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Discussion

DR. ROBERT ZEPPA (Miami, Florida): I would like to ask one question of Dr. Jarrell who is going to close.

Have they tried the experiment with plasma-rich material onto the collagen substrate, which would be yet another intermediate, and I wonder what the timing would be on that?

DR. ROBERT W. BARNES (Little Rock, Arkansas): I appreciated this presentation and the invitation to review the excellent manuscript by Dr. Jarrell and his colleagues. They have made substantive contributions to the feasibility of endothelial cell coating of vascular prostheses, which may become a practical clinical reality in the near future. Although one of my former mentors expressed ambivalance about the clinical utility of endothelial cell coating, stating that "grafts don't fail in the middle, they fail at the ends," I believe that the explosive advances in this field not only may make a small vascular prosthesis available soon, but also will advance our understanding of endothelial cell function in our native circulation.

Dr. Jarrell and his colleagues have appropriately initiated their exciting study in an *in vitro* model system. Although he alludes in his manuscript to many further logical steps at expanding this research, I hope that Dr. Jarrell might comment on some of the obvious questions raised by their paper: (1) What practical source of autologous endothelial cells do you envision for future clinical application? (2) Do you feel that freshly harvested cells can be used for graft coating without antecedent culture? (3) Does amnion have any antigenic or other potentially adverse properties for human use? (4) Do you think your pipette washing model adequately reflects the shear stresses expected in pulsatile blood flow circumstances? (5) Do you have evidence of normal function of the endothelial cell monolayer?

Again, I laud the authors for their valuable contribution, and I look forward to further creative developments from their laboratory.

DR. B. E. JARRELL (Closing discussion): We and others have spent considerable time trying to examine whether establishing an endothelial monolayer on a prosthetic surface in humans was possible and whether it would ever be useful. Most investigators have taken the approach that one can collect a few endothelial cells from a donor vessel and then seed them on a graft at low density and wait. What you are waiting for is the endothelial cells to grow out and ultimately cover the surface.

Although there may be advantages to this approach, we have had concerns about this as a practical methodology in humans. Among these concerns, certainly there is the well established fact that even in properly designed animal trials, 6 to 8 weeks are required from the time of seeding to the time that a stable endothelial monolayer has been generated.

There are some problems with that. If you compare that time period to most clinical series, you will note that the majority of graft failures in small vessel prostheses occur within the first several weeks. Thus, obviously with seeding experiments, the monolayer is not present at the critical, most thrombogenic period of the graft.

We wanted to determine in this study, and as a major thrust in our lab, whether an alternative approach was possible. You have seen the beginning part of that approach today.

Basically, could we set up a confluent monolayer within an hour or two that was compatible with most operating room scenarios? For that to be possible, we had to look at three different areas.

The requirements to do this would be, first, to have a large number of endothelial cells available at the time of implantation; two, to have a graft surface that was receptive to these cells; and three, knowledge of the temporal factors and other chemical parameters that allow this monolayer to form and completely cover the surface.

We will address Dr. Barnes' question of the source of endothelial cells in a later paper. However, I would add that we feel that it is possible without culture and without using a large vessel as a source of endothelial cells to get enough cells to put on a graft. The way that we are accomplishing this is to isolate capillary endothelial cells from fat. We can actually do that process on a routine basis. We do it several times a week in the laboratory from several grams of fat; thus, we feel that we have the source of cells. We will tell you more about this technique in the future.

We feel now that this study demonstrates what the temporal dynamics of monolayer formation are. I would point out that the work presented today represents these specific surfaces. But there are many other surfaces that are available that have not been studied. We have begun to look at some already, and we do feel that these temporal dynamics can be reproduced.

I would point out to you that there is a major thread in this study different from the seeding experiments. We feel adherence is the critical variable to look at, not growth. We spent many months looking at growth but feel that this new approach has more merit.

This is a beginning study. It demonstrates that the cells have a remarkable capacity to sit down on a surface and to connect with one another and totally cover that surface and that this can potentially be done in the operating room at the time of implantation.

We have not completely examined the functional characteristics demonstrated by these cells when placed graft material. We are looking at that currently, but I would hasten to add that when you compare the platelet rich plasma cells with the amnion covered cells, I would certainly be willing to guess that the cells on amnion are going to function better than the cells on platelet-rich plasma. We think that morphology is a very important first step in predicting what the functional characteristics of these cells will be.

In answer to Dr. Barnes' specific questions, the amnion that we use is human amnion. It does not appear to be allergenic, although this would be examined if it were going to be a potential graft application.

We are in the process of looking at flow experiments with this surface. Using freshly isolated cells, it appears that they have a remarkable capacity to remain adherent in fairly high physiological flow conditions. We have not looked at pulsatility yet.

Lastly, to Dr. Zeppa's question, we have not done a time sequence of plasma on collagen. We do routinely use platelet-rich plasma, but my suspicions are that it will not be as good as amnion. In our extensive growth studies with plasma it works, but it is not nearly as good as amnion.

I thank you very much for the privilege of discussing this paper.