Questions and Answers

Okay so let's take a few minutes if you some questions. I can't see anything up here so maybe we can turn house lights up. Can we turn the house lights up? And I ask you to go to the microphones. There are microphones the center if you have questions. And all the speakers if you kind of congregate in the front, we can get you a microphone. It's been a long day. Are there any questions? Okay, go to the microphones. Audience: Good evening. Okay, so this is a question for Dr. Pepperberg about his amount of technology that basically will replace the…can you hear me now? Speaker: That's better. Audience: Sorry. Now it's a question for Dr. Pepperberg, um he described a system where he was going to replace photoreceptors with a transducing molecular complex placed on a azobenzene. I just wondered whether, it seems very interesting, and I hope it works, but have you given any thought to the dynamic range that you might have with a system like that because you've got no provision there for light adaptation which you would have in the photoreceptor. Sir, I know this is a long shot but can you think of ways that you might be addressing that in the future? Speaker: Yeah, your questions touches on several very important issues and at this point we are just at the initial stages of trying to work on some of these. If I can touch briefly on two elements of your question. First for the ultimate requirement of transient activation of the receptor to which the structure is covered, is going to require considerable engineering of that photosensor molecule. You were also asking about light adaptation, keep in mind that the target neurons of the retina that we are seeking to modify are post photoreceptor neurons. And through conversions and through what we know are several stages of adaptation in the retina; there will certainly be the need of how best to mimic the effect of amplification that in the normal system has obtained at the level that we're trying of the several elements of your question, but these are certainly important considerations. Audience: Now I have a question, this is I think a very basic question. When I saw the lovely images of the liquid being converted to a gel, I think the whole room immediately thought of injecting something into the vitreous body of the eye and watching it polymerase. Is there a thermal reaction associated with polim, cuz it's not polymerization, where it's not, your not generating heat or cold or something. Speaker: Right it's not, not the thermal process. It is triggered by the formation of hydrogen bonds between the molecules. Audience: So we don't have to worry about heating the tissue when this happens? Speaker: Right. Audience: Is there a way… Speaker: And there is lot's of water around too, I mean it is mostly water so if there is any heat released it would be dissipated very easily. Audience: Right. Is there a way to engineer in sort of oxygen capacity? Because for instance I use three-dimensional MATRA gel for growing you know my capillary endothelial sups. But I am limited by their depth and the length of time because they simply run out of oxygen. Speaker: Right. So here again, well, one of the advantages of the nanostructure nature is that you can build the gel with only one percent by weight content of fiber. And so most of it is water and it is much easier than to have access to both oxygen and nutrients. Audience: Thank you. Audience: Hi. This is a question for Dr. Grinstaff. You said you've used your polymer for a sealing corneal autografts so I was wondering if you've done this with allografts, and if so if you've seen any problems with corneal survival of those grafts. Speaker: Sure. Thank you for the question. Ah what we've done today is just simple enucleated eye experiments to look at how well we could actually seal and to think about the surgical procedure itself. And so right now with the materials that we have, uh we could do something like, um eight sutures instead of sixteen sutures and get a good sealing and what we're trying to work on is both the application methods as well as asking questions how much more does a polymer need to do to get to a stage where we could possibly do a suture less, uh PKP. But that will come with time. Speaker: My question is straight out to you again; we know enucleated human eyes are very agreeable with respect to re adherance of the tissues in terms of suturing or whatever. But in reality, in a clinical setting, you have blood, you have the iris, you have vitreous, you have lenses, and many times you are pushing things back in while still trying to close the wound. This doesn't work well with any kind of adhesive at all. The second problem is that when you adhere tissues, ah with an adhesive of some kind, ah you're going to adhere the anterior tissues. But inevitably even with the suture, unless you put a predestinate suture you're going to have a gap of the endothelium, through with aqueous fluid will flow into the cornea and really interfere substantially with the healing process. So in particular when you have a corneal transplant, that would be the case. So these are some very practical matters. Speaker: Sure for something like a corneal transplant, it's gonna be very important for us to understand what the design requirements will be for that material in that type of wound setting, which is very different than, at least in my mind, a corneal laceration. And of course starting with a corneal laceration was a good place. Now the question is, what do we need to do for the material for an application a little more challenging, that being a PKP. That's exactly right. Audience: This is for Dr. Murphy, ah I was amazed by the, what's the magic with the four hundred to eight hundred nanometers? That, that, that, that… Speaker: You want me? Audience: Yes, yeah, that results in the fast formation of the cellular phenotype. The pitch when it changes from four hundred to eight hundred nanometers. You said, maybe you said that I missed it that the phenotype of the cells is completely changed. Speaker: Yes, in terms of when you look at the transition. My point was that at around featured dimensions of six hundred to eight hundred nanometers, which is twelve hundred to sixteen hundred nanometer pitch, right in that range as you drop below that size scale, the cells start behaving differently than above that size scale. Audience: Uh huh. Speaker: And that was the point. The only behavior I showed where that was not true was in the orientation. The cells that we looked at, the human corneal epithelial cells is what, and we've looked at fibroblasts and vascular endothelia cells. They all seem to align down to seventy nanometers. All the way up through, two, you know two micron features. So they align okay but what all the other behaviors that we've looked at, they had that transition zone where they behaved differently above that size scale than below that size scale and below that size scale is where we start entering the biomomedics scale, based

upon our description, or quantitative descriptions of the native base of the membrane and I should, I didn't have time to talk about it but there's a couple of things. One is that the base of the membranes are similar across species. That base of the membranes are similar across locations. MATRA gel has the same dimensions as the human corneal base of the membrane. The only difference we found, and it's for recent studies, is that vascular cells, vascular base of the membranes are different. They have the same sort of felt like topography, but they are smaller scale and more tightly packed. Very different than any other base of the membranes we've looked at. Audience: Thanks. Audience: That's a question to almost all of the panelists, I mean this isn't an equal question but, is there a size limitation in terms of the monomer for cell assembly units?
This is number one? And number two, can you predictably polymerize multiple monomers to control the suprastructure, like to frack with the mases of close fibers? Speaker: Well it would probably not be right to say that there is no limit in terms of the size of the monomer. The size of the monomer is something that can be tolerated by the system that it is very large, very small, provided that you have the right geometry in the structure. Provided you have the right , you've designed for all of the intermolecular interactions you need. Ah, sometimes if you think about monomers that are too large, it gets more challenging, because you have to fight the entropy much more and it is difficult to make it work. I think you have the best chance with smaller size monomers, but of course these are not monomers that, of the size that is typical in making polymers, these are much larger to begin with. So if you though of monomers as the size of polymers that is molecular weights in the range of fifty thousand to a hundred thousand that would be a challenge, but I think monomers in the range of five hundred to a thousand daltons is possible. Now your other question is can you mix monomers and the answer is yes. Audience: (Inaudible) Speaker: Yes. More than one monomer, one different types of monomers? Yes. You can definitely do that, and again, what you have to do is to essentially engineer the monomers to have a way to interact with each other, ah regardless of the different information that they bring in. So the typical approach would be, you use part of the monomer, to make sure that all of them, let's say ten different types of monomers, want to self assemble at the supermolecular structure. Ah, and then you can integrate those ten different functions in the same structure. Audience: Does the biological environment impact the cell self-assembly. Speaker: Yes. Very much so in fact in RK in our platform, it is the biological environment that triggers the self-assembly. And it does so because you have all these electrolytes in the medium and those electrolytes screen the charge of our molecules. Audience: Exactly, so does it, does it vary from one cell type to the other cell type? Speaker: Well the assembly was taken place in the extracellular space. Of course it could also take place in the intracellular space, in fact we are real interested in that topic. Ah but, uh, so it could occur in either place and if it is intracellular, definitely it might be cell dependent, ah in the extracellular space, there is also the same issue, right, depending on what proteins are around and that sort of thing. Speaker: Dr. Maiser. Audience: Dr. Fisher, those are spectacular. I was going to ask Dr. Murphy though; you have now dimensions that you are working with in

terms of the pitch. What corresponds to anything intracellularly some of the supermolecular assemblies that we tend to look for in terms of signal transduction.   And you threw up some of the diagrams. Does anything correspond to this? A minimal size for example of a focal adhesion that would have fat and junk and things like that in it? Speaker: Well, we've worked down to seventy nanometers, due to the fabrication constraints that we have faced and especially in terms of using the soft lithographic procedures that I described. We have just finished character. I have spent a year of characterizing. What can we, what are the limits at our hands and what we can do? When you get below seventy nanometers in terms of soft lithography and even that dimension you end up with a lot of collapse. So we're having to go to other methods now and I should point out that the transition zone that we've identified, that's the upper limit. We don't know what the lower limit is but there is going to be a point where the cells don't care anymore. Let see, you can think of it as cellular acuity, cus there is going to be some point where the cells can not distinguish two points in space. The sun setting is not going to be there. The other interesting thing is that there is a lot of work that is going on in terms of uh, compliance, modulus elasticity, Fisher's work out of Pennsylvania is looking at that, and it's gonna be intriguing as we start dissecting molecular pathways, whether or not we're sharing the same pathway in terms of biomechanical cuing, or is it truly that topography is unique among biomechanical inputs, we don't know. So does that answer your question? We haven't gotten down small enough to fully answer the question. Speaker: Okay, I think we will uh, close it there. Thank you everybody for coming and thank you to the speakers.