PACT Web Seminar: May 8th, 2014
"Scaling Up Cell Culture: Application of Closed Cell Culture Systems in Clinical Research"
Question & Answer Session Transcription
Speakers: Dr. Adrian Gee, Dr. Juan Vera, and Mr. Darin Sumstad

Question 1: What is the rationale for using fibronectin? Is it a requirement for cell propagation or is it a requirement for a disposable system?

Adrian Gee: Our understanding from Terumo is that it is a requirement for enhancing cell attachments to the fibers in the bioreactor. We have never run it without fibronectin so I can't say that we verified that this is an absolute requirement in order to get the cells into the bioreactor. And certainly when we used the flasks we did not pre-coat the flasks with fibronectin but it may be just the different materials that are used to make the fibers within the bioreactor that requires it.

Question 2: Thank you Adrian and you actually answered the next two questions about fibronectin. One was about- it was "did you try seeding the cells into the bioreactor without pre-coating with fibronectin?" And that was 'no' and also, "in your study were the flasks pre-coated with fibronectin?" and that was also 'no'.

Adrian Gee: We basically took the basic procedure from Terumo to use the bioreactor which required the use of fibronectin and we did not drop it just to see what the effect would be. The bioreactor cells are fairly expensive so we were following instructions as much as possible to optimize the chances of success.

Question 3: Great, thank you. And the next one I will open up to all the presenters but I'll start with you Adrian. Can this closed technology be used to produce dendritic cells from mononuclear cells?

Adrian Gee: We have not tried that. It's a very good, potentially it's a good application for using the bioreactor but we have not tried it. We have only tried it as I've said with the cells that produce retrovirus but I think it may well be worth trying.

Juan Vera: In regards to the G-Rex we haven't personally tried the G-Rex for the expansion of dendritic cells. I think it's worth a try. I would just go see if the materials are hydrophobic and adherent cells won't attach very well. That may affect how dendritic cells get activated. But as far as I know it hasn't been tried for that specific application.

Darin Sumstad: So as far as the Wave goes, the Wave can certainly be used with the addition of micro-carriers to grow adherent cell cultures. We have never tried it. There is publications on that but there are some available, but I would totally expect somebody to put some effort into that and work it out in the very near future.

Question 4: Thank you everyone. Darin Sumstad, the next question sort of follows along what you just said. What is the rationale for using hollow-fiber bioreactors instead of micro-carrier cultures?

Darin Sumstad: So as far as it goes for us, we haven't ever used a hollow-fiber but some of the drawbacks of micro-carriers that I've heard of some of them are extremely hard to get the cells off of because they're not necessarily a spherical and smooth surface. Some of the cells can really attach on and grab on so I think recovery of the cells from a micro-carrier culture is a big issue. Maybe Adrian can talk a little more about the hollow-fiber application.

Adrian Gee: I think the hollow-fibers are analogous to the system that Juan described in that you essentially have a different configuration: as opposed to growing cells on a flat surface with overlaying medium you've got medium coming at the cells from multiple directions. And I agree with Darin in terms of the micro-carriers. That seems to be traditionally one of the problems is getting good yields of cells back off them. Whereas with the hollow-fibers at least in the system we've used, detachment from the hollow-fibers seems to be very good. You get good recovery.

Question 5: Thank you everyone. Juan the next question is for you. Were any other markers compared to the glucose consumption to monitor cell expansion?

Juan Vera:
We initially focused on glucose simply because it was easy for us to measure the consumption by using a glucometer, used for patients with diabetes. The glucose strips are available in any pharmacy. We have also done a similar analysis with lactate. Those are the parameters that we basically measure and can be correlated with cell number easily. Glucose consumption and lactate build-up basically.

Question 6: Great Juan and the next question is also for you. I understand how glucose can predict yield for a cell line but how well does it work for primary cells? Is there too much variability to make it useful?

Juan Vera: That is a very good question. We initially were concerned that those calculations would only work for cell lines which have a very predictable kinetic of growth. Despite what we thought, you can use the same prediction or formulas to calculate primary T cells, and antigen-specific T cells. Again more details of the formula will be available on the manuscript. One can use it for other cell lines.

Question 7: Darin the next question is for you. Can the Wave bioreactor be used for both adherent and suspension cultures?

Darin Sumstad: Yeah we've talked about that previously. It definitely can be used for both with the addition of micro-carriers to provide surface area for the adherent cells to adhere to. Like we've discussed the issue seems to be with those is getting the cells to release from those micro-carriers efficiently. We've primarily used the Wave at our facility for suspension cultures. And

with the Wave's rocking motion that it provides it keeps the cells in suspension quite well. We've had pretty good success using that device.

Question 8: Thank you Darin. And someone entered a comment saying that Terumo BCT Quantum can be used for maturing monocytes into dendritic cells. A poster was presented last week at ISCT in Paris, following up on an earlier question.

Adrian Gee: I figured somebody had to be doing it.

Question 9: Adrian the next question is for you. Do you perform batch (1:05:44) testing internally on your cell production reagents?

Adrian Gee: Traditionally no. We have not done that routinely. We have not yet used the system to grow cells for clinical use. We've done all the validations to show it can be, but there hasn't been a patient enrolled on the protocol yet. I think we would probably do an upfront sterility check; we certainly do it on the platelet lysate which was our major concern. And we thought the platelet lysate would be a huge issue with the FDA but it apparently was not which quite surprised us. We have done some upfront testing on the final medium before it is diffused into the system, but we have generally used the system at risk before we get the results back of the sterility testing. So far, again, with all the validations run we haven't had anything come up positive.

Question 10: Thank you Adrian. Juan, next question is for you. If the G-Rex system is used for suspension cells, how are the cells differentiated from the media and harvested separately from the media?

Juan Vera: That's a good question. Basically the way it works is after 10 days of culture you will see such a high density of cells on the bottom of the divide that the cells are almost growing as a carpet, the cells are pretty much pellets the day of collection. So what we do is we simply remove the media which resides above the cells. You basically will have to try it or I think looking at the video you can probably see it but I'll describe it at this point. The pellet is actually quite thick and hard to break so you can actually remove the media above without disturbing the cells that are on the bottom of the divide. There is not any physical component that actually separates the cells from the medium. We've done experiments where we have measured the lots of cells by doing this sort of procedure versus doing regular sort of precipitation of the cells by collecting all the volume of media. What we have seen is that it is basically identical. Again those results will be available on the paper.

Question 11: Adrian the next question I will address to you. If contamination occurs in a closed system due to contaminates in the initial seeding culture, how is it detected?

Adrian Gee: What we did was we did cultures up front, on the bone marrow and then we did cultures on the harvested cells so if anything was in the bone marrow we would detect it up front. Obviously there is a risk that can happen but in the experiments we did we were very fortunate that all our marrow harvests were negative.

Question 12: Adrian the next question is also for you. During growth of stem cells, is it usually supply of nutrients or accumulation of toxic byproducts that would limit the growth of cells?

Adrian Gee: I think it depends on the kinds of stem cells you're talking about. I think the advantage of a bioreactor in this system is you've got continuous supply and removal of the medium whereas in the flask system the waste products accumulate in the overlying medium. But as you saw in the data from the flasks versus the bioreactor we got very similar results but it's just the medium was changed and the cells were split more often in the flasks. Presumably if we had taken the flasks and left them for the same time that the cells took to grow in the bioreactor we would not have had such good results. So in a way we compared apples and oranges in that we harvested the cells based on appearance in the flasks and/or changed the medium or passaged them based on their confluence whereas in the bioreactor we were looking at the accumulation of waste product. We did discuss going back and actually measuring the lactate levels in the flasks but we never got around to it in the end.

Question 13: Thank you Adrian. The next question is for all three presenters, and I'll start with you Darin. It seems likely that each of these technologies would differ from standard methods in the material used for culture contact surface. Have any of the presenters looked at impact of extractables or leachables on culture performance?

Darin Sumstad: So we have not personally looked at any leachables and its impact on culture performance for these bags. I think most of the bags are made out of a routinely used material as far as it's been in the blood banking system for quite a while. Some of that might have been done years ago but we haven't look at anything here as far as the Wave Bioreactor containers go.

Juan Vera: I know in regards to the G-Rex that the manufacturer Wilson Wolf has looked for leachables and they have seen that is not the case in any of the components used in the device. And we have measured the biological characteristics of the cells which are expanded and we haven't seen any change either on the final product. And these are products that now are basically being used in the clinic.

Adrian Gee: The answer I think is the same as Juan gave for the Terumo device. We have not done it at CAGT but I think in fact that Terumo has done that. And again as Juan said in terms of the final cells we have not seen any differences in functional activity or characteristics between those and those produced in the flasks. That's not to say that there are not things being released by the flasks that have not been detected.

Question 14: Adrian the next two questions are for you. Is there a reason why you moved from the initial Quantum seed and passage into another Quantum? Why not continue in the original device?

Adrian Gee: I think that was based on work that was done by the manufacturers. The cells will reach confluence in that device. Once you get to those lactate levels and those flow rates you've kind of maxed out the ability of the device and the feeding system to provide nutrients to let the cells expand and you've run out of room to let them expand. When you get into the second passage you can go a bit further because you got rid of a lot of irrelevant cell populations that

you have in the original bone marrow so you can stretch it a bit further and get a little bit better expansion. That's my understanding; you've basically maxed out the system's capability to provide room and sufficient nutrients to let the cells expand any further.

Question 15: And also for you Adrian do you use recombinant fibronectin or human derived fibronectin?

Adrian Gee: We used fibronectin. I've got it right in front of me here, let me just give you the source. We used the one from BD Biosciences and that's the only information I've got in front of me on it here. I can follow up with you, if the person wants to contact me. I can tell them exactly what we used.

Question 16: Thank you Adrian. Darin the next question is for you. When you use Wave bioreactors with micro-carriers is there any unwanted cell adhesion to the Wave bags? Does the Wave bag need to be treated for reducing unwanted cell attachment?

Darin Sumstad: So though we don't have a whole lot of experience with micro-carriers here I would imagine there's not a whole lot of adherence to the bag. They're an EVA bag which is typically used in your regular cell holding bag and I don't know of any cells that like to attach to that. Obviously there are but you would have to evaluate it with each cell type you're going to look at. We have not used micro-carriers here so unfortunately I can't give a very solid answer for that one.

Question 17: Thank you. The next question is for all speakers. Can anybody comment on the impact of cell aggregates and how it would affect the safety of the cell therapy product? Any ideas on characterizing or reducing cell aggregate formation? Any comments from Adrian, Darin, or Juan?

Darin Sumstad: I don't have too much to add to that. I guess we do occasionally see some cell aggregates forming. Typically they break up during processing and if they don't we normally do a terminal filter process with a standard blood filter and they would be pulled out at that point. Other than adding surfactants to the media to reduce the shear stress on the cells while they're rocking which would possibly promote the aggregate formation, that's about the only thing I can comment on I guess.

Juan Vera: And in regards to the G-Rex most of the cells we expand are suspension cells. Although particularly for the expansion of T cells although they can form a gentle aggregate of clusters, they go in single cell suspension very easily once they are collected so we have never seen that to be a problem.

Adrian Gee: On the appearance of the MSCs the triple select does a good job of producing a single cell suspension. I did get the information, in the validation studies that we did we used natural human fibronectin. In our other clinical applications where we used fibronectin we do use the recombinant form. I think when we go into the clinical study we will be using the recombinant form and use 5mg to coat the bioreactor**.**

Thank you everyone.