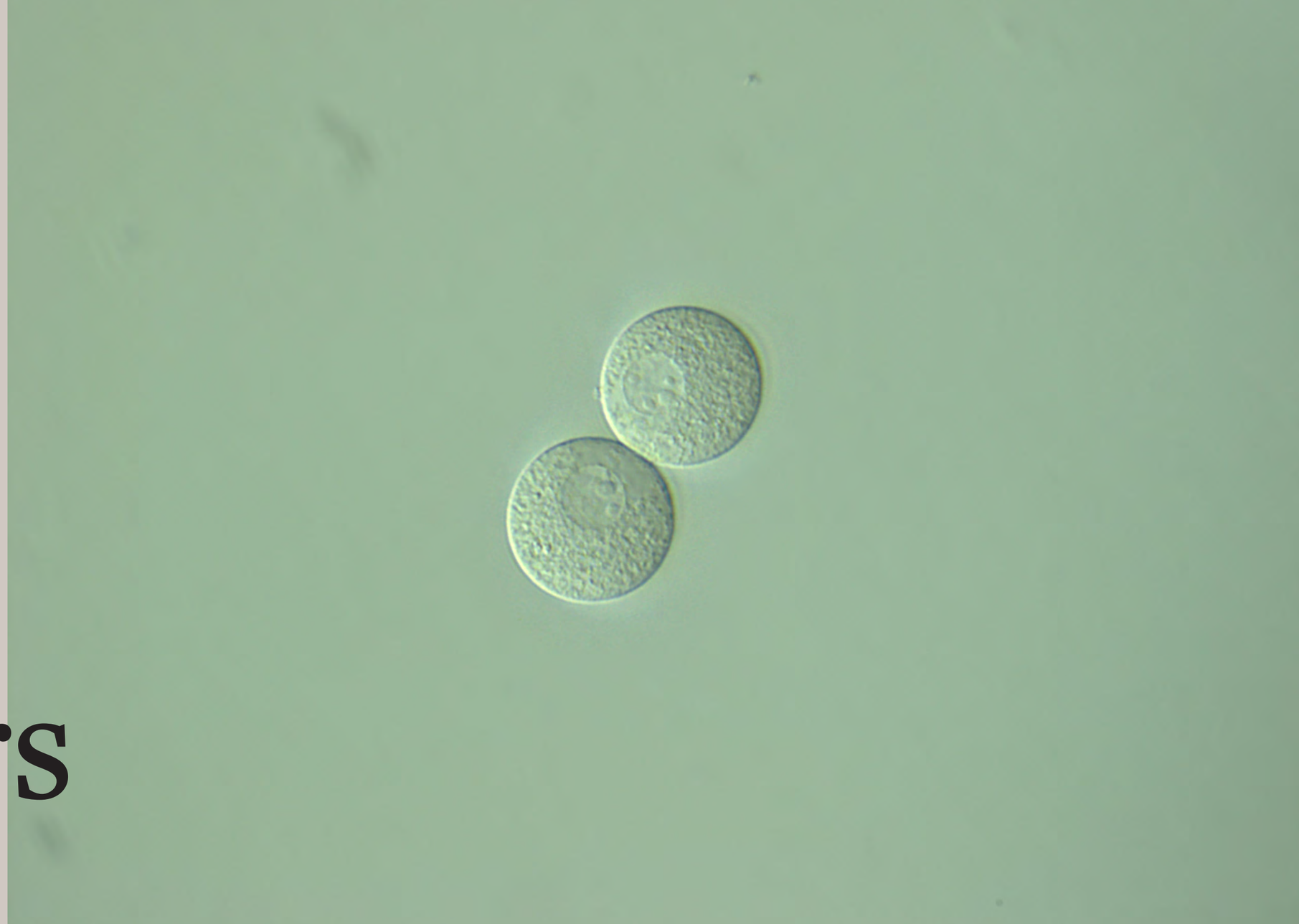
BioLamina
REVOLUTIONIZING CELL CULTURE

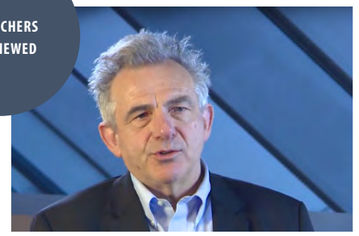
This text is based on interviews with leading stem cell researchers, pioneers within pluripotent stem cell research. Those who have been along for the entire ride.

hESC 20 Years

A trip down memory lane



RESEARCHERS INTERVIEWED



MARTIN F. PERA

Ph.D., PROFESSOR AT THE JACKSON LABORATORY, BAR HARBOUR, MAINE, USA

Professor Martin F. Pera is a pioneer and leading stem cell researcher with interests in neuroscience and regenerative medicine. His laboratory at Monash University was the second in the world to isolate embryonic stem cells from the human blastocyst, and the first to describe their differentiation into somatic cells *in vitro*. His work on neural differentiation of human pluripotent stem cells helped lead to the development of a new treatment for macular degeneration, which is now in clinical trials in Israel. Prof. Pera joined the Jackson Laboratory in 2017 where his research focuses on the biology and regulation of pluripotency and the genetic basis of individual differences in the response of the central nervous system to injury.

TENNEILLE LUDWIG

Ph.D., DIRECTOR AT WICELL STEM CELL BANK IN MADISON, WISCONSIN, USA

Dr. Tenneille Ludwig is the Director of the WiCell Stem Cell Bank overseeing the banking, distribution, and operation of the core facility at WiCell. Between 2001-2007 she worked in Dr. James Thomson's laboratory where her work on the optimization of cell culture conditions resulted in the development of the first defined, feeder-independent culture system for human embryonic stem cells (TeSR/mTeSR). Dr. Ludwig is a member of the Stem Cell and Regenerative Medicine Center at the University of Wisconsin and has served as a Scientific Advisor to multiple boards. She serves on the steering committee for the International Stem Cell Banking Forum (ISCBF) and operates one of the Core Laboratories for the International Stem Cell Initiative (ISCI).

OUTI HOVATTA

Ph.D., PROFESSOR EMERITA IN OBSTETRICS AND GYNECOLOGY, ESPECIALLY ASSISTED REPRODUCTION, KAROLINSKA INSTITUTET, SWEDEN

Professor emerita Outi Hovatta has been researching fertility for nearly 40 years. She was the first to apply for an ethical license to derive and grow embryonic stem cells in Sweden. Her group in Stockholm is also among the foremost in the world when it comes to developing completely clean lines that can be used for future treatments. Prof. em. Hovatta has helped countless infertile couples and derived over 30 different human embryonic stem cell lines.

BENJAMIN REUBINOFF

M.D., Ph.D., PROFESSOR, HADASSAH UNIVERSITY MEDICAL CENTER, JERUSALEM, ISRAEL

Professor Reubinoff is one of the pioneers of human embryonic stem cell (hESC) research. In collaboration with scientists from Monash University in Melbourne and the National University of Singapore, he was the second in the world to derive human embryonic stem cell (hESC) lines and was the first to show somatic differentiation of the hESCs in culture. Prof. Reubinoff is the founder and director of the Sidney and Judy Swartz Human Embryonic Stem Cell Research Center and a full professor and chairman of the Department of Obstetrics and Gynecology at the Hadassah University Medical Center in Jerusalem. The focus of Prof. Reubinoff's research is the exploitation of human embryonic stem cells (hESCs) in regenerative medicine for the treatment of neural and retinal degenerative disorders. He is the founder of Cell Cure Neurosciences Ltd. and has been the Chief Scientific Officer since 2006. He is also a member of the Scientific Advisory Board at Kadimastem Ltd. His work on human pluripotent stem cells helped lead to the development of programs that are now in clinical trials in Israel.

PETER ANDREWS

B.SC., MBA, DPhil, ARTHUR JACKSON PROFESSOR OF BIOMEDICAL SCIENCE, UNIVERSITY OF SHEFFIELD, UK

Professor Peter Andrews has devoted his research career to studying the biology of human embryonic stem (ES) cells and their malignant counterparts, embryonal carcinoma (EC) cells. Prof. Andrews was the first scientist in the UK to work with human ES cells, following the first derivation in 1998. Prof. Andrews' laboratory studies the causes and consequences of the non-random genetic abnormalities observed in human ES cells after prolonged culture, as well as the progression of stem cell-based cancers. Further work is focused on using induced pluripotent stem (iPS) cell techniques to establish models to study pediatric cancers. Prof. Andrews was a co-founder and director of Axordia Ltd., one of the UK's leading hESC companies (now a subsidiary of Pfizer) and has been involved in the derivation of several clinical grade hESC lines (the Sheffield lines), deposited in the UK Stem Cell Bank. Prof. Andrews coordinated the International Stem Cell Initiative and was the director of the Pluripotent Stem Cell Platform, a hub under the UKRMP. He is also on the editorial board of several stem cell journals.

PLURIPOTENT STEM CELLS

Pluripotent stem cells have virtually unlimited capacity to self-renew and have the potential to generate any type of tissue in the body. There are two different types of pluripotent stem cells: human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs).

hESCs are derived from the inner cell mass of the early embryo, from eggs that have been fertilized *in vitro* in an *in vitro* fertilization clinic and donated for research purposes, with the informed consent of the donors. The hESC are a powerful resource for the investigation of human developmental biology, for toxicity testing as well as for drug development. The immunogenicity and tumorigenicity are issues that need to be addressed.

iPSCs are artificially created pluripotent cells, derived from adult cells, that have been genetically reprogrammed to an embryonic stem cell-like state. The iPSC can be created from the tissue of the same patient that will receive the transplantation, thus avoiding immune rejection and are considered less ethically controversial. These patient-specific cells also provide an effective tool for genetic disease modeling. However, many problems still must be addressed, such as their potential to form tumors after transplantation and the low reprogramming efficiency of the technology.

HOW IT ALL STARTED

In November 1998, the news of an extraordinary discovery was broadcasted; researchers had been able to isolate stem cells from human embryos (Thomson et al. 1998). These human embryonic stem cells (hESCs) became world news due to their virtually unlimited capacity to self-renew and their potential to generate any type of tissue. This gave high hopes of finding new ways to treat or cure many diseases for which there are currently insufficient or nonexistent treatment alternatives, such as diabetes, cardiovascular diseases, and neurodegenerative diseases. These hopes were further enhanced by the generation of induced pluripotent stem cells (iPSCs), first from mouse fibroblasts in 2006 (Takahashi K. and Yamanaka S., 2006) and then from human fibroblasts in 2007 (Takahashi K. et al., 2007).

It was the development of mouse ES cells in 1981 (Evans and Kaufman, 1981; Martin, 1981) that provided much of the technology that enabled the development of human ES cells. The mouse ES cells, in turn, developed from previous, thorough studies of embryonal carcinoma (EC) cells, the stem cells of teratocarcinomas, tumors that arise in the gonads.

The teratocarcinoma research field boomed during the 1970s. “I think, for me, it started back in late ‘60s early ‘70s following Dr. Stevens’ work on the 129 mouse and the teratomas,” says Peter Andrews, Arthur Jackson Professor of Biomedical Science, University of Sheffield, UK. He continues, “Stevens noticed that the primordial germ cells that gave rise to teratomas looked a lot like the cells of considerably earlier embryos and that they could differentiate. Because these cells could give rise to cancerous as well as normal cells, they became known as embryonal carcinoma, or EC cells. A lot of developmental biologists got interested in teratomas as a route of looking into the mechanisms of embryonic development. There were various groups around that got hold of tumors and were growing out cell lines, trying to define what mouse embryonal carcinoma (EC) cells were and trying to define pluripotency.”

Peter Andrews continues, “I moved to the Wistar institute in ‘78 to work with Barbara Knowles and Davor Solter. That is really when I got hooked. Barbara and Davor had made one of the first monoclonal antibodies to recognize the key cell surface antigen, SSEA-1, which is expressed by both mouse ES and EC cells. People were starting to think that if you could get information on mouse embryos from looking at mouse teratocarcinomas, maybe you could get information on human development by looking at human teratocarcinoma cell lines. There had also been a couple of papers published describing cell lines derived from human teratocarcinomas. These expressed an antigen known as the F9-antigen, related to SSEA-1, so everyone thought they had found human EC cells. So, I really got involved in the human pluripotent stem cell activity in the late ‘70s, trying to identify human EC cells, what they were and if we could get a pluripotent one which would differentiate. I think it was Martin Pera and I who were the two people who made their career on it.”

“I have always been interested in human development and developmental tumors,” says Martin Pera, Professor at the Jackson Laboratory, Maine, USA. “Back in my early research career, I had an opportunity to join a clinical unit at the Royal Marsden Hospital in the UK. They had, and still have, the biggest testicular cancer clinic in Europe. So, we established cell lines from those cells which were pluripotent, and we studied the biology of those pluripotent cancer stem cells because we thought they provided a striking example of the fascinating connection between cancer cell malignancy, proliferation, and differentiation,” Martin Pera explains.

It was known that human EC cells had limitations, such as chromosomal abnormalities, and that they had limited ability to spontaneously differentiate into different somatic tissue types. >>

HIGHLIGHTED ARTICLES

Embryonic stem cell lines derived from human blastocysts.
Thomson J.A. et al. *Science, 1998. doi: 10.1126/science.282.5391.1145*
The first report of the isolation and cultivation of human blastocyst-derived stem cells.

Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.
Takahashi K., Yamanaka S. *Cell. 2006. doi: 10.1016/j.cell.2006.07.024*
First iPSC article in which the authors demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only four factors, Oct3/4, Sox2, c-Myc, and Klf4.

> "In the early '80s, just after Louise Brown was born and IVF clinics were started, we and others were wondering, 'What's the possibility of getting human embryos and deriving human ES cell lines?'"
>
> Peter Andrews

Moreover, the human EC cells were rather different in phenotype from mouse ES cells. When it became clear that teratocarcinomas could be induced by grafting blastocysts to ectopic sites, researches reasoned that it might be possible to derive pluripotent cell lines directly from blastocysts rather than from tumors. That is the work that Gail Martin and Martin Evans indecently published in 1981, the derivation and culture of mice embryonic stem cells in a laboratory. Could the same be done with human embryos?

"In the early '80s, just after Louise Brown (the world's first "test-tube baby") was born and IVF clinics were started, we and others were wondering, 'What's the possibility of getting human embryos and deriving human ES cell lines?'" Peter Andrews explains. He continues, "At the time, there was very limited access to human embryos, it was just logistically difficult. When I moved to Sheffield in '92, Harry Moore joined the university at the same time. He came from an IVF background with interests in reproduction. And so, we started talking and trying to work with local clinicians here to see whether we could access human embryos, with the idea of deriving ES cells. Just, we couldn't get it together. No-one managed to do it until Jamie (James Thomson) did, and that really set the ball rolling."

In 1995, James Thomson's team derived primate ES cells from rhesus monkey blastocysts (Thomson et al., 1995). "Jamie Thomson was a Ph.D. student with Davor in Wistar when I was there and he later ended up in the primate center in Wisconsin, with access to primates and an interest in early development. That access to the monkey embryos allowed him to derive the monkey ES cells, which was then the key to him getting access to some human embryos. So, he tried what he had done with the monkey embryos on the human ones and derived his human ES lines," says Peter Andrews.

Martin Pera explains, "Jamie Thomson's most important paper was not actually his human cell paper. It was his paper a few years before in which he describes stem cells from the rhesus monkey. Strangely, I don't think anyone much noticed the monkey paper. When we read about that monkey study, we realized immediately that these cells were very similar to our human embryonal carcinoma cells and we thought, we will be able to do derive normal ES cells in the human. However, access to embryos was a bottleneck, and we could not get ahold of enough embryos of quality. We also tried to make cell lines from primordial germ cells, and really didn't get anywhere with that. So, when Alan Trounson offered me the opportunity to come down to Australia, we decided to have another go at deriving human ES cell lines. Alan had gone on a sabbatical with Ariff Bongso in Singapore, an outstanding IVF embryologist. So, I went to Australia, joined Alan's Institute, and managed to get things off the ground with Alan, Benjamin Reubinoff and Ariff."

THE FIRST HESC LINES ARE BORN

James Thomson and his colleagues at the University of Wisconsin-Madison became the first researchers to report the isolation and cultivation of human embryonic stem cells. To most people this was breaking news, but for the researchers already working in the stem cell field, it was the vital next step in exploring the potential of stem cell science. It had been a tight race, and other researchers had been working effectively in parallel to their colleagues in Wisconsin. Drs. Martin Pera, Benjamin Reubinoff and Alan Trounson had been working for several years to see if they could make embryonic stem cells from donated human embryos, and their research group at Monash University (Australia) was the second in the world to isolate embryonic stem cells from the human blastocyst, and they were first to describe their differentiation into somatic cells *in vitro*. "We actually had a human embryonic stem cell line ready when Jamie published. I always tell people if you have to be second at something, it might as well be something like embryonic stem cells," says Martin Pera.

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What do you think has been the biggest breakthrough during these last 20 years of stem cell research?

TENNEILLE LUDWIG, Ph.D. DIRECTOR AT WICELL STEM CELL BANK IN MADISON, WISCONSIN, USA:

"I would love to say that it is the advent of xeno-free, feeder-independent media. It's not, but I hope that that helped enable some of the other work, and I hope that it will make some difference to the work people do in the future.

It's got to be first iPSCs, right? It allows the development of disease models which may make a tremendous difference, and philosophically and politically it solves a potential ethical dilemma. Then behind that, the more recent development, CRISPR/Cas, to be able to edit and modify. Although all the little incremental steps that got to those places have to be recognized, but for major breakthroughs, it's got to be iPS and then new genetic technologies."

Read the full transcript of the interview here:

What do you think has been the biggest breakthrough during these last 20 years of stem cell research?

JO MOUNTFORD, Ph.D. PROFESSOR AND HEAD OF CELLULAR THERAPEUTICS AT THE SNBTS JACK COPLAND CENTRE IN EDINBURGH, UK

"I'm sure you're going to hear this from everybody. It really was Yamanaka. That work was just astounding. It seemed like alchemy and I really wasn't sure that it was going to be true. The fact that they're already doing trials with these cells in Japan is amazing. So, yeah, for the iPSC particularly I think that was game changing because this whole ethical issue with ES cells was still big. At the same time there would often be somebody with a religious take on a situation – endless debate about this – and countries where it wasn't possible. So no, I think iPSC was as earthshattering as it seems, still. But then, the thing in the last 20 years that's probably surprised me the most was when bone marrow or cord blood stem cells seemed to become a panacea for all diseases. The idea that poorly defined "stem cell" preparations from these tissues would directly rebuild another different tissue when placed at that site. There is no solid scientific basis for this, clinical trials in heart attack have shown it to be safe but there is little evidence of persistent efficacy, but these kinds of stem cell therapies are still touted by many unregulated clinics all around the world for a huge variety of disorders."

Read the full transcript of the interview here:

Benjamin Reubinoff, Professor at Hadassah University Medical Center in Israel, also shares his thoughts from that time. "Back in the 90's, I was looking for a topic for a fellowship period, something which would have broad horizons beyond the area of infertility. So, I approached Alan Trounson, a well-known embryologist and *in vitro* fertilization (IVF) expert from Monash University, with vast expertise in stem cell research. He offered me to work on the derivation of hESCs. I was very excited about the project because of its potential for applications in regenerative therapy, but it was a risky project because there had been attempts to derive human embryonic stem cells which had not been successful by that time. Nevertheless, I decided that I would devote myself to deriving human embryonic stem cells. We (myself, Martin Pera, Alan Trounson, and Ariff Bongso) were the second group in the world to derive human embryonic stem cells."

He continues, "We were working together in Australia at that time but since there were ethical constraints to perform any research on human embryos in Australia, we had to perform the initial derivations in Singapore. So actually, after the first derivation of human embryonic stem cells, which I did in Singapore, I flew back to Australia with the isolated ICM in a small flask in my pocket. These were the cells from which our first human embryonic stem cell line was derived and established." Professor Reubinoff laughs at the memory. "It was a very exciting adventure, I must say! The story was covered within a review on the contribution of Israeli scientists to the field of human embryonic stem cells in Science Magazine."

Outi Hovatta, Professor emerita in obstetrics and gynecology, especially assisted reproduction at Karolinska Institutet in Sweden, was chief physician at the fertility unit at Karolinska University Hospital

"It was a very exciting adventure, I must say!"

Benjamin Reubinoff

Huddinge in Stockholm when the news about the first hESC line was published. "I had good access to embryos that were left over from in vitro fertilization treatments," says Outi Hovatta. "So, when we saw that it was possible to grow embryonic stem cells, I applied for an ethical permit and became the first researcher in Sweden to derive hES cells from donated IVF embryos."

Peter Andrews says, "For me, it was quite comforting when Jamie derived his human ES cell lines that actually they expressed a pattern of antigens that we had described in human EC cells, being different from mouse EC and ES cells. So, a lot of the markers that we had originally characterized, for characterizing human EC cells, turned out to be the ones that everyone uses today for characterizing human ES cells. Everyone looks back at EC cells and the clinical pathology of germ-cell tumors in the pursuit of trying to understand whether ES cells can be dangerous or not. We keep referring back to EC cells to try to understand whether particular genetic changes may be significant in terms of tumorigenicity, and whether they might be good, bad or indifferent. So, I think the two cell types have always gone hand-in-hand, but it's probably how I grew up and how my own career developed that I've always had this considered the close link between the two."

ENABLING STEM CELL RESEARCH

Recognizing the potential of Dr. James Thomson's human embryonic stem cells and being aware that regulations surrounding their use in a university setting were unclear, the Wisconsin Alumni Research Foundation established WiCell in 1999 as a haven for the advancement of stem cell research in the politically charged environment of the time. WiCell is a nonprofit, supporting organization of the University of Wisconsin–Madison and is a global leader in cell banking, characterization, testing, and distribution of stem cell lines. The organization also provides clinical grade pluripotent stem cell lines, quality control testing, and cell banking services.

"I lead the WiCell Stem Cell Bank portion of WiCell, and I see it as more of a service position," says Tenneille Ludwig, Director of the WiCell Stem Cell Bank, Madison, USA. "I spend a lot of time working with people at the university and other people in the general scientific community trying to find out, 'What do you need? What cell lines do you need? What characterization do you need? How can we help you get where you're going? What can I do to make your job easier?'" Tenneille Ludwig explains. She continues, "I'm actually very surprised by the amount of routine characterization that doesn't happen in many laboratories. Thirty percent of the >>

materials we receive from investigators, that they tell us that they have screened, that they believe is high quality material, has a significant problem. We see a wide variety of issues, from failure to thaw, to contamination, misidentification, mycoplasma and other terrifying things. Mycoplasma, karyotype, and STR screening, in my opinion, should be part of routine maintenance for lab cultures. So, based on what we are seeing, even in good quality labs from well-known institutions, if you're getting your materials from a colleague or researcher down the hall and not characterizing them before use, there is at least a 30% chance you don't really know what you're working with, and it could significantly impact your research or results. Yeah, it's scary. People need to know."

She continues, "To use a defined and animal component-free culture system creates a cleaner platform for research and ultimately clinical translation. Any time you're using feeders or serum, you've got a biological product that's going to be highly variable lot to lot, batch to batch that's going to affect research. You have more confidence that the differences that you're seeing are due to your treatment and not a random effect of a variability in a biological product."

> "To have generated and curated a collection this vast with the high level of quality we have is something I'm very proud of."
>
> Tenneille Ludwig

When I started, unlike now, Jamie's lab and WiCell were very interconnected," says Dr. Ludwig. "It was just off campus in a little residential area. The room itself was terrible – it was like a bunker. Only small windows, way up high, so practically no natural light and no way to see what's going on outside. But it was close to campus, easily accessible to the research community, and fully outfitted with all the equipment you needed, with plenty of space for all the researchers that wanted to try this new technology. So, as rough as it was, it was a gift, and it was the place to be.

"WiCell then opened another lab at University Research Park off campus to teach the education course, and bank and distribute cell lines, and my group migrated along. After a few years, Jamie asked if I would consider working directly for WiCell, and I made a sort of a seamless slide into WiCell at that point. The job has changed over the years, from more of a research position to more of a service position. We've moved from having 21 lines available to more than 1,400 at the moment, with more arriving all the time. To have generated and curated a collection this vast with the high level of quality we have is something I'm very proud of. And with the additional services we've grown to offer – contract banking, quality control testing – we've come a long way in the last 20 years. As much as I loved the active research, I love what I'm doing now. It's a really nice environment to work in. This is the job I hope to retire from," Tenneille Ludwig says with a smile.

AIMING FOR REGENERATIVE MEDICINE

It is only 20 years after the first report of the growth of human ES cells *in vitro* and just 10 years after discovering that somatic cells can be reprogrammed to pluripotency as iPS cells, and it's quite remarkable that clinical trials of human PSC-derived cells are already under way for conditions, such as diabetes, Parkinson's disease, and heart disease.

"When Jamie Thomson published his paper on human embryonic stem cells, the degree of attention that that attracted was enormous," remembers Martin Pera. "But you should know that the idea and interest in the regenerative medicine applications of stem cells were there already from before Jamie's publication. There were even some clinical trials of cells made from teratocarcinoma stem cells. It seems crazy in retrospect, but people did it. No harm came from it, but no good came from it. Anyway, so that notion was already there."

I remember Jamie was thinking in terms of more defined systems early on," says Tenneille Ludwig. "He instantly recognized that defined media formulations would be essential to the progression toward clinical application. So, we developed TeSR and the thing that Jamie did was that he published the whole formulation, and step-by-step directions for how to make it. A lot of people were surprised by that at the time, but Jamie's real focus with the media work was to enable research, to give the researchers the resources and the information to be able to advance the field. Clearly, I am biased, but I like TeSR and it makes me happy every time I see

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What do you think has been the biggest breakthrough during these last 20 years of stem cell research?

MARTIN F. PERA, Ph.D. PROFESSOR AT THE JACKSON LABORATORY, BAR HARBOUR, MAINE, USA:

"I would say the actual discovery of human ES cells. It had an impact that is hard to overstate. It opened peoples' minds to a whole different range of possibilities. And I would say it also triggered a lot of work on tissue stem cells in adults. If it were not for the discovery of IVF – which was an enormous breakthrough - we might not have produced human ES cell lines like we did and might not have been able to pursue it so quickly. The discovery of induction of pluripotency by defined factors is of course also a great achievement. Another huge development, without any question, is in the field of 3D organoids and co-cultures where I see enormous potential."

Read the full transcript of the interview here:

What do you think has been the biggest breakthrough during these last 20 years of stem cell research?

DAVID C. HAY, Ph.D., GROUP LEADER AND PROFESSOR OF TISSUE ENGINEERING, MRC CENTRE FOR REGENERATIVE MEDICINE, UNIVERSITY OF EDINBURGH, UK

"Yamanaka's discovery got to be up there. Fantastic! From the endodermal field point of view, I would say Kevin D'Amour's work finding activin made a huge difference. A real positive move for the field. Before, we were producing endoderm, but in relatively low amounts. With the use of activin you're going from 10–20% efficiency up to 80–90%, so we were very glad to jump on the back of that nice work by Kevin."

TILO KUNATH, Ph.D, GROUP LEADER AT MRC CENTRE FOR REGENERATIVE MEDICINE, UNIVERSITY OF EDINBURGH, UK

"In biology, things are usually easier in mouse models than in human cells, but in the case of neural induction from pluripotent cells, it's the opposite. A big surprise to me is one of Austin Smith's concepts, that mouse ES cells and human ES cells are in different states. Mouse ES cells are in a naïve state and human ES cells are in a primed state. For making neural cells, human ES cells get more efficient conversion and better purity of neurons. In terms of a practical application of the cells, the human cells are in an ideal state, because they are already in a primed state in comparison to ES cells from mouse. The 2006 Cell paper by Takahashi and Yamanaka was pretty significant as well."

Read the full transcript of the interview here:

> "Compared to working with feeder cells, it became so much easier to culture hESC on laminin substrates."
>
> Outi Hovatta

someone give a talk and mention that they used it in their research and I know it's working well for them," says Tenneille Ludwig and smiles.

Already from the start, professor emerita Outi Hovatta also had the idea to use the pluripotent stem cells for cell therapy applications. "That is why we wanted to get rid of all animal-derived components," says Outi Hovatta. "Most stem cell researchers were using mouse fibroblasts as feeder cells, but we chose to use human foreskin fibroblasts. However, feeder cells were quite hard to work with, and I was looking for a way to culture hESC without the need for feeders. My colleague, professor Karl Tryggvason, suggested that I test the recombinant laminin proteins that he was producing in his lab. So, we did a test, culturing hESC on laminins, and it was a great success. Compared to working with feeder cells, it became so much easier to culture hESC on laminin substrates. We got much more robust results, and we did not have to put time and effort into growing human fibroblasts from different donors. We have been using Biolaminin substrates since then, and I consider them essential for clinical research."

Benjamin Reubinoff was also working towards more controlled culture systems from an early stage. "We have been very focused and systematic from the start in our work on developing human embryonic stem cells suitable for clinical applications, and that has been a significant advantage. In the early days, the available cell lines were only of research grade, so the first step that we took was developing clinical grade human ESC lines. Progress that has been made along the years in understanding pluripotent stem cell biology and differentiation has really been important and helpful in developing the hESCs for clinical applications. It helped us to overcome manufacturing hurdles, such as growing on a larger scale in the GMP facility, and to better understand the biology of differentiation, and to be able to control it. Then, of course, it was a great challenge was getting pure populations of functional, differentiated cells. And it was crucial also to show safety and efficacy of the differentiated cells in animal models. Then there was, of course, a lot of work in developing all of the regulatory structures for application to regulatory agencies," says Benjamin Reubinoff.

Benjamin Reubinoff continues, "I am proud of the work we do at the Hadassah University Medical Center. We were the first to develop GMP-grade, xeno-free human embryonic cell lines suitable for clinical applications, with the vision that they would serve the academic and medical communities worldwide as starting materials for clinical transplantation applications. It's very rewarding to see that our cell lines are performing well and that multiple groups worldwide are successfully using them. And just the fact that our cell lines are >>

What do you think has been the biggest breakthrough during these last 20 years of stem cell research?

BENJAMIN REUBINOFF, M.D., Ph.D. PROFESSOR, HADASSAH UNIVERSITY MEDICAL CENTER, ISRAEL

"There really has been a tremendous advancement and monumental progress but it's hard to point out one particular achievement. I think that during these 20 years there have been many, many small steps forward where we have learned more and more about the biology of pluripotent stem cells and how to successfully control them. All of this information has helped make a big change, and if you look back, there has been a revolution in the way of culture of pluripotent stem cells, in our abilities to direct differentiation, and how we now are able to genetically modify them. We could hardly keep the undifferentiated cells ongoing in culture in the early days, up to the stage we are at today where we are taking our protocols through to clinical trials."

Read the full transcript of the interview here:

What do you think has been the biggest breakthrough during these last 20 years of stem cell research?

PETER ANDREWS, B.SC., MBA, D.Phil ARTHUR JACKSON PROFESSOR OF BIOMEDICAL SCIENCE, UNIVERSITY OF SHEFFIELD, UNITED KINGDOM

"Well, of course, the development of IPS cells is a major change. It changes the landscape a lot, from many points of view. There's always the logistics issue of getting access to human embryos. For a lot of the early cell lines, ES cell lines were only derived by groups who had easy access to human embryos. When the iPS cells came along it suddenly meant that almost anyone could do it, and it opened up the field to a lot more people. This of course caused some problems, I think, because these cells suddenly became an off-the-shelf tool resulting in, in some cases, less good quality control, and a focus on potential applications without considering some of the underlying basic biology. Another major thing is that it overcomes ethical issues with the ES cells. And, of course, there's the basic biology of it, which is, I think it blew everyone's mind that we could reprogram a somatic cell to an embryonic state just by over expressing four genes. No one expected that.

There's a lot of things which have surprised people along the way, and one thing is simply how quickly the whole field has moved. That after 20 years, there are clinical trials taking place. That is actually quite remarkable. It's still very early and I think we still have to be very cautious, but it's still remarkable."

Read the full transcript of the interview here:

> "I think the problem we still have is understanding the basic biology of the cells."
>
> Peter Andrews

already in clinical trials with two projects; for macular degeneration at Cell Cure Neurosciences Ltd. and for ALS at Kadimastem, that is amazing!" However, I must confess that, in the early days, when we derived the human embryonic stem cells, their utilization for clinical transplantation felt like a dream and not like something that would actually become a reality," says Benjamin Reubinoff.

Peter Andrews wasn't thinking about cell therapy applications either. "Back in my early days of stem cell research, I was focused purely on developmental biology. There was a pluripotent human EC cell line that I characterized, NTERA-2, which I published in 1984. It turned out that this line made neurons and group at the University of Pennsylvania who got hold of the cells developed further methods for purifying neurons from those cells, and actually did some trials with stroke patients. I don't think they were particularly sensible trials. Nothing good came out of it, but fortunately, nothing terrible happened either. They published that but I wasn't involved. That was probably the first clinical trial, for what it's worth, of derivatives of pluripotent cells. It hadn't really crossed my mind, until that point actually, that there was a potential in regenerative medicine."

THE BIGGEST HURDLES TO OVERCOME TO REACH THE CLINIC WITH PLURIPOTENT CELLS

To reach the clinical stage with pluripotent cells there are still hurdles to overcome.

"I think pluripotent stem cell therapies have the potential to be big, but I think it's going to take a long time," says Peter Andrews and continues, "In 20 years' time, I'm pretty sure that in some areas, for some diseases, it will be important. I think the problem we still have is understanding the basic biology of the cells. That's always my hang-up, as I am a basic biologist. How cells make decisions, how do you get the right sorts of cells out? And one of the big problems there is the relationship of these cells to the human embryo. Of course, we know an awful lot about mouse embryos at this point, but we don't know nearly as much about human embryos except that there's a lot of differences. Still, people try and squash the data about human ES cells into information that is from the mouse embryo and not the human embryo. There are now a few groups that are actually doing real embryology on early human embryos, which I think is really important to give us some better insight into what human ES cells correspond to and the mechanisms that control their differentiation."

Peter Andrews adds, "One of the big issues is how derivatives of these cells mature. I mean, a common observation is that the cells people were getting out of human ES cells tended to have immature properties as opposed to mature properties of whatever cells people were trying to make. Another issue is getting the correct cells from ES cells. I think Malin Parmar's work on getting the right sort of dopaminergic neuron is interesting. The discovery of two regions in the brain from which dopaminergic neurons behaved differently has allowed them to develop ways of treating the ES cells to get the right sort of dopaminergic neurons out so that they get better engraftment. That's really quite interesting and probably going to be reflected in what people do elsewhere later in other systems."

"I am optimistic that there will be ready-made, off-the-shelf products that will come from pluripotent stem cells, but it's not going to happen tomorrow," says Benjamin Reubinoff. He continues, "There is still a way to go. I think that the big challenge for the next 5 to 10 years will be to cross the barrier of phase II and III clinical trials. If proof of efficiency and therapeutic effects will be demonstrated in these clinical trials, the vision of off-the shelf product for regenerative therapy will become a reality."

Tenneille Ludwig shares her thoughts, "Depending on the treatment, the hurdles are going to be different, biologically or scientifically. The biggest hurdle that everybody's going to face is financial. The amount of money that it takes to go through the clinical trial process and the need for venture capitalist investors to see a short-term return on dollars makes the funding harder and harder to come by even when it's very promising. Tumorigenicity testing is another thing, we've got to come up with a better way to do that. Right now, having to do it in small and large animal models takes years and hundreds of thousands of dollars. So, if we could develop a predictive test that didn't involve large animal models, that would be a major breakthrough." •