

EXPERT INSIGHT

Harnessing the power of cardiac stem cells



Christine Mummery studied physics at the University of Nottingham, UK and has a PhD in Biophysics from the University of London. After holding positions including a postdoc and tenured group leader at the Hubrecht Institute, she became professor at the UMC Utrecht in 2002. In 2008, she became Professor of Developmental Biology at Leiden University Medical Centre in the Netherlands and head of the Department of Anatomy and Embryology. Her research concerns heart development and the differentiation of pluripotent human stem cells into the cardiac and vascular lineages and using these cells as disease models, for safety pharmacology, drug discovery and future cardiac repair. She is a member of the Royal Netherlands Academy of Science (KNAW), and board member of the International Society of Stem Cell research (ISSCR), the KNAW and the Netherlands Medical Research Council (ZonMW). She was recently awarded the Hugo van de Poelgeest Prize for Animal Alternatives in research. She co-authored a popular book on stem cells “Stem Cells: scientific facts and Fiction” in 2011 (2nd edition 2014) and is Editor-in-Chief of the ISSCR journal *Stem Cell Reports*.

Q You pioneered early studies on cardiomyocytes derived from human embryonic stem cells, can you describe this work and what it has taught us about the use of cell therapies in cardiovascular disease?

I come from a background where I have been working on physics and developmental biology. When the first human embryonic stem cells were derived in 1998, I had one experiment that I wished to do and that was based on an observation in mice that if the endoderm

in mouse doesn't develop very well, its heart was abnormal and I thought probably the signals coming from that area of the embryo to the region that should become the heart, would be turning into heart cells. So I thought that would be a really good idea to try and turn human embryonic stem cells into heart cells.

The first papers on human embryonic stem cells turned them into neurons and following these publications I contacted Martin Pera, a good friend of mine, who was in the group who derived the second set of human embryonic stem cells, and he kindly agreed to provide me with these cells. Three months later in 2001, we performed an experiment that turned embryonic stem cells into heart cells. These cells looked spectacular under the microscope and appeared as beautiful

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beating clumps; but upon closer analysis only about 5% of the cells in these clumps were cardiomyocytes. We spent the next 10 years trying to increase these numbers because my dream was to treat heart patients – the rationale being that if you suffer a myocardial infarction 10^9 cells get destroyed and after that the rest of your heart starts to work harder and you get

heart failure. I thought in a very straightforward way: if we replace those 10^9 cells then you wouldn't get heart failure, even if you've only replace to 10^7 . We spent several years trying to increase the efficiency of cardiomyocyte production from hES cells and we finally managed to get very good cultures with about 25% cardiomyocytes and millions of these cells. Our next experiment was to inject these cells into the hearts of immunodeficient mice that had a myocardial infarction, which is performed by pinching off a vessel. In parallel to our work, there were also various other studies being performed using different stem cells such as bone marrow- and adipose-derived cells – all being injected in the same way as our study.

We injected our heart cells and observed that the cells had engrafted beautifully. The cells comprised 25% cardiomyocytes and the remaining were non-cardiomyocytes which we were unable to do anything about – but fortunately this non-cardiomyocyte population actually die off very soon after injection. We were left with this beautiful population of cardiomyocytes in the heart and by tagging them with a fluorescent label we could see where they were and confirm they were human cells. The death of non-cardiomyocytes fitted well with previous findings about bone marrow stem cells which don't become cardiomyocytes – they disappear as well. So we felt confident that we were streets ahead of the groups who were working with bone marrow as we had these beautiful cardiomyocytes. When we looked at cardiac function which was measured by the ejection fraction – amount of blood that's squeezed out of the heart in one contraction – in the infarct sham mice, we observed that the efficiency with which the blood is

pumped by the heart goes down as you would expect; and in the mice that had received our cardiomyocytes, it went down much slower at the 4-week mark. However, at 12 weeks we were surprised to observe that the sham mice and the mice that had received the cardiomyocytes were displaying the same level of recovery and cardiac function. So all of our efforts to get these heart cells in and survive in the heart had actually, at 12 weeks, made no difference. If we had looked only at four weeks we'd have said hallelujah we've done it! But of course you wouldn't just be monitoring a patient for 4 weeks, and you want a sustainable cure; but our competitors at the time had published an interesting paper in *Nature Biotechnology* with positive results and had only looked up to 4 weeks. When we mentioned the possibility of looking at 12 weeks, the journals weren't really interested because they had found a positive result.

We continued to investigate whether we could improve the outcome by increasing the cell numbers. We did a huge amount of work in trying to improve this but still saw that the cardiomyocytes did not line up properly and that was the major issue. In a normal heart, all the cells line up together and they all pull in the same direction at the same time. But what we were seeing in our mice was similar to a tug of war with cells being pulled in opposite directions and thus not creating a net work effect. Now the cardiomyocytes we were putting into the mice had to be very young and beating – if you put them in when they were not beating and more mature they died. Unfortunately, however whilst they were beating and continued to do so post injection, they created these isolated spots of beating cells that were not listening to what the rest of the heart was doing. In a human this would lead to cardiac arrhythmias. Following multiple experiments, we could only conclude that not only did very few injected cardiomyocytes survive, but also they didn't do what we wanted them to do.

Q At that point, looking at the data you had obtained, what did you consider as a viable next step in moving this research forward?

Our next question then was to determine whether this was a case of an 'engineering problem' - we are performing these experiments with human cells in a mouse or rat and that's not going to tell us a lot of what happens in humans because the human heart beats at an average of 60 times a minute and the mouse heart at 500 beats/minute. So the ineffectiveness we were observing could be an incompatibility issue and therefore the only way forward was to do these experiments in large animals. We initially extended our studies to mini-pigs which have comparable hearts to humans, but a remaining challenge was having to immunologically suppress the pigs, as they were still receiving foreign cells i.e., human cardiomyocytes since there were no

pig embryonic stem cells at that time. Immunosuppression is extremely expensive – imagine, administering immunosuppressants to pigs, every day for 12 weeks, that created a huge cost. So we moved more towards non-human primates pluripotent stem cell in non-human primates i.e., monkeys. Our original competitor in the US, Chuck Murray, is currently doing these experiments but still he's got 10 years of work ahead of him before we even know anything about how this is going. So I thought I can do something sensible and more immediate with my cardiomyocytes and that's how I changed course.

Q And where did this course change lead you?

I realized that a lot of cardiac diseases are caused by genetic defects – not all of them, some develop at adulthood and can be caused by spontaneous genetic mutations. But I thought it would be interesting to look at some genetic forms of cardiac disease because they can teach us a lot about non-genetic forms. Following on from our work with cardiac transplantation I was feeling very pessimistic that this particular line of research would prove fruitful and so I went on a sabbatical to Harvard and it was at this time that Shinya Yamanaka had discovered induced pluripotent stem cells. These are pluripotent stem cells that you can create by reprogramming the cells from any individual. At Harvard in 2007 they were working hard to get around the restrictive laws and guidelines in the US surrounding embryonic stem cells and to this end had established the Harvard Stem Cell Institute where they had derived their own cells lines. However, with the announcement of Yamanaka's work on iPS cells this could very quickly have made their initiative redundant so they had to make a quick course change.

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iPS cell technology was very rapidly adopted in Boston, which is the second hub of iPS technology in the world, and I was infected by this enthusiasm and thought this would be a great line of work to develop back in Leiden. I obtained materials to do this while I was in Boston and set it up back in my group in Leiden using the background I already had in human ES cells – my group was very skilled in making cardiomyocytes from human ES cells and we used the

same protocols to derive iPS cells from patients with specific types of mutations. In the first instance we studied mutations in ion channels which give rise to cardiac arrhythmias. When we derived cardiomyocytes from these individuals' iPS cells, the cardiomyocytes had abnormal heart rates as well and therefore, we had a very nice disease model with which we could look at drugs that triggered arrhythmias, which

were relevant for humans. It allows us to identify an individual's susceptibility to a drug and it also allows us to create disease models for heart failure. The pharmaceutical industry has no drugs at all in the pipeline to treat heart failure at this time. But using iPS cells from patients with genetic forms of heart failure – from which we derive cardiomyocytes – we can create a platform to quantitatively study the difference in cardiac cell strength by how they pump and contract, and we can now screen for drugs that will improve the force of that contraction. So the idea of developing disease models is not to put the cardiomyocytes in the patients but to utilize these cells to determine which drug might work and give that drug to the patient.

Q What are the potential limitations in creating these disease models?

One of the main challenges is that we still can't get the cell to mature. Many heart diseases and many diseases in general, develop when you are middle aged, yet our iPS cells, however you treat them or whichever tricks we use to make them mature they are still fetal or prenatal. Sometimes we are lucky when the disease symptoms are evident in these cells early on. In normal conditions, you wouldn't see the disease in the fetus but in the stressful circumstances of a lab they can develop some of the symptoms sufficiently. But what we would like to do is grow them into adult cells to replicate the stage at which we usually see disease in humans, and we would like to be able to expose them to the stress of alcohol, obesity and all the factors known to contribute towards the development of heart failure. This is a major challenge and we will need engineers to do this.

Another challenge is genetic background. If you are looking at how drugs or even how genetic mutations affect different individuals, you'll find that an Asian or a Caucasian or an African or an African-American population will be affected in very different ways. And the drugs will also potentially affect them differently and we don't know to what extent we can reflect these differences in iPS cells. Most iPS cells you read in the literature are from patient X but they never disclose their ethnic background or anything about them at all, except that they are male or female and their age. So we need to join this data together to really enable advances and this is where the development of iPS cell banks, which are being set up in various parts of the world, could really deliver value. In future I would hope we will just be able to call a bank and say I need this cell type from this ethnic group with this specific mutation which interests me; but for most basic laboratories it's quite hard to access patients and crucially to get the informed consent right. The ISSCR, the International Society of Stem Cell Research has provided some guidelines that you could have a template if I get my informed consent right then I'll be able to share the cells



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with industry; but everybody's afraid of having a situation like a HeLa cell line where the donor of the cell didn't know it would be used for commercial purposes. That's unfortunate, you never make money on this, it costs you money but the perception is if you use my cells you may make money. So we have to do this properly this time but it's still one of the hurdles, among others.

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