collagen materials." So, this is a graph where we're looking at the 3D gelatian of our collagen hydrogels at 37 Celsius, over time. And then you're looking at the strength of the gels. You'll see the telocollagen gels are the ones that gel very quickly and form stronger gels. The atelocollagens gel much slower and much softer. And this was great for a little bit, but you can see that the upper range of these unmodified collagens is really about 10,000 pascals. There're methods to super concentrate the collagen, and it's much more expensive and difficult. And maintaining sterility and all that. So, this was good a little bit and there's some tunability, but it didn't really address the full range that was needed to recreate heart, muscle, tendon, cartilage, cornea, bone.

So, that led us into the creation of these methacrylated extracellular matrices. And so, the ones that we started on, I think, are the ones that really help recreate the human body. We started with collagen, gelatin and hyaluronic acid. And collagen being the most abundant protein, hyaluronic acid being the most abundant glycosaminoglycan. It kind of covers most of the body. So we have PhotoCol, PhotoGel, PhotoHA, PhotDextran, and PhotoAlginate. You'll also see that with the gelatin, there's two different degrees of methacrylation. 95% high and 50%, say, medium. With the methacrylated hyaluronic acid, we run two different reactions which results in a stiffer and a softer hydrogel. And really the goal is to allow tunability, right? Methacrylation allows these materials to be photocrosslinked. And so you can now photocrosslink these materials and have a huge range of stiffnesses, soft to really stiff. And they're being widely used for extrusion printing, inkjet, DLP printing molds, basic 3D hydrogels, tissue models, all sorts of things. So, it's a lot of fun.



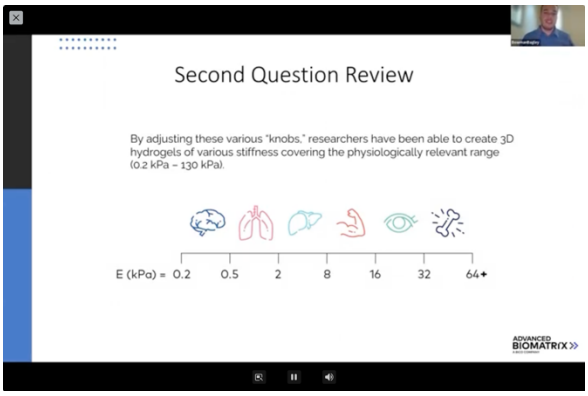
Now let's talk about the tunability and how these materials are so tuneable. The first one I'll talk about is the concentration of the protein or sugar. So, I'm not supposed to plug Millrock, but we just bought one of their lyophilizers because we lyophilize all these products, all the methacrylated ones. And we just bought their massive seven tray. They'll probably say it's small because they had even bigger ones. But we bought their seven tray lyophilizer. We ran a LinkedIn poll to get some feedback on what we should name it, because we have a couple other lyophilizers, and we need to keep them separate. So that one we've called 'The Rock' because it's big and strong, and it's a little play off of Millrock. I'll post a picture soon of the big Rock's face that we have on our lyophilizer. But we lyophilize all these materials to give you this tunability.



So, the gelatin, for example, the PhotoGel, you can concentrate that from 1% to, I probably wouldn't go much higher than 20%, but you can go higher. 1% to 30%, we'll say. You get this huge range of concentrations. We can look at the degree of methacrylation. The GelMA has the two different high and medium methacrylation. How does that affect the stiffness of the environment? Bunch of different photoinitiators. I think the three most popular right now are LAP, Irgacure and Ruthenium doing different photoinitiators, different concentrations of those photoinitiators. On our website, we typically recommend the lowest range of photoinitiator to be on the safe side. But we've seen with LAP for example, we've seen researchers using 10 or 50 times higher concentrations of LAP. And then it also depends on if you're using cells or not. You can get away with higher concentrations and avoid photo toxicity, cell toxicity.

You can tune the photocrosslinking time, how long you crosslink it for. What's the temperature of the protein when you crosslink it. The intensity of those lights. You can mix and match different ECMs. We start thinking about, "I want to recreate a native environment. I should probably have some collagen and some hyaluronic acid. I want to mix these two components together." And the list of the

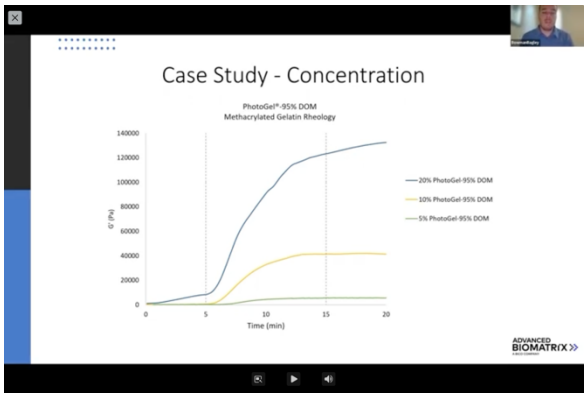
methacrylated ECMs we have, that's going to continue to grow this next year. We're putting a strong focus on it because the materials are so versatile, and there's so many fun knobs to turn, so we're having a lot of fun with it.



So really by adjusting these various knobs, researchers can create a 3D hydrogel with the essentially correct ECMs in the physiologically relevant range of stiffness.

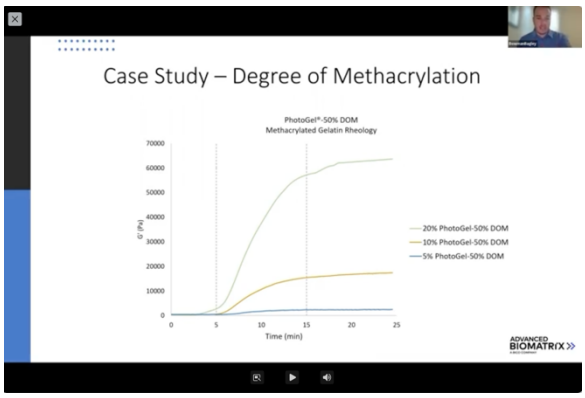
We're going to show some very basic case studies, all of the data, how we ran these tests, the parameters, the machines we used, all of that is found on our website on the 'White Papers' that are tied to these studies.

And you can also reach out to me, I'll just send them to you directly if you want. And keep in mind with radiology, my radiology results are going to be different than your radiology results, which is kind of funny to say in science. But radiology is so dependent on testing methods. Methodology. Are you using a machine that uses vibrations or lasers? Are you pulling the sample? Are you straining, twisting, compressing, stretching? All these different methodologies, as well as just user handle ability, temperature, humidity of your room. All these things play an effect. So, I want you to focus on, generally the values, but then also relative values between the different concentrations within this one. Or these values, compared to the next study I show. This particular test was performed at 25 Celsius. So, let's dive into this. So, we took the methacrylated gelatin, the 95% methacrylated, we allowed it to equilibrate for five minutes, and then we crosslinked it for 10 minutes. And then we let it sit for five more minutes. And that's the data that we're looking at.

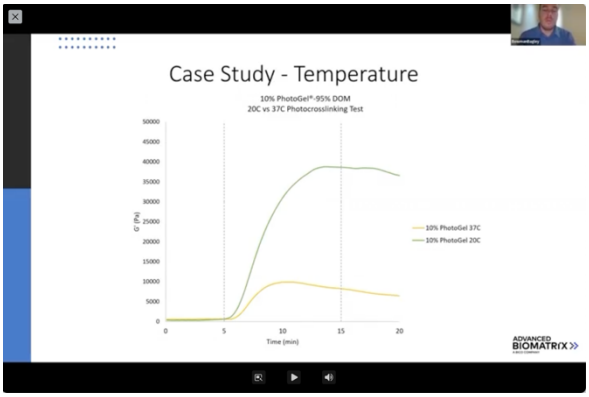


This particular one is used with LAP and 405nm blue light. And again, all the data is on the 'White Paper'. So, you see that just changing the concentration from 5% to 10%, you're increasing the stiffness from about 5,000 to close to 40,000 pascals. And then again, from 10% to 20%, you're going from 40,000 to, we'll say, 120,000 to 140,000. So, you get this one protein, this methacrylated gelatin, can almost by itself be tuned to cover essentially almost the entire physiologically relevant range of stiffness that you might need for a product. Which is really cool.

The second one. Now we said, "Okay, well, that's the 95%. Let's take a look at the 50%." It's half as methacrylated. And in a beautiful science, the stiffness values are essentially half as well. So, the 20% gelatin, crosslinked the exact same way, but with the 50% methacrylation, had a stiffness of essentially half of the 95% methacrylation at 70,000. And about 15,000 to 20,000. And then two-ish thousand at the bottom. So just by adjusting the methacrylation, you can have a huge play on tunability. And now the question I know someone's going to ask is, "Well, if you're changing the concentration, you are also changing the porosity. How does the porosity affect the cells?" That's a great question. And we haven't measured porosity yet, or really dove into that. We're just strictly looking at stiffness at this point. But we recognize that the concentration will change the porosity and that will induce some additional questions, of course.

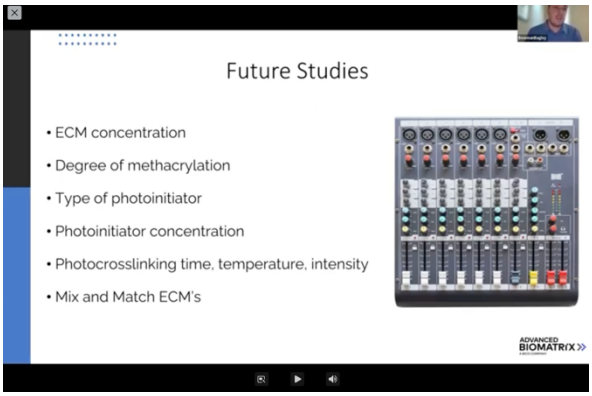


The third thing we wanted to look at, is temperature. So, the reason we did this study is that we had a customer call and say, I followed your protocol, and I made a gel, I crosslinked it, and it was way softer of a gel than I thought it would be." And we said, "Okay, well, walk us through what you did." And they warmed it up 37, they kept it at 37 and then they crosslinked it. And we said, "We've never looked at crosslinking, the resulting stiffness at 37, versus 25 or 20, room temperature." So, we just quickly went back to the lab, ran some tests, and got some really fun data. Where simply by cross-linking the gelatin at the higher temperature, they got a significantly softer gel. Whereas, at the lower temperature, you get a much stronger gel.

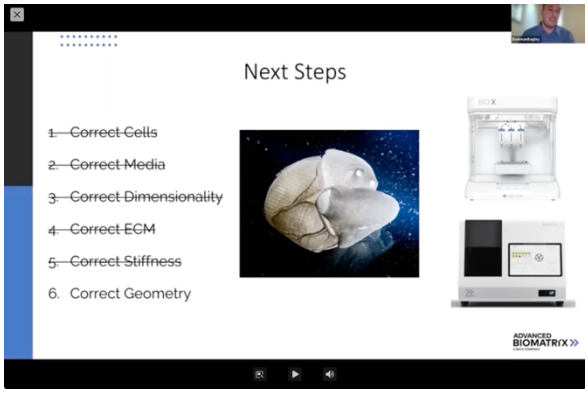


And with the gelatin connects of gelatin, it makes sense. And so, the results made sense. We just had never thought of it before. And so, we're extremely grateful for researchers just asking us these questions because it gives us an excuse to go play in the lab, run some studies, and get some of this data. And every time we do any of these studies, we post it on our website. We'll share it on LinkedIn. We want to share as much data and information as we can, because I see a tremendous value in sharing data, so that... All the mistakes that I've made in the past, you don't have to make those same mistakes. And everything that we've learned, you can learn as well. And hopefully start at a level higher than where we started. I think it's important for science to continue to build off each other. So, if you have particular radiology requests or questions like that, we'd love to work and try to just answer some more of these questions together. So those were kind of three quick case studies.

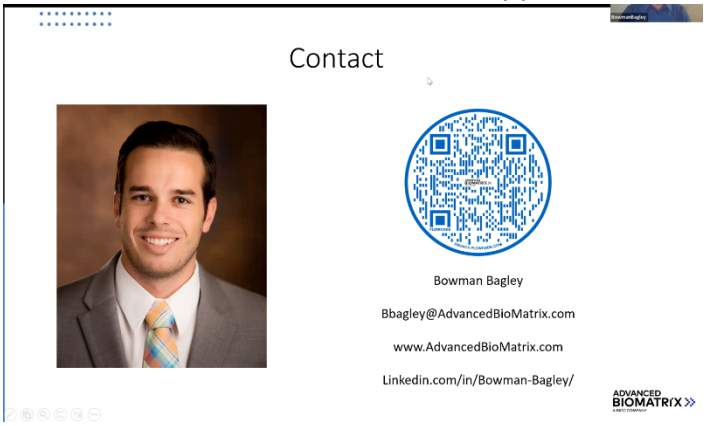
Our future studies. Again, we're diving further into ECM concentration for more of our products. We're looking at a broader range of methacrylation. Really just trying to have more tunable materials for researchers. We're going to do some more studies comparing photoinitiator. Which one is the most efficient? LAP, Irgacure, Ruthenium, VAOA6. The photoinitiated concentration. What are the limits of that? How high can you go? Again, more crosslinking time. How long can you zap it before you either fry your cells, or it just runs out of crosslinking sites? And then we really want to start playing around with more of mixing and matching these ECMs. Again, being able to combine... If you want to recreate a native environment, you start thinking about the collagen and the hyaluronic acid really interacting together.



So, 1-2-3-4-5, we've addressed. And we're really actively addressing 4 and 5 right now. The ECM and the stiffness. The next step really, in my mind, is the geometry, right? We're looking at creating aligned fibers for cells to kind of align along these aligned materials. Or cellular alignment and cellular deposition, or multicellular, highly organized geometries of the cells. And when you start combining cells and media, and the three dimensionality, and the right proteins, and the right stiffness in the right shape, we're just that much closer to having the most physiologically relevant human tissue models for drug delivery, drug studies or toxicology studies. And we're just getting that much closer in advancing our science, just that much more. And a lot of this geometry is going to be done with 3D printing. I'm not going to pretend like I'm an expert at the actual equipment, but there's two photon and lasers and DLP, extrusion, inkjet, holographic.



There's even a new printer that uses sound vibrations. I'm not going to dive into the printers, but there's so many options out there. And these methacrylated-modified materials are really a key component towards really recreating an in vivo environment in our research. So that's me again, the QR code takes you to the website so you can see some of our materials. You can also go to the 'Contact us' tab on the website. There's my personal email, you can email me anything you want. love to collaborate. Ask me questions. Work together if you want some studies done. Or another great way is to reach out to me on LinkedIn. I'm on there every day and happy to connect through there.



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