

Nanotechnology Symposium

1-May-06 - 8:30 am - 10:15 am

Man: The next speaker is Professor Michael Young, who's going to speak about the role of nanotechnology in retinal repair.

Young: Thank you. I'd also like to thank Marco and Bob for inviting me here to talk to you today about some of the work going on in my laboratory. I'm going to talk today mostly about drug delivery and tissue engineering. And so most of the work that I'm going to tell you about today involves polymer constructs that start out at the nanoscale. But when they're polymerized they end up larger, most of them around the 10 micron scale as the polymer prior to biodegradation.

And so I'll summarize a few projects that have gone on in my lab in the past couple of years. First talking about drug delivery and the use of biodegradable microspheres and nanospheres as a means of drug delivery into the central nervous system and in this case into an animal model of glaucoma. And then I'll talk to you about injectable constructs that are polymerized in situ in most cases by exposure to light. So these are liquids that are polymerized into a gel after injection and then used for drug delivery. I'm going to tell you a little bit about the work we've done in the past on progenitor cell transplantation with out the use of constructs for bioengineering. Let's highlight some of the limitations of that approach and talk to you about some of the new things we're doing using biodegradable polymers and nonbiodegradable thin constructs using MEMS and electrospun techniques. And just sort of summarize where I think this work is going in the future.

Okay, so, biodegradable microspheres things used for a long time now as a source of drug delivery in various compartments of the body. We've been collaborating with Bob Langer's lab at MIT and Matt Ward and Ali Khoobehi in my lab working with Erin Lavik before she left to start her own lab at MIT. Started working with these constructs PLGA (polylactic-co-glycolic) acid so these are polymers of these monomer structures that can be used to load drugs into them and be released as this molecule degrades over time. You can achieve release of approaching zero order kinetics over a period of about eight to 12 weeks is the optimal time period, at least for the constructs we've been using. And so these are very small microspheres (we've also used some nanospheres as well) but to get that kind of long term release with this construct we need spheres that are on the order of eight to 10 microns in diameter.

The study that I'll tell you about briefly uses GDNF (glial derived neurotrophic factor) using a spontaneous single emulsion technique done by Erin when she was at MIT and we injected these – uh oh this is a step-wise slide, I'll go back. So these were into the vitreous of DBA2J mice. I'm sure most of you know about these mice. These are an animal model of pigment dispersion glaucoma. They get a rapid degeneration that begins at about eight to 10 months of age involving retinal ganglion cell loss. And so just a couple of – just one of two experiments we did – this was recently published in the *Journal of Pharmaceutical Sciences*, injecting these mice at either three or four time points with these microspheres containing GDNF gives a profound rescue of retinal ganglion cells. Almost three-fold of survival of retinal ganglion cells and the reason for that, there are a couple reasons for it. One is that this drug is indeed released continuously over time and in this case continuously over a period of about eight months during the life of these animals. The other attribute of slow release drug delivery is that

this drug is not available in a bolus amount like one would see in a typical injection. It's continuously available at a very low level, and I think that is critical. You don't see any up regulation of the receptor--down regulation-- of the receptors for these molecules that are the neurotrophic molecules that are delivered. And you don't see any of the sort of systemic side effects that one would see with a high dose delivered over just a short period of time.

Okay, onto another study. Again collaboration with Bob Langer's lab, working with Jason _____ in his lab, and Matt Ward in my lab, trying to do something a little bit different now again using injectable polymers. So these are photopolymerizable hydrogel, so they're water based, they're polymerized in most cases by ultraviolet light. You can imagine that that can be a problem when you're using these in the eye. It's a very low dose of ultraviolet light. This is kind of thing that's been used in dentistry for instance to polymerize structures using UV light. We've been working on some other photo initiators that are more in the visible realm and less likely to be toxic to the eye. But the liquid that you inject containing drugs that are bound to this--now I'll tell you about a bunch of different drugs that we've tried in the next slide. But they are polymerized *in situ* via the exposure to light and break down over time again by the same method that PLGA breaks down. This is not an enzymatic break down of the polymer, this is occurring by acid hydrolysis so it happens as soon as polymers are injected. And I'll just tell you about some studies we've done recently published as well using CNTF and BDNF in an assay here for neurite outgrowth from these structures. Just showing that these polymers do, indeed, release active drug that can have an effect on the growth of retinal ganglion cells.

Okay, now I'm going to move into some of the work we're doing in cell transplantation of tissue engineering and just a few slides from some of the work we've done previously, injecting single cell suspensions either into the vitreous or into the subretinal space. And what you can see quite clearly here is what one sees as widespread migration integration into the retina. In most cases, the target is really the photoreceptor layer in these transplantation paradigms and that doesn't really occur at a high level. Photoreceptor integration doesn't occur at a high level when you just inject these cells as a single cell suspension either into the vitreous or subretinal space. Another study we did now looking at early development model with Don _____'s group. This is a Brazilian opossum and again I think you can see widespread migration throughout the retina. Really nice integration and differentiation of these cells but not at targeting of these cells to a specific layer and that's one of benefits of tissue engineering is that you can generate a construct and target it to a specific region with some hope that those cells are going to stay anchored into that location.

And this is a slide from _____ who was in my lab before showing--really what we are trying to achieve and what we achieve very rarely with this approach which is this is the outer nuclear layer here, here is a transplant of GFP positive progenitor cells, integration here right at the outer layer without migration into the rest of the retina. So that's really the target for photoreceptor replacement strategies.

I'll just take you through a few studies that we've published again with mostly Bob Langer's group as well, this is Erin Lavik who is now at Yale, with our initial work using the same polymer monomer PLGA (polylactic-co-glycolic) acid but rather than form microspheres, we're forming sheets of these cells with a quite different technique. Porous sheets of polymer that can then used to seed progenitor cells onto. And this is an example of an EM – a scanning EM shot – and on the top here you can see the polymer with progenitor cells seated on it and on the bottom is a retinal – is a slice of

retina. And so this is actually a transplant, we can do these in culture as well in an explant preparation. And this is a transplant just showing the integration that can occur between the graft and host without widespread migration of these cells into the host.

And another aspect of tissue engineering that can have a profound impact on graft outcome is the survival of the grafted cells. So this is largely the work of Minora Tomita in my lab showing that when you graft progenitor cells on a biodegradable construct as opposed to making a single bolus injection you get a massive increase in the number of cells that survive. So you see a tremendous _____ death in neurotransplants when they are performed as a single cell suspension for a number of reasons and we highlighted a few of them in this paper: the presence of growth factor anchor to the polymer; the lack of cell substrate contact at the time of transplantation is another principle reason why you see about 90 percent of the cells that are transplanted die within the first few days. And that's true of just about any neurotransplant of progenitor cells or other cell types. And that's improved greatly with the presence of the polymer construct.

Okay, so these grafts that we're using here and here are shown here in the mouse eye. They're really thick, okay. I think you can see that. Here they are 200-300 microns thick using this technique. And I think you can imagine that that's really a non-starter in terms of transplant to the subretinal space. So we started moving quickly onto a new technique using some different technology to get very thin polymers. This is using--this is a collaboration with _____ and Sarah _____ who are now at UCSF, used to be in BU, and Conan Young--no relation--and Yinquan Zhang in my lab. Using, in this case nondegradable PMMA (polymethylmethacrylate) scaffold and the MEMS (MicroElectroMechanical Systems) similar to what is used to create microchips to devise very thin polymers on the order of six microns thick. And here you see a few images of that, let's see if you look here upi can see here is the scaffold. It's almost invisible, even in histology at six microns with a layer of cells on them. And I'll just point you to this image here, if you look back these are porous structures with holes in them and here is an example of a neurofilament positive cell sending its process through this pore in the scaffold.

And just lastly, another technique that we are interested in is electrospun fibers that are biodegradable as well PLGA fibers. This is a technique being developed by Gary Winick (?) at Case Western and Chi Wui (?) in my lab is doing some of this work as well using these thin biodegradable porous and very sticky constructs. So that important that these polymers actually maintain attachments to progenitor cells that we're grafting. And I'll just show you an image of these constructs from Chi Wui (?) work. Now we can see how this is sort of an X,Y,Z plot of cell seeding density and you can see basically that these cells are widespread all over the surface of the polymer. And that's something that's very difficult to achieve with thin constructs. And this shows the morphology of these cells. I didn't have time to get quite enough free glasses to pass out today, but if this is the work of Chi Wui (?). If you had 3-D glasses the polymers are actually maintaining attachment to these cells and with the processes that these cells are entering the construct as well.

I'll just sum up now by what I think is sort of the future of tissue engineering in retinal repair and that is really--a lot of questions are open about the materials and the methods. It's really just the beginning of this work. Some of the polymers I've mentioned today are biodegradable, some are not. I think that's a really important issue. And related to that is biocompatibility. The eye presents positive and negative attributes. And so the eye is a very non-inflammatory site by nature and that's good when inflammation does occur it can be sight robbing and so that's something that one always

has to balance when one is putting constructs into the posterior chamber of the eye. And again, polymerization *in vitro* versus *in vivo* is another sort of open question now I would say from both drug delivery and for tissue engineering. And so I talked about these two issues separately, drug delivery and tissue engineering. The goal is really to combine these two together and generate what are known as smart polymers, polymers that are containing drug delivery vehicles that can actively control the differentiation of the progenitor cells on them. And I'll finish now and thank those who supported this work. Thank you.

Zarbin: Once again, if Professor Konig is in the room, please come forward immediately. Are there any questions for Mike Young? Yes, please.

Man: Want to take the microphone? Come on up and grab this one or that one.

Q: When you put inside the ___ polymers they might attract or bind because of some low affinity or high affinity. Many of the unwanted proteins, which are biologically imparted. What do you say about that?

A: Well, I think it depends upon the polymer. Really, some of these polymers can be very hydrophilic; some could be very hydrophobic. I think one of the benefits that we saw in the PLGA polymer graft with the progenitor cells is that they can bind proteins that are present in the culture medium prior to transplantation and maintain those proteins close to the progenitor cell that are grafted. And we found that this is particularly true with EGF in which the polymer can act as a sink for this growth factor that can promote the survival of the grafted cell. We haven't looked at all about what is happening to the polymer in terms of host protein proteins present.

Man: What I meant to say that there might be enzymes that could get deactivated in binding with polymers.

Young: Okay, thank you.

Q: Yes, I have a very naïve question. Regarding the possible potential therapeutic applications of all this type of technologies--a practical thing-- whenever you will be successful to have a viable, let's say injectable product for treatment inside the eye, what would be the -- let's call it the shelf life? Like whenever you get this thing ready in a lab then from the time it is prepared to the time it has to be, let's say injected to make it simple. Do you foresee any specific problems or how you keep this motor functioning from the moment it is prepared to the moment it is injected or delivered?

A: That's a great point. And that's always a challenge that we have in--you know when we talk to--I'm not a tissue engineer. I work with people that are chemist that generate these constructs and then provide the progenitor cells and those techniques. We've got requirements. We want--we tell them our dream scenario--we want it to be very rigid for two weeks, biodegrade in two weeks to nothing and that would be perfect. And so the question really is once you see this polymer, in most cases it begins to degrade okay so you're generating these constructs in a degradable polymer that starts to degrade from time zero and so one always has to be mindful of the rate of degradation of that polymer. And so you can--if you chose to optimize that aspect -- you can do that but you sacrifice other things such as rigidity and transplantability to deliver this polymer itself. So yeah I would say we've been pretty happy so far with the PLGA construct, being able to possess the best attributes of rigid structure that allows you to deliver this polymer into the eye and then have it degrade in a reasonable period of time. And so I mean that's something that can be engineered into the polymer itself through the alteration of

molecular weights and the seeding density as well. So these are all things that have to be worked out I would say in the future.

Q: So your answer is that as we stand now the shelf life is really almost nil?

A: So what do you mean by shelf life?

Q: Yeah, like from the moment you prepare it--ready to inject--to the moment you actually have to inject it or to deliver it to the living tissue, the degradation starts whether you inject it or not. Therefore, the formal--in pharmaceutical terms--the shelf life, is not there yet? You don't have any time in which you say this is stable and I can wait one day, one month, one week before I inject it, is that correct?

A: No. What I tried to say is that we do know what that time is. And for constructs that we're using it's on the order of weeks. Okay?

Man: Okay great, thank you.

Zarbin: I think we have time for one last, very quick question.

Woman: If you have some _____ injections of the microspheres do you know about the biodegradation? Have you studied it?

A: The biodegradation the microspheres?

Q: Yes, inside the vitreous?

A: Yes. The ones that I talked about today, eight weeks.

Q: Eight weeks?

A: Yes.

Q: But are you sure that the microspheres, the PLGA, is disappearing from the vitreous at this time or not?

A: Yeah. By eight weeks the microspheres are gone.

Woman: Uh huh, Okay. Thank you.

A: Sure.