Myocardial Protection: The Science and Pathophysiology of Myocardial Ischemic Injury

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All right. Thanks again. I’m glad you guys are awake, and here, and present. I’ll try to keep you all engaged as much as I can. So let’s go through some of the slides. The first thing I’ll go through is an overview of coronary blood flow, how that contributes to cardiovascular disease, and myocardial infarction, and really, how did we get to this point? I’ll go through a little bit about the current medical treatments, which you all are probably more, even, familiar with than I am. Then, I’ll go into a little bit about my expertise about cell therapy, and really, the importance of timing for that. And then, I’ll finish up with the future areas of research.

This is a slide I used yesterday, but I think it emphasizes how important and unique the heart is, compared to other organs. Coronary blood flow is tightly coupled to cardiac metabolism. Since the heart already has a high oxygen extraction rate at rest, that really leaves very little wiggle room in terms of ischemic tolerance. So the heart is very unique, but really, it only has about 20% room to go from basal to maximum oxygen extraction, compared to a skeletal muscle, which really can increase by about 70% in its blood flow and ability to deliver oxygen to the tissue. So I highlight this again, because I think it’s important. We are seeing a decrease in the mortality rates for men, shown in the blue. We see this blue line, back from 1979, decreasing all the way to 2004, in terms of deaths for men. However, for females, shown here in the red, we’re really still at the same spot we were back in 1979 (1) (Figure 1).

The current clinical treatments for an myocardial infarction (MI), if a person were to come in, they would be diagnosed through an angiograph. It would show narrowing of a large artery, and typically, this mostly happens with men. They would show this narrowing of a large artery shown here in the yellow circle. And then from there, what they typically will do is a percutaneous coronary intervention, so they would go in and stent this large artery. Another thing they can do is a coronary bypass. These patients would be put on thrombolytic therapy, beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, anticoagulants, and really, they get a whole tirade of drugs put at them after this point. However, there is a post-myocardial infarction prognosis that’s termed “no-reflow,” which is very typically found in women. And what happens is that they have reopened a large artery that was blocked and shown on an angiograph, and they’re still not getting distal flow to the tissue. And what they’re thinking is that it’s an adverse manifestation of coronary microvascular dysfunction. I talked about it yesterday, how the microvascular bed could really determine how much blood flow gets into the endocardial tissue. So, we’ve got a couple of things going on here. We’ve got the large arteries that can be blocked and then opened-up, and hopefully can reperfuse the tissue, but if not, we still have this microcirculatory bed that’s dysfunctional.

So in an angiograph, of course, what you’re seeing here is the large arteries. However, an angiograph is unable to tell you anything, really, about the microcirculation, and the microcirculatory bed of the cardiac tissue is very profuse and very profound. So what we’re seeing is that the microcirculation is key for a lot of patients. Not just women, but men as well. In terms of the anatomical differences between men and women, really, in terms of a vascular bed, they look exactly the same. We always have an artery coming in, branching off into smaller arterials. This is the area, as we can see, that blood flow distribution occurs in the arterials. Going down into the capillaries, where that oxygen extraction actually occurs, and then of course going into the venules, and then out into the veins. So nothing’s different there, in the hearts of men and women. But, it’s the function of those vessels that are involved that are different, typically, between men and women. When you develop coronary artery disease, for men, typically you’ve got that open artery. Over time, the plaque builds up, and that’s when you get that hardened

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artery that shows up on the angiograph. Typically, for the women, what you’re seeing is not really a clogged artery. What you’re seeing is over time, these open microvessels become more constricted at rest (2), and when being called upon to open up and deliver more blood flow, they fail (3). You can possibly get a very, very thin level of plaque in some of the larger arterioles. We’re talking about 200 microns in size. But, when we’re getting down to about the pre-capillary arterioles around, say for 30 microns, you don’t get plaque in there. Really, what you’re seeing there is just the ability of those vessels to open up and deliver more blood flow is not occurring (4).

All right, so we are moving on to why we think it’s important to stop MI progression. When researching this talk, and trying to make it to where you guys would be interested in what I’m doing, I came across this review article that I’ll show in just a minute, but upon getting to that, I saw that, reperfusion therapy is really aimed at reducing that infarct size (5). The reason that it is so important in our field is because if we’re not able to stop part of that infarct from forming, what happens is the heart’s going to take it upon itself to compensate, and try to deliver blood and oxygen like normal. And when it takes it upon itself to do that, it’s going to start going down a road where it’s really hard to come back from. You start to get this left ventricular remodeling of the heart, and you start getting this dysfunction in the way that the heart pumps blood. And over time, that can lead to congestive heart failure, and episodes of acute decompensation.

So, this review article is from Kloner, back in 2013, in Circ Research, and what it really shows is why you all are so important. You get an infarct of approximately 40 minutes before the patient would come into the operating room (OR), and administering infarct size-reducing agents will prevent that infarct area from getting bigger, and hopefully prevent that remodeling that I showed you from occurring too much. However, if the infarct lasts for too long, or if the patient doesn’t come in until later, that infarct starts to grow, and it goes from the endocardial region, out to the epicardial region. And so, then you get this infarct that’s formed, shown here in the white, and after a long time, you start to get very thin left ventricular wall, because of increased scarring, and collagen deposition in that area. Whereas, if you were able to administer the infarct size-reducing agents, you’re able to keep the infarct from not getting as large, and you’re able to reduce the size of that scar tissue in the left ventricular free-wall. From that same review article, they highlight some of the different methods that you guys have tried to use to reduce the size of the infarct right after an MI. And this just lists a lot of the different things that has been tried in the past—adenosine, clopidogrel, intravenous (IV) cooling, different things like that. So I’ll refer you to this review article to learn more about that.

So, I’ll start talking about what I’m the most familiar with, the cell therapies for MI. Now, the current field has been using stem cells, and that’s what we hear a lot about in the media. Of course, in the media, they portray these promising results that could potentially restore cardiac function. And what’s most widely used right now are bone marrow cells, or cardiac-derived stem cells. With these, we don’t really know what the optimal cell type is from these cell populations. A lot of times, what they’ll do is to cling onto one or two markers and will culture these cells to express these markers, thinking that these cells, once implanted, would de-differentiate, or differentiate into a particular cell lineage. So they would take bone marrow cells, culture them to express one or two markers, and then they would put them back into the heart, hoping that they would then turn into cardiomyocytes. What the field now shows is that doesn’t really happen. So they don’t really know what the optimal cell type is, how to prepare it, and how to deliver it. One of the things that they’ve looked at is using intramyocardial injection of these cells, which results in very, very poor cell survival (6). They don’t know exactly where you deliver it. If you do already have an ischemic zone, do you put the cells directly into the ischemic area? Do you need to put the cells along the area that’s the border between the infarct area and health tissue? What they also don’t know is the optimal cell concentration. Really, the way that most of these studies have gotten about using one million cells per injection was really just, I think they threw up some numbers in the air, and chose one, and said, “This is what we’re going to use.” They also don’t know whether a single injection would be efficient, or if a patient might need multiple injections.

So, what all of these show is that none of these studies look at a microcirculatory endpoint. With most of these studies, what they look at is ejection fraction. But of course, what I’ve shown you earlier in this talk today is
that ejection fraction isn’t all there is to it. You’ve also got a microcirculation that needs to be attended to, and none of these studies really focuses on the function of those vessels, and how they contribute to an MI. Because, if you just look at the total vessel density, that does not always indicate perfusion (7). So we’ve done some previous cell therapy studies, where we’ve used a three-dimensional fibroblast patch, and that’s shown here in the Figure 3 (A, B, C, and D). What this is that fibroblasts have been cultured onto Vicryl, which is a slowly-degrading material that can be put onto the heart. And we’ve shown improvements in ejection fraction, using this therapy (8,9).

The other therapy that we’ve used in the lab, prior to this was isolated adipose-derived microvessel fragments. These aren’t single-cell populations, but small vessels that have been combined with collagen, and put onto the side of the heart. What’s interesting about these vessels is that they will connect with each other, and then they’ll inoculate with the host circulation. And what ends up happening is you get this bypass effect over the heart, which is very cool, especially if you’ve got an infarct underneath it. You can just bypass it with a microcirculation over the top, to help continue to have perfusion to the infarcted area (10). We combine these two therapies that we’ve previously used in our lab. We’ve continued to use an epicardial application for cell therapy, and we wanted to use those adipose-derived cells.

So, we took this stromal vascular fraction, isolated from adipose tissue. And again, it’s a heterogeneous cell population; containing endothelial cells, smooth muscle cells, blood cells, and mesenchymal cells. It’s everything in the adipose tissue, except the adipocytes. It has regenerative and stem cell-like qualities. It has the capacity to differentiate into many different cell types (11,12). Really, the way that we’re using it, we’re not asking it to differentiate into a different cell type. We’re really using it as a whole cell preparation. We’re asking the cells to really just do what they know how to do best. And of course, these cells are easier to derive, compared to bone marrow cells, or cardiac-derived stem cells. Everyone’s willing to give me a piece of his or her fat.

So we make this epicardial patch from stromal vascular fraction (SVF). We think it’s well-suited to treat microvascular dysfunction, because it slowly degrades. And that means that the cells stay on the heart longer, compared to if we were to do an intracoronary injection, or an intramyocardial injection. As I show here, about 1.3–2.6 of transplanted cells could only be detected in an infarcted myocardium, just 50–75 minutes after an intracoronary injection (6). We think these cells are a pharmaceutical factory, too. We think it’s proangiogenic, and also pro-vascular (13). The way that we’re using it, it’s an allograft application. But, this technology has been in use for quite some time, and they already have a machine that’s in the operating room. We can get fat cells out of the patient, put it into a machine, an hour and 10 minutes later we have a syringe that’s already good laboratory practice (GLP), good manufacturing practice (GMP) certified, ready to inject these cells back into the patient. So it really does have an autograft clinical future. We wanted to know, if we implant this SVF patch onto a myocardial infarcted heart, could we preserve the heart function specifically through a microcirculatory mechanism?

This is how we create our patch. We mince up the fat pad from a rat. We combine it with collagenase, we spin it down, and then we remove those adipocytes. The fat cells are gone and what we’re left with is this heterogeneous cell population. Combine it with that slowly degrading mesh, culture it for 14 days, where we’re really just replenishing the nutrients, we’re not trying to change the cell type at all. After 14 days, we get this three-dimensional patch (7) (Figure 2). This is something that looks very similar to this picture. If you were to put this onto a human heart, this is similar to what it would look like, and then onto a mouse heart, it would look something like this. So this is our patch characteristics. What I want to highlight here is the top left panel, in showing that the cells that are pointed with the black arrows are going all the way through the patch, so this really is a three-dimensional patch.

All right, so here’s our experimental groups. We perform our coronary artery ligation. And at that time, we have one group that receives the SVF patch implant, and another group that receives a patch with no cells to control for putting a material onto the heart. We also have a sham-control surgery. Take that out for 4 weeks, and then we explant. So then, we’ve got three groups here that I’ll show you data from. I think I’ve left out some of the data from the patch with no cells, because in almost every
single instance, it looks exactly the same as just an MI only, just an untreated heart.

These are some of the morphological changes we have, 4 weeks after treating with our MI patch, shown here, on our third column. These are our sham animals, the ones that did not undergo an MI ligation. After MI, and after 4 weeks, you start to see that thinning of the left ventricular free wall. The collagen deposition has set down, you get a very, very thin free wall. The same occurs with our MI Vicryl group, so this is the one without cells, but it had a patch. However, if we were to put the patch on with the cells, we’re able to keep some of the integrity of the left ventricular free wall.

We also did pressure-volume loops, to look at overall cardiac function. This graph shows pressure here on the y-axis, and volume here on the x-axis. The loop in black is showing what a sham animal looks like in terms of its pressure-volume relationship. Now, if we were to leave a heart untreated for 4 weeks after we create an MI, or if we put the patch on that didn’t have any cells, we see this relationship shift to the right, and it gets more narrow. This is what you’re seeing when you see cardiac dysfunction. However, if we were to put the patch on at the time of infarct, what we see is that we’re able to prevent that shift to the right. This is exhibited by our differences in ejection fractions shown at the bottom. In our MI group, it goes all the way down to about 37%, but our MI with our SVF application, we’re able to keep it around 65%.

We also wanted to look at the microcirculation, and one way that we can do that in the lab is we can inject microspheres into the heart. We do this during baseline, and after we’ve induced higher contractility through the use of Dobutamine. Then we calculate that as a percentage. What I’m showing here is our baseline conditions shown in the top, and then our dobutamine conditions shown in the bottom. It’s on a scale, so the dark blue is the least amount of blood flow, whereas the red is the highest amount of blood flow. If we’re looking down towards the apex of the heart, distal to the ligation, we see that after MI we get less perfusion. This occurs at baseline, and this is even more noted after we’ve asked the heart to work hard, the heart is unable to increase perfusion in the area of infarct, and also in the hearts that have been treated with no cells, but the patch alone. But, if we put the SVF on at the time of infarction, what we can do is to make it look very similar to our sham animals. We’re able to preserve this microcirculatory function after MI. And this correlates to the amount of blood vessels in the myocardium.

However, what we weren’t really expecting to see was that our MI group and our MI Vicryl group were also going to show such high vessel density in the area of infarction. What this tells us is that there’s a ton of vessels for these groups, but only our MI with SVF group display a higher perfusion in that area (Figure 3). So, these other two groups—our MI, and MI Vicryl group—are just showing us fake vessels. If we use vessel density as an endpoint in the clinic, or in the laboratory, this may not be a true indication of perfusion. We also wanted to see if these cells were going into the epicardium. Four weeks after our intervention, we explanted the hearts. Cells have been labeled with GFP, or green fluorescence protein. What we see is that these GFP+ cells are indeed incorporated into the epicardium. If we also stain for Griffonia simplicifolia (GS-1), which is shown in red, that is a vascular label. What these cells are doing is that they’re actually going into the epicardium, and colocalizing with a vascular element. So we really do think that this is helping out the microcirculation specifically, rather than just going in and being a support cell for the myocardium and cardiomyocytes.

To sum up this study, immediate placement of the SVF patch after MI results in our pressure volume relationship staying very close to what our sham animals look like. We see decreased fibrosis, we maintain our left ventricular wall structure, and if we look at the immunohistochemistry, we’re able to see a colocalization of our implanted cells with our vascular elements in the infarcted area. What this tells us is that this therapy sustained coronary blood flow reserve. And what’s important that came out of this is that vessel density does not equal vascular perfusion.

Then we wanted to look at a chronic model of MI. This is because current Phase II trials, such as the Transplantation in Myocardial Infarction Evaluation (TIME) and LateTIME had goals of determining the best time to transplant bone marrow stem cells following an acute MI (14,15). They looked 2–3 weeks post-MI. We’ve shown an immediate application of this SVF patch did well, let’s see if it would do well on an already-established infarct. So what we did is basically performed the same study as before, except we waited 2 weeks after we created the MI to put on the SVF patch, and then we took that out for another 4 weeks (16).

Again, we are looking at pressure volume relationship to look at overall cardiac function. If we let the MI progress, we start to see it shift to the right. Four weeks, 6 weeks, it starts to go even further to the right, and even narrower, indicative of decreased ejection fraction. However, if we put on that SVF patch at 2 weeks, we’re able to halt that progression from happening. We’re stopping that relationship from shifting over here, to where the 6-week untreated heart is shown. So we’re stopping it in its path, at the time of intervention, which is where the 2-week MI loop occurs. If we look at some of the hemodynamic parameters, we see an improvement in systolic volume and diastolic volume in our treated rats. We’re also seeing an improvement in our Emax performance.
The slope of the Emax line indicates systolic performance. If it’s less steep, it indicates decreased systolic performance. Our MI and MI Vicryl group have a less steep curve compared to our MI SVF group.

We also wanted to look a little bit more at a clinical readout, so we did some positron emission tomography (PET) imaging. This was where we infused fluoro-deoxyglucose (FDG). The cardiomyocytes will uptake...
FDG, and it is an indicator of glucose metabolism. If the cardiomyocytes are alive and functioning well, they take it up. So you see here in the middle, this is the area of infarct for all three groups. You can still see an area where there’s not a lot of uptake of FDG. But, what I hope you can appreciate is that we see a lot more uptake throughout the rest of the left ventricle in our MI SVF group compared to the MI Vicryl and MI groups. Well, then we also wanted to look at the vascular density. What we saw was that our treated group at 2 weeks resulted in increased vessel density in the infarct region. But, from our previous study, we showed that vascular density didn’t always equal vascular perfusion. So we injected a fluorescent label for vascular perfusion, and that’s shown in the red. The blue is our vascular stain, and what I hope you can appreciate, it may be a little hard to see, but our MI SVF group has more red, and it has more blue, which means it’s got more vessels, and it’s got more perfused vessels, compared to our MI group, shown on the left, and our MI Vicryl group. We also went back and looked at just the 2-week time point, to see exactly what was happening at the time that we put on this SVF patch. What we see here is that at 2 weeks, the infarcted volume is exactly the same between these two groups. What this shows us is, we’re able to stop the progression of the MI at the time we put this cell therapy on the heart.

So it halted the progressive decline and overall left ventricular function and metabolism compared to our untreated MI hearts. The SVF-treated hearts significantly increase the number of vessels, and the number of perfused vessels in the area of infarct, whereas our untreated and sham-treated groups suffered vessel dropout. Now, vessel dropout is very important when you’re talking about a remodeling microcirculation. As we go through an angiogenic stimulus such as an MI, of course, the heart wants to create more vessels because it needs to increase its perfusion again. Once we get blood flow to restart, what we go through is this very complex program of arteriovenous specification, we get vascular pruning, and we get structural adaptation (17) (Figure 4). All of this goes on at the same time to then create a microcirculatory bed. What we think is going on with our MI hearts is that they’re really unable to get through this point to create a functioning microcirculation. But, if we put on that SVF patch, I think what it’s doing here is it’s helping the heart to sort this complex time period out. And, this takes about a couple weeks in our hands.

So to conclude, we have a three-dimensional epicardial delivery of SVF that can potentially stabilize the actively remodeling microcirculation that arises following an infarct. What we think we have is a microcirculatory support device. Therapeutic treatment of MI at different time points looks like we can halt that progressive decline. We think of it kind of like a pause button. We were using an epicardial construct to deliver SVF. Some of the other clinical uses of SVF is, of course, we think that we can do this in an autograft manner, like a point of care approach, in the same operating room. We think that this SVF is also very applicable to peripheral ischemia, of course. I mean, vessels aren’t all the same all around the body, but vessels are everywhere around the body. So if SVF can prove to be good for the coronary vessels, we think

![Figure 4. Proposed timeline of events related to post-ischemic revascularization.](image)
that it’s also going to be good for the vessels in the periphery. We’ve also taken it upon ourselves to start looking at this in an IV manner, and injecting these SVF cells IV to see if that’s safe, effective, and it looks like cells are able to go and incorporate into the vascular wall everywhere (18). But, if you have an injury onboard such as an MI, they will home more to that area of injury than they would anywhere else (19). Another way that we think this could be a potential therapy is a catheter-based approach, to deliver pieces of this construct endocardially, so that you do not have to open up the chest to put this on.

I guess I’ll finish up; what we pointed out yesterday during the panel discussion is that most animal models of MI remodeling and therapeutics are using young animals. So we’re using a heart that’s almost primed to resist MI, yet in the clinical situation, of course, we don’t really have 20-something-year olds exhibiting MI. We’re having the 50-, 60-, 70-year-old people who are undergoing this. That’s something that my lab is trying to change, and looking at older models of MI. So for future studies, we think with an alternate delivery, we’re wondering if SVF could potentially prevent ischemic damage. And that’s one of the studies that we’re actually getting ready to start here in the next couple of weeks. And then another question that we have is, is re-dosing with SVF necessary? It might not be necessary, but it may be even more beneficial. And so then I’d like to finish up, and just acknowledge my lab that helped create a lot of the figures that I presented today, and I’ll take questions during the panel discussion. Thank you all.

REFERENCES


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