Brain Reconstruction: the next biomedical breakthrough, or a biological impossibility?

Transcript

Date: Thursday, 19 April 2012 - 6:00PM

Location: Barnard's Inn Hall
So, let me start, in the spirit of full disclosure, by saying that I stand before you as an Institute of Psychiatry professor, but also, I work as a consultant for a company called ReNeuron Ltd, which is a stem cell company based in the UK, so you should bear that in mind in terms of my provenance.

What I want to talk to you about today is brain reconstruction or the attempts to try and do it, and I have sort of separated the lecture into three sections.

One is very to the point, so the first section is all about brain repair and projects that I have been involved in to try and achieve brain repair, and I am going to give you a progress report on where we are up to with that.

Then I want to slightly change gears because what I am going to say to you is that, although we are making progress I like to think, it is not quite all it seems. It is a bit like the phrase “Don’t believe everything you read in the press” – well, you also should not believe everything a scientist tells you. So I am going to try and put the whole business in a bit of perspective and suggest that some of the things that we are trying to do, and people are claiming they are making progress on, might be pretty close to impossible and certainly very difficult. So that is the public health warning in the middle.

With the final section to my lecture, I want to slightly reverse that and say there actually are a couple of avenues that are coming through that might just be the breakthroughs we are looking for, and I am going to tell you about one particular technology, a stem cell technology, that is really, I think, a really wonderful possibility for the future - not my technology, I should say, I did not invent it, but I think it is potentially very important.

So, those are the three sections.

Let me start at the top: why is brain repair or brain reconstruction, even an issue? I want to say there are two issues really: one is obvious, and the other one perhaps is slightly more cryptic.

The obvious one is that there is an enormous unmet medical need. This is just a list of some of the brain disorders that lead to serious profound brain damage, and there are a lot of our compatriots out there, suffering considerably because of these neuro-degenerative disorders. So that is I suppose the obvious point.

Brain Disorders:

Stroke
Traumatic Brain Injury
Alzheimer’s Disease
Parkinson’s Disease
Batten’s Disease
Cerebral Palsy

Let me just drill down slightly deeper with reference just to one disorder. I could have picked any of those on that list, but this is one that I am going to refer to again later on, and this is stroke. So, stroke, as you are all aware actually comes in two forms: a haemorrhagic stroke, which is the consequence of a bleed; but the kind of stroke I am talking about is what we call ischemic stroke, and that is a consequence of a type of blockage in the arteries supplying the brain, and typically it is in the middle cerebral arteries and as a consequence of this blockage, a whole area of brain tissue gets deprived of blood and there is therefore an ischemic event, and as a consequence of that ischemic event, you do not have to be a radiologist to see exactly where the stroke has been, a big chunk of brain tissue becomes considerably distressed as a consequence of the ischemia.

What is happening at this stage is that there is a battle going on, that the brain is desperately trying to save some of that tissue in the afflicted chunk, and for a lot of it, it is losing, and the tissue will eventually be lost and will be cleared away, and if we come back a little bit later and scan this individual, we will find there is effectively a big hole there - there is a fluid-filled cyst where brain tissue was, and that brain tissue has simply been lost and cleared away. And then, the bit round the periphery, the brain might win the battle and manage to save some of the tissue and maybe that is tissue that can go on and function normally, with a bit of luck.

Stroke is the third largest killer in the UK, and in the Western world generally, behind heart disease and cancer, so it is a major disorder, and it is the primary cause of serious disability amongst those of us in the West here, so it really is an area of considerable interest.
That is the obvious way in to brain repair and brain reconstruction, this unmet medical need, and we have got to try and do something for patients with those kinds of problems. The other one, as I say, is slightly more cryptic, but it is nonetheless important, and certainly you will not have to Google very far on the Internet to come across the concept of brain enhancement. There are a lot of people out there who think that the techniques are just going to fall in our lap in terms of brain repair, that we are currently working on and I will tell you a bit more about in a moment, those techniques are also going to allow us to intervene with normal individuals and make them better. Some people think that this is really the glorious future and it is just a few years down the line.

Henry Markham is a professor in Lausanne, Switzerland. He runs the “Blue Brain Project”, and this is a verbatim quote, he says: “It is not impossible to build a human brain,” he says, “and we can do it in ten years.” So there is confidence and if you are interested in the debate about that subject, that Scientific American article is certainly worth a look into. That is not a mis-quote. Again, if you have a look on YouTube, you will have no trouble finding the presentation in which he says precisely that. So, for some people, this is just the start, and we are hoping to go very much further.

That possibility of human enhancement, of using these kinds of technologies not just repair damaged brain but make things better, has provoked quite a debate. So, a number of bio-ethicists, like John Harris in Manchester, think this is great. He is all for it, if you read his book, you will find statements that he sees no reason at all why we should not be able to use these brain repair approaches to enhance humans and the more, the better – he thinks this is terrific. Others are not quite so sure. In fact, others would like to regulate what we are doing because there is a danger that, when we go in and try and repair brains, we might change the personality of the person we are dealing with, in a way that is unpredictable, and we ought to be taking that a bit more seriously. A lot of people think brain repair is a good thing, but we have got to be very careful that we do not go too far and change and improve on stuff that people do not want to be improved upon.

There are these two elements running together: the unmet medical need; and then there is either this threat or a promise, depending on your perspective, of human enhancement. So we have to have both of these things in mind when we start to think about the potential for brain repair.

So that is the context. What is all this about brain repair? What really is possible and what really can be done?

I want to split this section into three really, and the main bit I am going to talk about is the middle of those three - I am going to talk about stem cell transplantation as an approach to rebuilding brain tissue.

Before I get onto that, I want to talk a little bit about endogenous neurogenesis and tell you what that term means, and explain why – that will also give us a way of defining the problem a bit more precisely. Then, finally, I want to talk a bit about this approach that I call stem cells “plus”, and that term will explain itself when we get to it.

Let us just drill down slightly, using the concept of endogenous neurogenesis, and try and sort of frame the problem a bit more precisely. What is the problem here? I mean the problem here is that this brain has done a very poor job of repairing itself.

If we think about a similar sort of phenomenon happening in another tissue, say your liver, or the example I want to pick upon, the blood, then the body does a much better job of repairing itself. Just think about blood. We lose blood all the time, blood tissue is lost all the time, and yet we never worry about running out of blood or not being able to refill, as it were, our blood supply. Why is that? Well, the reason is quite simple: it is because we have, in the bone marrow, a population of stem cells, and the job of this population of stem cells is to replenish blood on a continuous basis – that is what it does - and lots of other tissues can do something similar.

Sometimes the cells are slightly different, they are not always called stem cells, but the phenomenon is the same. If somebody were to cut off a chunk of your liver, which actually might well happen if you are having a tumour removed or something like that, then the liver has quite a good capacity to re-grow and re-form itself. But the brain is pretty useless in this regard. It just cannot do it. And that is what we are looking at here: we are looking at the fact that you have lost a chunk of brain tissue and your brain is not able to do very much about it.

Why is that? What is about the brain that is different from blood or liver or skin or various other tissues that can replace and regenerate themselves? I would suggest to you there are two answers to that: there is an evolutionary answer; and there is a cell biology answer.

So, the evolutionary answer is, or the evolutionary view of the question is a very interesting one, but it is not one we have a lot of time for today. The answer is, for some reason, we have lost the ability to regenerate brain tissue. It is not that regenerating brain tissue is impossible because some animals do it - so those vertebrates we like to disparagingly call “lower vertebrates” like otters and frogs and goldfish and things like that, if you damage their nervous systems, they can actually replace part of it. But we mammals, and birds, have somehow lost that, and it is an interesting question as to why that should be. I would suggest that it is probably something to do with the fact that we have lost the ability to continue to grow our nervous system right through life. So if you look at, for example, a goldfish, it continues to grow and grow and grow, as it gets older and older, and its nervous system grows with it. That is something we do not do. Our nervous system reaches pretty much full compliment soon after birth, and if you lose brain tissue after that point, you are stuck.
That is the evolutionary answer, but let us put that to one side and concentrate on the cell biology, and the simple reason is that the brain does not have an equivalent population of cells, like the bone marrow that the blood has, to keep replenishing it. Now, note that I did not say that it does not have any stem cells at all. If we were having this discussion fifteen or twenty years ago, that is precisely what I would have said because, at that point, we did not think there was such a thing as neural stem cells. But one of the discoveries that has really transformed this field over this last decade or two has been the discovery that there actually are stem cells in the brain.

What we now know is that there are two populations, stem cell populations, in the adult brain. As far as we know, all mammals have them.

What they are is simply this: there is the hippocampus, so there is a population of cells in that bit of brain – for those who do not know, the hippocampus is an important part of the forebrain and it is involved in memory and lots of other really crucial functions. The important point for this discussion is that there is a population of cells within the hippocampus that are really quite equivalent to the blood, by which I mean it is being continuously replaced, there is a population of stem cells, exactly equivalent to bone marrow, that keeps regenerating this particular type of nerve cell all the time, a particular type of granule cell – we call it the dentate gyrus granule cell. So it is having continuous turnover like that.

And then there is a second population that feeds the olfactory bulb. So the olfactory bulb is stuck on the front, the forebrain, very important to the rat, not quite so important to us, and again, there is a population of stem cells that continuously feeds this olfactory bulb, granule cell population.

Why did it evolve that these two bits of brain manage to make neurons perfectly happily, whereas the rest of the brain, the cerebral cortex, the corpus striatum, the thalamus, all those other important bits, fail miserably to make themselves? The simple answer to that is we do not know. There is a slight suggestion there is a particular kind of plasticity involved in hippocampus and olfactory bulb that makes it unique and requires these new neurons, but at this stage, that is a slightly shaky explanation. The truth is we do not really know.

It raises the question: could we not divert these stem cell populations to do something about the stroke? So, in the stroke, we have lost chunks of striatum or chunks of cerebral cortex – could we not induce these cells to make those kinds of neurons?

The simple answer, at the moment, seems to be it does not work, that they are a bit like blood cells – it is a bit like asking the blood cells to make liver or asking the blood cells to make kidney. They are happy to make blood as blood is lost, but they cannot transfer to a different function. And just as that is true for the bone marrow stem cells, it seems to be true for these. That is not to say there are not people who are trying to do that, and there is a lot of work going on along those routes, trying to work out exactly how you would have to re-programme the cells to make them divert into these other routes, but suffice to say, at this present moment in time, that is not something we have managed to do. So, this is a possibility, but we are not very far with it yet.

Where we are making progress is with stem cell transplantation. So let me explain what stem cells really are.

Adult stem cells have two seminal properties: the first is what we call self-replicative – they make more cells like themselves; and the second is they are multi-potential – they make a whole range of cells that make up a tissue. You can immediately understand why they are the seminal properties, again, by reference to our bone marrow stem cell population that replenishes the blood. They have to self-replicate because they have to keep the population of cells alive for the entire lifetime of the organism, and they have to be multi-potential because when you make blood, you need to make all the blood cell types. Obviously, it is no good if you make red cells and no white cells or some other combination. You have to be able to make the whole lot. So that is why they are the seminal properties.

In neural stem cells, it is exactly the same: they are self-replicative, and they are multi-potential – they can generate the neurons and the different types of glial cells that make up the adult brain.

The concept is this: can we do real, true regenerative medicine with these cells? Can we do the equivalent of a bone marrow transplant? Could we take such cells that have this multi-potentiality, could we put them into the bit of the brain where the damage has been, in the stroke, for example, and can we get them to replace the missing cells? If we could do that, that would be a true regenerative medicine of the brain, so that is the real nub of it. That is what my colleagues and I have been trying to do for about twelve years.

The first problem we had is you have got to get enough cells to work with. It is not too difficult to find neural stem cells. We have already said you can find them in the adult brain, although there are not very many. A better source of them is the foetal brain, so if you take a chunk of foetal brain, it is quite easy to come up with quite a large number of cells that have these properties. But the problem you have got is this: if you take these cells and put them in a tissue culture dish to try and grow them so you can have lots and lots, that you can transplant lots and lots of people, what you find is that the cells in culture, they like to do this, but they also do not like to do this. In other words, when you try and grow stem cell population, you find they just differentiate on you – they just do that and they turn into nerves and you end up with no stem cell left.
So your first problem is to come up with a technology that will keep them in the stem cell phase, such that you can generate a whole bucket-load of them, so that I could treat 1,000 stroke patients – that is what I would like to be able to do, each patient getting essentially the same population of cells, that is the key.

We have managed to do that, and how have we done it? Well, I am not going to go into the molecular biology, but this is the schematic of what we have managed to do. We have come up with a strategy we call conditional immortalisation. What that means is we take these foetal neural stem cells, these multi-potential neural stem cells, and into them we introduce a genetic construct, and this is a genetic construct that is basically an oncogene. An oncogene is a gene that causes cancer, so we have got to be pretty careful with it, but the way we control it is we put a switch on it, a molecular switch on it, and we can switch it on and off. If we put the gene into the cells and we switch it on, then what we find is these cells will expand, basically ad infinitum. So, almost literally, we can generate billions and billions of cells, starting from a single cell, and we end up with a massive population of cells, all of which are essentially identical. Then the key is we can take these cells at the end of this expansion process and we can switch the gene off.

The question is: are they still multi-potential? Can they still generate the entire range of brain cells that the starting cell could do? The answer is they can. What we have got here now is a way of generating enough cells to treat 1,000 stroke patients, all from a single cell, all frozen down in separate vials, all identical, so we can go in and test them on animal models or any other models. We can test them for sterility and identity and all the other things that regulators like to be able to see, and that is what we have done.

Well, that is all fine, but do these cells do anything – are they any use?

This is a result of an animal experiment, and what we have done here is we have taken this particular line, which is our favourite, CTX0E03, and the CTX stands for “cortex”, so this originally came from human foetal cortex, cerebral cortex, and we have made the line, in exactly the way I have just described to you, and what we do is we inject it into the brains of rats that have undergone a stroke, essentially an identical stroke to what I have just shown you in patients. This is a middle cerebral artery occlusion, so you block the middle cerebral artery, and, just as with the people, these rats have lost a whole chunk of brain tissue. So we inject them in.

Do the rats get any better as a consequence of this therapy? We test that in a number of different ways, and there is a number of different disabilities, just as with human stroke patients, but the one that we really wanted to hone in on and one we spent most time thinking about is the sensory motor deficit. Stroke patients, as you are very well aware, are typically hemiplegic – they lose the use of a limb, one limb or both limbs, on the side contralateral to the afflicted side. Rats do exactly the same. So how can we measure improvement in that?

Well, we do a test we call the sticky tape test. This is primitive but it works beautifully. We put a piece of parcel tape around both the animal’s front paws. If you do that to a normal rat, it does not like it very much and it rips it off pretty quick, and because rats are not particularly handed, left or right, they rip it off one side pretty much as fast as they rip it off the other. So, if you look at the performance, the speed it takes for the animals to rip the tape off, and compare right with left, you find there is no difference, so one side divided by the other side is pretty much zero – there is no difference between the two. The blue line is the control, over several weeks, and these are control animals – you can see that it does not change.

Now, the red line is what happens with an animal like this, that has gone through the stroke, and what you can see is, following the stroke, now suddenly there is a big difference between left and right, and that is exactly what you would have predicted. So the side contralateral to the stroke side, the animal has lost sensation, so it does not even feel the sticky tape is there, and when it does realise it is there, it is less able to rip it off, so it rips off this side much faster than it rips off that side, and that is what you are looking at here. That is this difference. You can see, again, this is a fairly stable phenomenon – these rats, the disability is pretty stable over time.

What the grey lines are is a dose curve, where animals have received increasing doses of these cells, in exactly the way I have just described to you. If we just concentrate on the black line, which is the highest dose of cells, you can see that something like four to six weeks following the engraftment, there is a substantial improvement in this ratio between right and left and that, over the twelve weeks of the experiment, it is really a stable improvement, that these animals are essentially indistinguishable now from the control animals, the animals that did not have the stroke, and substantially and significantly improved upon the animals that did not receive any cells.

This is a very robust finding, so we have done this multiple times, and we always get this result. It is also a slightly staggering finding. There are not very many agents that you can administer to an animal or a person with severe brain damage of this type that gives you this kind of functional improvement. So this is not a trivial observation.

The other thing about these CTX cells is that they were developed in conjunction with my colleagues at ReNeuron and these are clinical grade cells, so these cells were grown, for example, in line with the Human Tissue Act and with various issues about sterility and identity and all the rest.

These have been through regulators: so, in 2006, 2007, we went to the MHRA (Medicines and Healthcare products Regulatory Agency) and we asked permission to put these cells into a clinical trial; and in 2010, we
finally started the trial, and that trial is now underway. This was the first trial of a neural stem cell product, a regulated stem cell product. I think it is the first anywhere in the world, and certainly the first in the UK, in attempt to treat stroke with a stem cell therapeutic.

The situation is that we are doing patients one at a time. That is what the regulator wanted, perfectly appropriately, so we are up to patient six now. What I can tell you that it is very early days, there is not very much to report yet, but actually, all the previous five patients have all shown some improvement. But the point I want to make strongly is it is very early days. These patients are still getting a very low dose of cells. We were asked to start at a low dose and work up, again totally appropriately, so we are still on quite a low dose, but for what it is worth, that is where we currently are.

So, so far, so good! What I said at the outset was this was an approach we were taking to come up with a therapeutic to meet this unmet medical need that I had told you about at the outset. But what I also said was that we were perhaps not quite as far along as we thought we were or some people would have you believe we are. So, what did I mean by that?

Well, what I meant is that injecting these cells into rodents, and hopefully into people, works. The recipients show an improvement in these very important behavioural measures. But we are right, if we are right at all, for the wrong reason, and it is become increasingly clear that we are not achieving what we set out to achieve.

I have shown you the behavioural outcomes. If we look at the histological and anatomical outcomes - in other words, we ask what happens to the cells when we inject them - what we find is we are not building any new brain tissue. The original hypothesis was, these cells are multi-potential cells, we will inject them into this scenario where brain cells have been lost, and these multi-potential cells will rebuild brain tissue. Well, whatever else is happening, it is not that. We can be quite confident these cells are not building any new brain tissue - that quite simply is not happening.

What do we think is happening and how can we sort of explain it? Well, let us back off a minute and let us just look a bit more into what exactly we are trying to achieve here. Let us just think about it and try and analyse a little bit more deeply exactly what we are asking these cells to do.

I have got a colleague, a regenerative medicine colleague, who says, "Jack, I do not know why you guys who work on the brain always think life has to be so difficult. Tissue regeneration is fundamentally straightforward: all you need to do is you go into the tissue, you inhibit those factors that are screwing up regeneration, like inflammation and various things like that, you put your cells in, you put a matrix in and other components that will help the cells do what you want them to do, and hey, bob's your uncle, they will do it!" He says, "Look, we can do it," and he points to a piece of tissue, and he says, "We had completely destroyed that and we completely rebuilt it." And I say, "Yes Steve, but that is bladder!" So he says, "Well, what is the difference?"

So why is the brain such a problem? Well, let us think about it because this is the nub of the issue.

The reason why it is different is its complexity. If you look at a piece of tissue like bladder or liver tissue or any other somatic tissues, what you are dealing with is - it is not simple, that would be a fatuous thing to say, but nonetheless, most of the cell/cell interactions that define the tissue are small in number, they are interactions between relatively small types of cells, and they are relatively short distances, so cells interact with other cells locally. Of course, there is a hormonal milieu and all those other things that we know about, but by and large, it is a small-scale problem. The brain has a level of complexity that is just several orders of magnitude higher than that, and the simple way to think about that is the brain has circuits.

If we look at a piece of cerebral cortex in a rat, what makes this so complex is the number of different cell types that this tissue is composed of is so many we cannot even really count them. There are several different types of neurons, but even that does not capture it. There is enormous diversity of cell types in the brain, not the simplistic diversity that you see in, say, liver or pancreas.

But there is also an incredible complexity of connectivity, and these connectivities have widely differing both space and time constants. What do I mean by that? Each cell is probably getting contacts from, and making contacts to, somewhere between ten to the three and ten to the four other neurons, and they are incredibly complexly organised. So, this cell is receiving other inputs onto its dendrites, but they are organised - some cells put synapses out, others put them in elsewhere - they are organised spatially and temporally. This codes for incredible complexity, of a type that you just simply do not see with other tissue.

What happens then in the scenario that we are envisaging, in our stroke brain, when we lose this? So, that is normal - what happens in our stroke now? Well, three different things happen.

Where we have lost the entire tissue, we have lost the entire circuits. We have lost all the different nerve cells, we have lost the tissue integrity, we have lost the inputs, we have lost the outputs from this tissue, so that tissue is completely gone.

But even in the neighbouring region, what is sometimes called the penumbra of the stroke, we see a substantial loss of tissue because, although tissue integrity is retained, some cells will have been lost. Right at the outset, I
said there is a battle that goes on following a stroke: the brain tries to protect and keep some tissue, and some tissue it is able to keep more or less intact; but others are at the borderline of the battle and some cells are lost and some are retained. So, the tissue has lost some of its cells, some of its input, some of its output, and so it is partly damaged.

But even tissue has suffered the consequences because this tissue would have had reciprocal connections, either directly with this bit of brain or via another bit of brain – there is a chain of connections. So this bit of brain will have lost its inputs and its outputs that would have linked up with these other bits of brain tissue.

If we are going to try and really rebuild brain tissue, we have got to somehow reproduce the entire process of generating this complexity. So, you suddenly start to see how naïve our original experiment was. We took these stem cells and we just squirted them into the damaged brain and said, you know, “Go on guys – do it!” But that is to make a really fundamental mistake: so, I told you these cells are multi-potential, meaning they can make all the different brain cell types that make up this tissue; the sleight of hand was that they are also histogenic, that they can build the tissue that is composed of all those different cell types, and that is actually quite a different thing. In fact, the only process that we know is truly histogenic is development, is when you actually build this tissue during the process of normal development, and that is a process we understand increasingly well. Over the last decade or two, there has been incredible improvement in our understanding of how brain development works, and we know enough to know that it is incredibly complex.

What is happening in the scenario where we have transplanted in our cells? What I think is happening is that as a consequence of engrafting those cells, we are getting some re-wiring, there is some evidence of plasticity, so we know this wiring process, although it is incredibly precise, it is also flexible. Cells are re-making and breaking and re-making connections all the time, so we think we are helping this process. We also think we are helping this process, but we are making no progress at all. You could say, two out of three is not bad, but the problem is, we got lucky – this is an entirely empirical subject. We squirted the cells in, they have given us a little bit of what we wanted, not all of what we wanted, but we do not understand how it is even doing what it is doing. If we are going to achieve anything more fundamental, if we are going to achieve anything beyond what we are just getting gratis by squirting the cells in, we are going to have to understand how to control these processes, and, at this present moment, we really do not.

Before I go on, I just want to give you a little bit of the evidence of why I think we are actually producing these two effects but not that one. I have already told you why we are not getting that one, because we do not see any rebuilding of tissue, and that is a straightforward fact. But let me show you some of the evidence because it is quite exciting that we are, at least, able to get these two phenomena to work.

So, in an experiment that we did, not with that same cell line but with a very similar one, and this is also not a stroke experiment – this was with a neuro-toxic brain lesion, but that is a small difference in the context of what we are discussing today. What we have to look at are dopamine receptors. Dopamine is part of the connectivity between these different brain cells, so if we could retain dopamine connectivity, that would be a big plus. This is a control animal in which we have injected this dopamine agonist, this drug that stimulates dopamine function, called bromocriptine, and the blue is the results of a neural imaging study. So, what you are looking at is a whole series of virtual sections, taken with a scanner through the brain of the rat. The blue signal is a positive response to the bromocriptine, so it is evidence of active dopamine signalling. In the control brain, there is quite a lot of dopamine signalling going on. The middle section is the lesioned brain, so that is a scan through the brain of an animal that has had this neuro-toxic lesion, and it has lost its dopamine signal. It is another way of saying that we have blown away a lot of that connectivity that requires dopamine. The last panel is the animal that got the transplant, and you can immediately see that that is much more like the control. So, we have either restored that dopamine function or the other possibility, in this particular experiment, is we have stopped the damage in the first place – we helped retain that dopamine function as a consequence in grafting the cells. We take this as evidence that if we engraft those cells, maybe we do not build new brain tissue, but what we do do is help the connectivity between the brain cells that are retained.
This is one piece of evidence that says we can form new brain cells that are fitting into that damaged tissue. The middle condition, where the tissue integrity is still there, there is still some cells survived but some cells have gone missing, so we think this is evidence that we can put new cells into that. What we have done here, you are looking at four conditions: this is a normal animal; this is a normal animal that has received the graft of cells; this is a stroked animal, and you can see the area of damage over here; and then this is stroked animal that has received the graft of cells. What we are looking at is these little brown dots. Those are the young neurons. We have used a particular stain, a way of recognising those new neurons and staining them brown. These are the numbers, over here, and you can see that this is the control, and very few new neurons have been generated, and that tells you what I told you right at the start. If you put the cells in, it does not make very much difference. If you stroke the brain, it does not make very much difference. But in this condition, where the animal had the stroke and then was engrafted with the cells, there has been a dramatic increase in the number of new brain cells that are being incorporated into this damaged tissue. This tells you that we can start to repair by introducing new cells into this area of damage.

So, it is exciting. There is two out of three we can do: we can rewire; we can introduce new cells where there is still tissue integrity. What we cannot do is build new tissue.

The good news is we have a potential therapy, and I would accentuate the point that our stroke patients do not care how we do it – if we could get an improvement in our stroke patients the same as we saw in the rats, they will be very happy, whether we are rebuilding new brain tissue or not. But the bad news, from a long-term perspective, is this is not brain reconstruction. We are able, just on this empirical basis, to make these various improvements in terms of adding new elements, but we have not been able to reconstruct new brain. So, to go back to our original problem, that hole in the brain that exists after stroke, that is still there and we have not done anything about it at all.
I said at the outset I was going to talk about three things. I talked a bit about endogenous neurogenesis, and I said that the brain does have stem cell populations and we are trying to work out how to get them to join in and create new brain tissue following a stroke, but currently we have not really managed that. Then I talked about stem cell transplantation, and as you see, that is quite promising and it is making progress, but we are still not able to really reconstruct brain in the real sense in which we are trying to do it.

The final point I want to say is that we have not given up on building brain tissue from scratch, but at this stage, it is slightly more of a game than a serious science because we really do not know what we are doing, but we are trying a few things, and one of the things we are doing is what I call the “Stem Cell Plus” strategy.

We teamed up with Professor Kevin Shakesheff at the University of Nottingham, and some of his colleagues, and they are very good material scientists and they can do all sorts of things with materials, and one of the things they can make are these little beads made from poly-lactic glycolic acid, PLGA. These are wonderful because you can grow cells on these little beads, but the other thing that Kevin can do is he can design them so that the beads hang around for a long time or a short time. What you can do put the cells on the beads, inject the beads into the brain, and then the beads will slowly dissolve and leave the brain cells. We had this idea which was maybe we could grow the cells on the beads, then put the beads into that big space, that big hole that we were not making any progress with, and maybe, in that scenario, there would be enough structure to get the cells to really start to indulge in tissue histogenesis and really build new brain tissue. I thought there is no chance this will work but it will be fun to try, so we did.

So, this is a picture of that experiment. So this is our beads, and the cells are growing on the outside of the beads. These, I should say, we have sized these beads and these are just 100 microns, which turn out to be the perfect size to get lots of cells on so you pack lots of cells in.

This is an animal that has undergone a stroke, so you can see the stroke developing over time, and then the really black stuff is the beads. It turns out it is what brain imagers called hypo-intense, meaning it gives you a black signal, a dark signal, when you see it on the MRI machine. So that is what you are seeing here. We injected these particles in here and then we left it a little bit and then we had a look to see what had happened, and this is where there was a big hole, here, and this is new tissue, so this is the result of our engrafted cells. As a consequence of growing them on the beads, we were able to put about two orders of magnitude more cells in than we were able to do without the beads, so that was great. We had built this big chunk of tissue, so there we were, shaking hands with each other, congratulating each other on success, but then we did the experiment we should have done before, which was to section the material and ask what have we got here, and what we have actually got is a jumble of cells. It is not really tissue at all. There are neurons in there and glia and various other things like blood vessels, and it is quite nice, but is it reconstructed brain tissue? No, it is not. In fact, this is exactly what you would expect. We know these cells make neurons and glia – they will just do that in a tissue culture dish, so what we have done here is provided a perfect tissue culture environment for them, so that is what they have done – they have grown and they have made lots of brain cells. But is it proper tissue? No, it is not, which just reinforces the point that I started off making: we really do not know yet how to make the main proper tissue.

So, in one sense, that is exciting – we have got approaches that work and are now in the clinic; however in another sense, it is slightly frustrating and we still do not know how to overcome the fundamental problems.
But there has been a development in stem cell biology in the last couple of years that I think really might give us a new impetus, and that is all around pluri-potent stem cells. I have talked to you a bit about multi-potent stem cells, stem cells that can make brain cells, neurons, glia. Now, let me explain what we are talking about here.

Here, we have to go back really to the very earliest stages of human development. So, we are a fertilised oocyte, an oocyte being fertilised by an egg. This grows, makes a little ball of cells called the morula, and then the first differentiation event that happens during development is that two populations of cells emerge. This is called the blastocyst. There is this outer trophectoderm, as we call it, and they are the ones that go on and contribute to the placenta. But the one’s we are really interested in are called the inner cell mass, and that population of inner cell mass cells are the pluri-potent cells that make the entire body. Pluri-potent, in this context, means the ability to make not just neurons and glia, or not just blood, or not just liver, but all of those things, so these cells make the entire body.

This has been known for a long time, and, just as I was saying with the neural stem cells, the trick was - so, you would really like to have these cells growing in the dish, you would like to work with them, but as I alluded to before, the real problem with stem cells is, the moment you take them out, they differentiate.

It took many years before Martin Evans, now in Cardiff, then in Cambridge, discovered the way to do this, and won the Nobel Prize for it a couple of years ago, and that led to this technology, taking these cells out and working out how to grow them in vitro, grow them in a culture dish, and that is what we call ES cells, ES being embryonic stem cells.

You will be aware of this technology because it has got masses of publicity over the last decade or two because this was the technology that people argued about the morality of and the ethics of because you have to destroy the embryo to make these cells, and, in the United Kingdom, we came to the decision that this was a technology we would permit, with very strict rules. Lots of other countries, most famously the United States, under President Bush, and other countries like Germany and Italy in Europe, decided that this was not an ethical approach and prohibited it or severely restricted its use.

The virtue of this, from a technical point of view, is that these are pluri-potent cells, identical in their potential to these cells here, and so these cells could be made to make all the different cell types that make up the body, just as the inner cell mass do during normal development.

That technology was restricted from an ethical point of view but it was also very restricted from a technical point of view because this was not a technology one could ever do on an enormous scale, and one had no control over the starting material because you simply had to use these excess embryos that happened to be left in a freezer after somebody had had IVF.

The situation changed very dramatically in 2006, thanks to Shinya Yamanaka who works in Kyoto. He will win the next Nobel Prize in this area, you can take it from me, because he came up with a dramatic new technology that has totally revolutionised this area.

He came up with a way of making IPS cells, induced pluri-potent cells, and this is as close to magic as makes no difference. So what he was able to show you could do was to take any old cell, any somatic cell – he started with skin fibroblasts – and into those cells, you introduce just four genetic factors, trivially easy to do technically, no challenge whatsoever. As a consequence of inducing those four factors into the cell, what emerged are these IPS cells that are essentially indistinguishable from the embryonic stem cells. So these are induced pluri-potent cells, pluri-potent in exactly the same way as ES cells, and they can give rise to the entire range of cells that make up the body.

An experiment that has been done now in lots of labs across the world, including our own, is where you can start from just a tissue sample. The way we do it is we start with a hair, pluck a single hair. From the bulb at the end of the hair, you can grow a population of cells we call keratinocytes. You introduce the four “Yamanaka factors”, and out come these IPS colonies, essentially identical to ES cells, and from those, you can make neural stem cells, or anything else come to that, but that is obviously our interest, and you can make neurons. Enormously powerful as a way of getting human neurons in a dish, and the main impact of this technology in the next couple of years is going to be our ability to model neural-developmental disorders. That is one of the things that we are doing at the Institute of Psychiatry. What it allows us to do is we can get those cells and we can turn them into brain cells, and unlike the neural stem cells that I was just telling you about, these cells, in vitro, undergo a process that is much more similar to normal developmental histogenesis.

Here is one example. When you build cerebral cortex, it is a highly laminated structure, and these laminae, during development, are built in a very specific order. So, the deep layers are generated first during development, and each successive cohort of cells forms the next more superficial layer, so it is built in an inside-out fashion: the deepest layers are built first, and then the more superficial layers are laid on top. If we grow our IPS cells and follow the markers and follow the technology through, these IPS cells do exactly that. So, in a dish, in isolation, they reproduce this inside-out histogenesis - they build the deep neurons first and then the more superficial neurons later.
I have told you really about a number of technologies. I have described the problem to you and I have shown you our attempts to repair brain using neural stem cells, conditionally immortalised neural stem cells, and I have shown you how, in one sense, we have succeeded – we have got cells going through to the clinic – but how really, when it comes down to it, we really have not succeeded, and this empirical approach of just shooting the cells in and standing back and hoping they are going to do what you wanted them to do clearly has enormous limitation. We need to put a lot more effort into that, and maybe these IPS cells will be the way to go.

As a conclusion: what is biotechnology like then in this region? If you read about it in the newspapers or you listen to scientists parading on the Today show, you get the impression that it is like an escalator – you know, you get on and it just keeps going up. Francis [Fukuyama], I do not know if any of you ever read his book, he talks about this all the time. He says, technology, you just start with the simple problems, then you go on to the more complex ones, and you build yourself up - you just keep going up that escalator.

So, is that the right analogy? Or is it more like salmon swimming upstream? You get in a pool, and you make progress against the current for a little while, and you think you are doing pretty well, but what you do not realise is you fell into a pool where there is not much of a problem. What you do not see is round the next corner is this enormous waterfall coming your way, and you are going to have real trouble jumping up that waterfall. It is a serious problem. It is not just more of the same. It is not an escalator. It is a real quantum leap.

I would suggest to you that brain repair now is facing that quantal leap: we really do have an enormous step-change, and if it is a quantum leap like this, then it stands to reason that all the technologies are not going to make it – we are not all going to make it to the top. Some are going to fall by the wayside, and on that thought, I will leave you, and thank you for your attention.