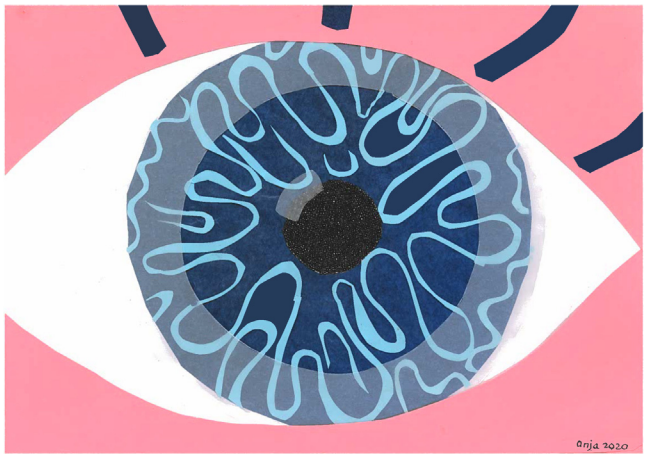
Chapter 16

Stem cell perspectives: a vision of the future



Chapter Outline

In this closing chapter, we will take a bird's eye view of some of the interesting new ideas currently in development that illustrate the diversity of applications that will be enabled by stem cell technology in the coming few years.

The first decade or so of research on stem cells since the first isolation of embryonic stem cells in 1998 revolutionized the way we think about regenerative medicine: the restoration of tissue and organ function by replacing its cellular components, not simply looking for drugs that heal. For cell biology

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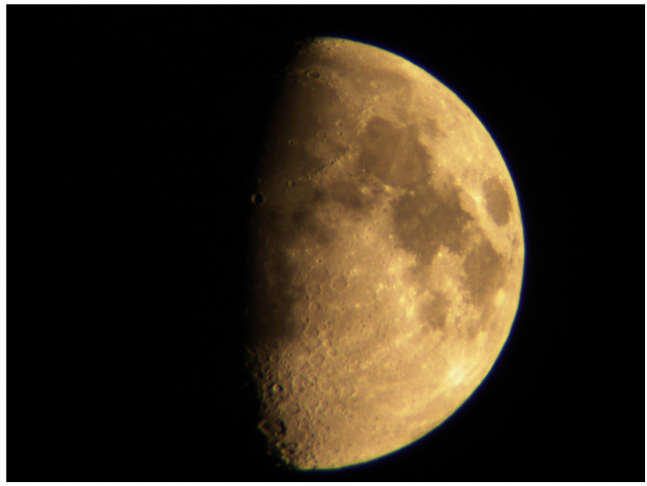

FIGURE 16.1 Stem cell research has been as challenging as the race to the moon.

this has been a little like "the race to the moon" was for engineering and computer science in the 1960s (Fig. 16.1). It has brought us to the eve of being able to treat a number of chronic, debilitating, and even fatal diseases in a permanent way. As we have discussed in this book, treatments for Parkinson's disease, diabetes, age-related- and other inherited forms of blindness, intestinal ulcers, and massive bone and cartilage damage may well be on the immediate horizon. Other treatments, for example, of the heart using cardiomyocytes from pluripotent stem cells, the inner ear for deafness, and liver or kidney may soon follow. Within a decade we will know from first clinical trials whether these diseases actually benefit from stem cell therapy. And we will learn so much from the new human disease models based on stem cells, that drugs will be available for diseases now chronic and untreatable.

But what else do we expect for the coming decade?

In such a rapidly developing field, this is of course difficult to predict. Who would have expected in 2007 that "therapeutic cloning" to generate patient matched embryonic stem cells would become obsolete, and within just 1 year be replaced by the concept of induced pluripotent stem cells? Or that adult stem cells would turn out not only to be identifiable directly in tissues but expandable in culture and on the verge of clinical application? Or that cells referred to widely as MSCs or mesenchymal stem cells would prove to have so few clinical applications with proven benefit even though they are safe. With such unexpected, not just rapid, advances how can we make any predictions for even the next couple of years, let alone a decade?

To begin with the pluripotent cells: three years after their generation in humans it is clear that human induced pluripotent stem cells are very similar to human embryonic cells, not only in terms of gene and protein expression but also imprinting. However, they might not quite be there yet in terms of therapy: their long-term stability during in vitro culture still requires some study. This is an issue high on the scientific agenda which we might expect to be solved in the coming few years most particularly because of huge investments in this area in Japan. Since being awarded the Nobel Prize, Shinya Yamanaka has changed the scientific horizon in Japan: whole institutes are devoted to research on induced pluripotent stem cells and much of the life science research budget is dedicated to keeping Japan at the forefront in this area, particularly with respect to stem cell therapy. Even the law in Japan has been changed to allow rapid entry into clinical trials although not all see this as a benefit given that there may still be associated risks. However, once we have human induced pluripotent stem cells as true equivalents of human embryonic stem cells, then it is likely that "banks" of these cells will be made as a resource for transplantation to patients with different HLA gene combinations. Japan will need a different bank for matching cells to their patients than China, and China a different bank from Europe or the United States, and so on. In addition, human induced pluripotent stem cells may be banked from individuals of mixed racial backgrounds for whom tissue matches are the most difficult to find. This is already the case for umbilical cord banks. An Asian father and African mother, for example, is a difficult combination to match. There may also be banking of cells from so-called "universal donors" who provide a match for a wider range of individuals. Or perhaps stem cells will become available that have been genetically modified such that they can escape notice of the patient's immune system. Differentiated cells from these "stealth" pluripotent stem cells could also be stored in banks and be readily available for immediate use.

How would these banks be used? For cell therapy, a clinician would have the HLA type of, say, a diabetic patient determined, but not know how to make the pancreas cells needed for treatment. This will remain a specialist activity. It is conceivable that specialist facilities will select the right HLA-matched cell line and produce properly differentiated cells in the required quantity under standardized GMP ("Good Medical Practice") conditions, which could be fully automated using robotics. Several biotechnology companies have been established exactly for this purpose. These differentiated cells would be delivered live or frozen to the hospital for administration to the patient. This will keep costs to a minimum, one of the major obstacles that need to be overcome before introducing cell therapies as part of standard patient care. Costs are the major reason that most scientists do not expect human induced pluripotent stem cell transplantation to be fully "individualized," meaning that it will probably not

be possible to generate a personal cell line for each patient that needs cell transplantation within a reasonable budget, even if in principle such lines could be produced. Hundreds of thousands of dollars as expected costs for the production of just one cell line under GMP conditions and the same amount again to produce the differentiated cells required for transplantation would be prohibitive for any health insurance plan and most individual budgets. Aside from use as a resource for transplantation, banks of induced pluripotent stem cells are likely to have their greatest potential in modeling diseases so that we learn more about what causes the disease in humans and how to treat it. In addition, in combination with whole genome sequencing and knowledge of the medical history and drug responses of patients from whom they are derived, it may well be possible to predict who is likely to get the disease (disease "predisposition") and whether there are any lifestyle factors which influence this probability. Prevention is always better than cure. Banks of induced pluripotent stem cells are already being established for this purpose in Japan, the United States (at the National Institutes of Health, the New York Stem Cell Foundation and the California Institute for Regenerative Medicine, among others), and the United Kingdom (by the Wellcome Trust and StemBANCC, an EU funded project). More will possibly follow. This will likely become an amazing source of information on health, disease, and longevity.

Transplantation with progenitor cells from postnatal tissues (adult stem cells) is likely to reach the clinic earlier than with pluripotent stem cells because the risk of forming tumors is lower. Questions of producing cells efficiently, at reasonable costs, and in sufficient numbers are issues for which there is partial overlap with pluripotent stem cells, although adult stem cells in general require less manipulation in culture. The two areas will nevertheless likely benefit from each other's expertise. Of the progenitor cells most likely to be in use for therapy soon, neural progenitors or progenitors in the eye are high on the list. It seems that neural stem cells have the ability to form derivatives that can migrate and extend neurites throughout the brain and central nervous system. Parkinson's disease is also a neural degenerative disease in which the last few years have seen real advances in producing the right nerve cells for transplantation; the start of a trial is on track for 2020 in New York, Japan started in 2019, and other sites in the UK and Sweden may follow as they learn from the experience there. Also making great advances as treatments in mice and now looking very promising is the use of the new stem cell types recently identified in the intestine. Ulcers healed with these intestinal stem cells and the first steps toward making this applicable to humans are underway. Regarding stem cells in other tissue types, the mammary gland, skin, hair, ear, sweat glands, sebaceous glands, pancreas, liver, etc., we will know in the coming years whether these cells can be expanded

from small numbers of cells in humans, either in the laboratory or in situ in the patients' own tissues and organs. And we will learn whether they have a clinical value in treating diseases of these organs and tissues. We will also learn whether the combination of gene therapy and stem or progenitor cells will work as a safe therapy: repairing the patient's defective gene and using his own repaired cells to treat the disease. We described one example of a serious skin disease treated by a combination of gene and stem cell therapy (Box 7.17). This approach is likely to expand in the coming years to be applied to more diseases, particularly as the methods for introducing genes into cells become safer and more precise so that other important genes that control cell behavior are not affected.

There is also increasing awareness that in many forms of cancer cells with a stem cell phenotype, called cancer stem cells, play a dominant role in disease progression and metastasis (see Chapter 12). It has proven possible to culture cancer stem cells from cancer tissue samples from many cancer types, including colon and breast cancer, for example, using the same organoid culture approach as used for healthy adult tissue and grow cancer organoids from a tissue sample of an individual patient. These are being explored for use in "personalized medicine" (see below) where a series of chemotherapeutic (or other anticancer) drugs are administered to the organoid cultures in different combinations and concentrations to see which is the best at killing the cancer cells or reducing cell division, and therefore which would be best to treat the patient with the tumor. However, a hurdle is that the real tumor in the patients consists of not only cancer cells, but also other cell types, such as fibroblasts and infiltrating immune cells, which modify the characteristics of the cancer cells, and influence their behavior and responsiveness to therapies. Creating an organoid-based tumor that really resembles the in vivo cancer of the individual patient will require additional research and is in progress. This sums up what we do expect from stem cell therapy in the coming decade, and at least some of the questions to which we expect an answer. It is important to realize that most new therapies of any sort (think of bone marrow transplantation to treat blood diseases, or monoclonal antibodies against cancer) in general take 30 years or more to become clinically useful and routine practice. Stem cell therapy in its recent form is in this respect still in its infancy.

Another question could be: what do we *not* expect of stem cells in therapy in the coming decade? This is always most difficult to speculate upon because scientific research often surprises us, but perhaps to mention a few of the more challenging diseases where expectations for stem cell therapy are lower: Alzheimer's disease, multiple sclerosis in adults, atherosclerotic heart disease and stroke. Reasons include challenges of cell delivery to the appropriate location in the body, inability to produce the required cell types, and the nature of the tissue destroyed.

Beyond direct cell therapy, we do have the exciting prospect of using stem cells (both from diseased and healthy individuals) for drug discovery: if we can model disease in a culture dish in the laboratory (not just cancer, but also many inherited diseases) we may be able to discover novel drugs, as necessary in combination, to slow down the rate at which a disease develops or to ameliorate the symptoms. This would ultimately also be of huge benefit to patients, particularly those with chronic progressive disease, since it may extend the period in which they live in relatively good health, prevent or slow down development of their most debilitating symptoms, or even reverse them after their onset.

Box 16.1 Human stem cell-based model systems to replace animal experiments?

Society increasingly voices reluctance to experiment on animals if there are alternatives, such as those based on cultured cells. Pluripotent and multipotent human stem cells offer opportunities to develop model systems for tissues very similar to those in the human body. The more these human tissues "in a dish" resemble *bona fide* human tissue, the greater the chance that they will be able to contribute to the "3Rs" in animal experiments (Replacement, Reduction, and Refinement) and become highly predictive models for drug discovery and safety pharmacology. As an example, tissue engineers in France, the Netherlands, and the United States synthesize model systems based on stem cells for skin which can be used in combination with inflammatory cells as alternatives to rabbit experiments to test chemical substances for skin irritation.

Combining technologies: new human disease models for drug discovery

Many diseases are typically human, so there may be no appropriate cell or animal models the pharmaceutical industry could use for developing and testing new drugs. With the rapidly developing technologies to culture and differentiate human pluripotent stem cells in a controlled manner, human disease models come within reach of pharma companies, eager to incorporate them into their drug development program for discovery and development of new drugs. Often though, cultured cells grown on standard tissue culture plastic, even if they are human, show very different responses to drugs than cells in a three-dimensional tissue composed of multiple cell types attached to a matrix protein scaffold. Tissue engineering, physics, microfluidics (technology to manipulate fluid flow in microchannels), nanotechnology, advanced microscopy, and microfabrication (organ-on-chip, the technology used to make "chips," electronic integrated circuits) are all techniques that will contribute to creating human tissue "look-alikes" in the laboratory in the

coming decade. This creates unprecedented opportunities for development of unique human disease models.

Tissue engineers in for instance Germany, the United States, Canada, and the Netherlands all have research groups very actively pursuing solutions to manufacturing human heart and vascular tissue. Not simply human heart cells derived from stem cells attached to the commonly used plastic surface, but mixtures of different heart cells layered and precisely aligned in patterned strips. In some cases, they are attached to flexible substrates that can undergo cyclic stretch and strain to mimic the mechanical environment of the beating heart, and even how it undergoes stress during physical exercise.

This assay system, and others like it for lung, liver, kidney, blood vessels, bone, and cartilage, for example, will likely be the new drug testing systems in the coming years. The Wyss Institute and its spinout Emulate in Boston and researchers at both Stanford University in the United States and Toronto in Canada are also making major investments and advances in these areas. In addition, model systems based on stem cells to investigate how drugs pass the blood–brain barrier, so that they can access the brain as their therapeutic site of action, are already under development. These exciting approaches could in the future be combined with "second generation" model systems based on human induced pluripotent stem cells, for example, from patients with relatively common and typically human diseases with a complex genetic background, like atherosclerosis and diabetes type II, but also, for example, ALS (amyotrophic lateral sclerosis) and some forms of Parkinson's disease. Induced pluripotent stem cell-based model systems are expected to provide an entirely new range of opportunities to understand how these human diseases develop and progress, and open a new era for drug target discovery and drug development.

Box 16.2 What could stem cells mean for future health?

A question often asked of stem cell researchers is will all of the research on stem cells lead to "immortality." Will we live forever? The consensus is probably not. If stem cell research is as successful as we hope it may be possible to repair vital organs repeatedly throughout a lifetime and, through knowledge of stem cell behavior, prevent damage from disease happening so quickly. The aim would be to prolong the period of health so that "healthy aging" allows people to spend more of their lives contributing to society rather than depending on it. Perhaps a good analogy are cars in Cuba: many huge American Chevrolets, Plymouths, Chryslers, etc. were imported during the heydays of the 1940s and 1950s. These cars still drive, the chassis is often in top condition but the engine? The original is long gone and usually replaced by another from Toyota, Kia, or Lada. Brilliant local engineering to replace the original engine with a completely different brand but it works fine. That is how we see stem cell and regenerative medicine

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at its best: the same old chassis, just new parts and a new motor! (Figure B16.2.1).

FIGURE B16.2.1 Regenerative medicine and the use of stem cells can be likened to a car in Cuba: old chassis, new engine. This classic model from 1949 is running perfectly well on a new Korean engine. *Photograph courtesy of Kelly Hosman.*

Personalized medicine and safer drugs

Many drugs have more or less serious side effects. However, not all patients receiving a drug for a specific disease will experience these side effects; in fact, they may be very rare. Likewise, drugs designed to treat specific diseases do not always benefit all patients. Of the 10 best-selling (most prescribed) drugs in the United States, some work in 1 in 4 patients, but others only in 1 in 20 (Fig. 16.2). This is an enormous waste of resources and frustration for patients and doctors follows. The reasons for this are often unclear, but there may be a genetic contribution, determined by the patient's own genetic makeup and predisposition. Drug prescription as a result is often necessarily empirical: "try this drug first and if it does not work, we'll prescribe another." Likewise, for side effects: "let's try another one—see whether that one is tolerated better."

It is currently not possible for pharmaceutical companies to determine individual risk of a side effect prior to starting clinical trials, since no model systems for screening toxicity of drugs are available that enable the investigation of the relationship between side effects and the particular variations in the genome of the patient.

However, the current trend in the development of novel drugs is based on the concept of "personalized treatment." This means that in the future, the

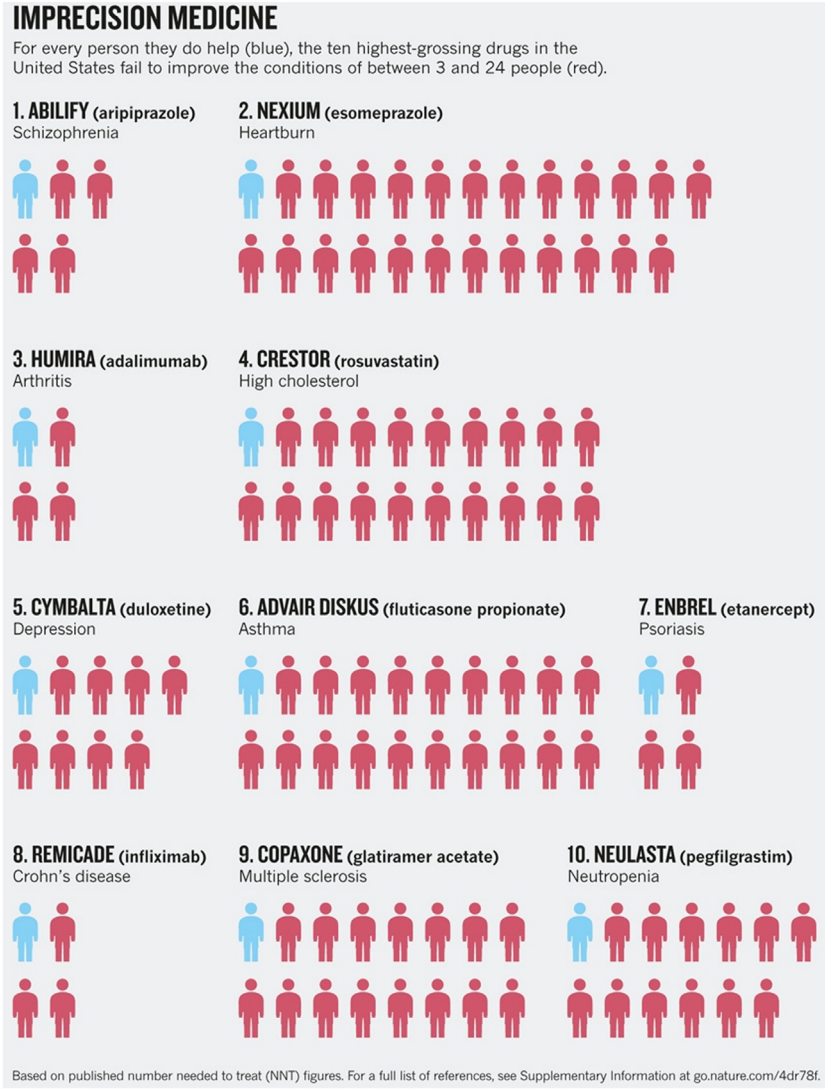

FIGURE 16.2 Although beneficial for some (blue), the 10 highest grossing drugs in the United States fail to improve the conditions of the majority (red) of patients. *Reproduced with permission from Schork, J., 2015. Time for one-person trials. Nature 520, 609–611.*

decision to treat a patient with a specific drug will become more dependent on whether the individual patient is expected to respond favorably to that drug. Toward this aim, diagnostic tests are being developed to predict the response of a patient to a drug prior to starting therapy, sometimes called "therapy response prediction tests" or, if they are directly coupled to a

specific drug, "companion diagnostic tests." Healthy and diseased tissue model systems based on human stem cells derived from patients with various genetic background, or having specific gene variations or mutations, which may potentially influence the response to a drug, may help pharmaceutical companies to already develop these companion diagnostic assays prior to starting clinical trials involving real patients. One could imagine building a collection ("library" or "bank") of genetically different human stem cell lines, with their genome (DNA) well-characterized using DNA sequencing, which as a whole could be to some extent representative for the human population, and use this as an "in vitro laboratory-based clinical trial." Cardiotoxicity (the side effects of a drug on the heart) can be used as an example to illustrate this concept. A side effect of many drug compounds is that they can block *ion channels* in heart cells, which increases the risk of life-threatening arrhythmias. Arrhythmias usually only occur in a very few patients and may be due to their specific genetic predisposition. It would be useful to have a test which could identify patients at risk for developing such an arrythmia, who would be excluded for use of the drug. For the purpose of developing such a test, a cell line-bank could be created of genetically identical pluripotent cell lines, in which only the genes coding for the proteins of the ion channels have been changed to represent the genetic variants found in the general population. The modified cell lines could be differentiated to

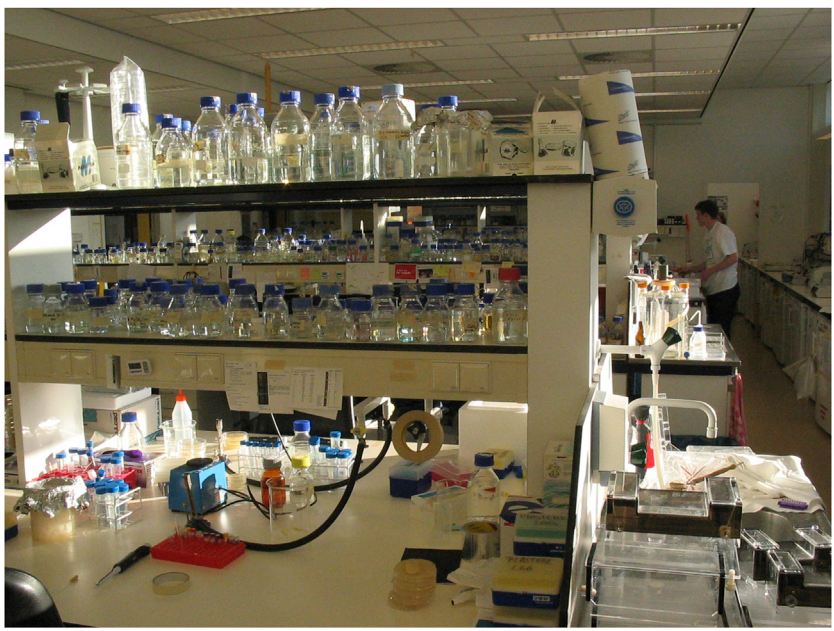

FIGURE 16.3 A lot of fundamental and applied research is still necessary to make full use of the potential of stem cells in health.

heart cells and used to screen for toxic side effects of a drug compound on the heart. Alternatively a cell line-bank could be composed of pluripotent cell lines of a large number of individuals, that together represent the relevant genetic variants found in the ion channel genes in the general population. If an association with a specific ion channel gene variant were found, a simple blood-based test to identify the genetic determinant could be developed to identify patients at risk, prior to administering the drug. This is already becoming a reality. Similar banks could be developed for a variety of cells and tissues to predict predisposition to toxic effects of drugs on liver, brain, blood, nerve; in fact, any cell type that can be made from a pluripotent cell (Fig. 16.3).

Box 16.3 Human organs in animals?

One possible new application of stem cells could be the growth of whole organs from stem cells inside the body of an animal. If this worked with human stem cells, these cultured organs could in principle be collected from the animals and transplanted into human patients.

For tissue that consists of one or only a few cell types it may be feasible to develop these outside the body from stem cells just simply in laboratory culture and use them for organ repair, but organs have much more complex structures and are composed of various cell types that need to be at the correct position for the organ to function. To create these outside the body would be extremely challenging. What scientists find virtually impossible in the laboratory, however, takes place routinely in developing embryos: embryos make organized tissues and organs all the time. For this reason, scientists are now exploring the possibilities using animals for the development of human organs from stem cells.

This is how it works: when pluripotent mouse stem cells are injected into a blastocyst stage mouse embryo, the cells can contribute in the formation of the developing fetus. If the embryo is transferred to the uterus, or womb, of a female mouse, a baby mouse will be born that is composed of two different cell types: cells derived from the original embryo and cells derived from the pluripotent cell types. Such an animal is called a chimera, a word that originates from Homer's Iliad where the Chimera refers to a ferocious fire-breathing animal that is partly lion, partly goat, and with a serpent as a tail. Japanese researchers then took this a step further. They injected rat pluripotent stem cells into a mouse blastocyst that was genetically modified in such a way that it could not produce a pancreas. The resulting embryos were transferred to the womb of a recipient female mouse, which carried the embryos and fetuses to term. The resulting pups were predominantly of mouse origin, with some rat cells in various organs, but the pancreas was entirely of rat origin, so completely formed from the injected rat stem cells. Similar experiments have resulted in mice with a rat thymus and pigs from one strain have been generated with a pancreas completely derived from a different pig strain. These experiments open the possibilities of more extreme engineering: could it be possible to develop and grow a human organ, say a

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pancreas, in a large animal, say a pig? For this one would need an embryo of a pig that cannot form a pancreas from its own cells, and human pluripotent stem cells. Cells of a pig that is genetically unable to form a pancreas could be used for cloning by somatic cell nuclear transfer (Chapter 6), resulting in embryos that because of the genetic engineering cannot form a pancreas. Simultaneously, skin cells could be derived from, say, a diabetic patient, with a defective pancreas function, and turned into pluripotent stem cells. These human stem cells could be injected into the pig embryo, and then the embryo transferred to a sow so that it could develop to term. The resulting piglet would have a human pancreas

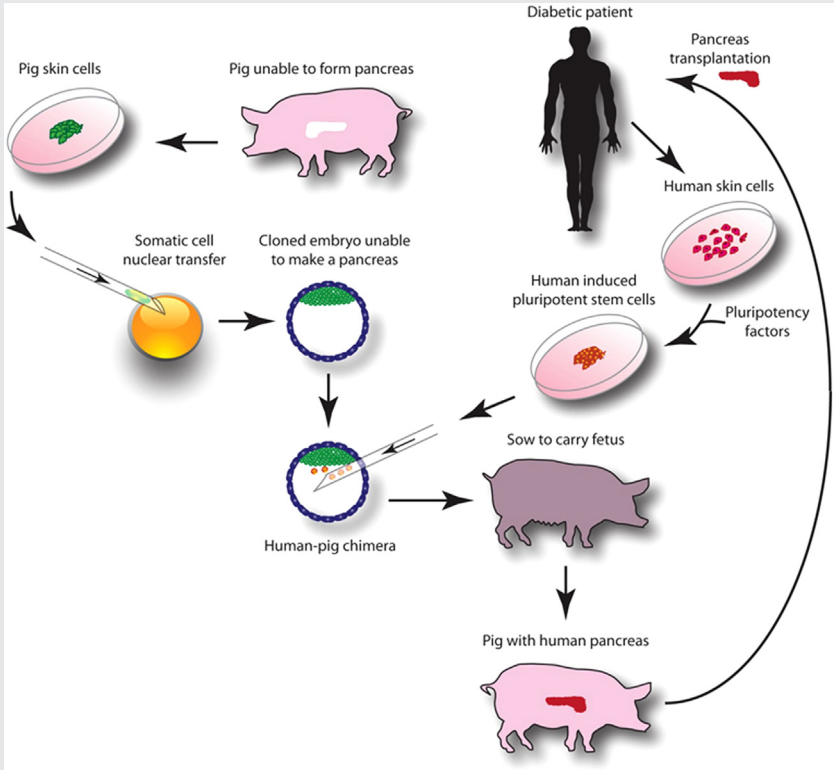

FIGURE B16.3.1 Skin cells from a pig genetically unable to form a pancreas are used for cloning by somatic cell nuclear transfer to make an embryo that is therefore unable to form a pancreas. Skin cells from a diabetic patient are used to make patient-specific induced pluripotent stem cells. These cells are injected into the pig embryo, where they are able to contribute to the formation of the fetus. The embryo is transferred into a carrier sow, in which the fetus will develop. Since the fetus cannot form a pancreas, the human cells will form this organ: a human organ in a pig. When the pig is born, it will have a human pancreas that is genetically identical to the patient and can be used for transplantation.

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that could be used for transplantation to the specific diabetic patient from which the stem cells were derived. Since the pancreas is made of cells that are genetically identical to the patient, the pancreas would be recognized as self by the immune system and not be rejected (Figure B16.3.1). One of the issues that would need to be solved if the whole pancreas were transplanted would be how to make the blood vessels be of human origin. If only the islets containing the insulin secreting cells beta-cells were transplanted to the patient though, this would not be a problem. For other organs, like kidneys, it would of course. Of note though, chimeras can actually occur in humans, albeit rarely, but in this case one individual originates from two individually fertilized eggs which fuse early in development, before implantation into the womb. These two embryos would normally have resulted in fraternal twins, but because they stick together, just one baby is born. When two "brothers" or "sisters" embryos fuse, this is usually not noticed throughout the entire life of the person and might only be discovered when they need medical treatment like a bone marrow transplant.

Since early embryos are sticky, embryos of two different species can even be fused artificially, something that would obviously never happen in nature. This has for instance been done with sheep and goat embryos. The resulting animals had characteristics of both sheep and goats.

Currently scientists, policy makers, and ethicists are discussing whether an animal with a human organ would be beneficial for the community and whether it is ethically and morally justifiable? Should we allow the breeding of animals with human organs? How much can this be controlled, or would this result in animals that are "humanized"? What happens when, for instance, a pig develops with human brain cells? Or how could we exclude that such tissues are formed? Is it acceptable to "instrumentalize" the use of animals?

All questions that need to be addressed before human organs in animals become reality even if the procedure is feasible.

Box 16.4 The "oids": blastoids and gastruloids.

A new concept: in addition to "organoids," we now have "blastoids" and "gastruloids." Clusters of cells that self-organize into structures that are similar to early embryos. All made from pluripotent stem cells.

Pluripotent stem cells have the capacity to form all the cell types of the fetus and adult. When allowed to differentiate freely in suspension cultures, these cells form disorganized aggregates containing all kinds of specialized cells and tissues, without "polarity" or axis formation. However, when derived from a mouse and introduced into an organized structure like a preimplantation mouse embryo, these cells can contribute to, or even entirely form, functional organs. They are said to be "patterned" by the embryonic and extraembryonic environment.

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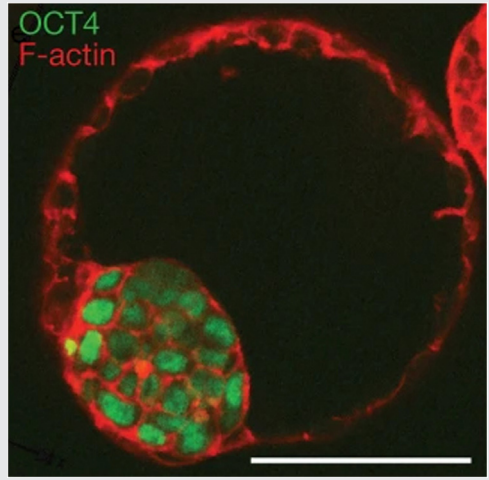

FIGURE B16.4.1 A blastocyst-like structure (blastoid) generated from stem cells. Green indicates pluripotent cells expressing OCT4. Red indicates cellular microfilaments. *Reproduced with permission from Rivron et al., 2018. Blastocyst-like structures generated solely from stem cells. Nature 557, 106–111.*

Regular pluripotent cells such as ES and iPS cells cannot contribute to extraembryonic structures such as the trophectoderm needed for implantation into the womb and formation of the placenta. ES and iPS cells therefore cannot alone form the whole "conceptus." However, if aggregates of mouse pluripotent stem cells are combined with mouse trophoblast stem cells in culture, they can develop into blastocyst-like structures that are now called "blastoids." Both in their morphology and protein expression these blastoids resemble real preimplantation mouse blastocysts (Figure B16.4.1). The inner cell mass-like structure however does not further turn into epiblast and form the fetus or into hypoblast (primitive endoderm) and form the yolk sac. In an attempt to promote further development, blastoids have been transferred to the uterus of mice, in the hope that they would implant and form a healthy fetus that would develop to birth. Although uteri that received blastoids responded similarly to uteri that received real blastocysts (swelling at the implantation site), advanced development of the blastoids did not occur and the structures disintegrated.

In parallel, attempts are being made to make more advanced (postimplantation) embryonic structures, again, in the first instance, with mouse pluripotent stem cells. In this case, mouse pluripotent stem cells are combined with trophectoderm stem cells but then allowed to form aggregates in a three-dimensional scaffold of extracellular matrix protein with physical properties of a gel. Under these conditions, the cells can self-assemble into an elongated structure typical

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of the early postimplantation mouse embryo, including the hollow structure (cavity) in the middle that is characteristic for these embryonic stages. Most importantly, provided that the structures were cultured in the presence of a suitable mixture of growth factors, mesodermal cells developed in these embryos, much as they do in gastrulating embryos after implantation in the uterus. Such structures were hence termed gastruloids. Even in the absence of extraembryonic cells it has been possible to generate mouse gastruloids that had already started to show left–right asymmetry and a rudimentary head–tail pattern could be identified.

This research field is developing rapidly and researchers have also generated blastoids from a new type of pluripotent stem cell, called "extended pluripotent stem cells," which in contrast to regular pluripotent stem cells can also differentiate to trophoblast cells. This has enabled the generation of blastoids from a single cell line, rather than the combination of two different cell lines (creating a chimaera). Blastoids from enhanced pluripotent stem cells form trophectoderm-like structures and cells that resembled hypoblast cells, although these did not form an epithelium covering the epiblast.

Research on human blastoids or gastruloids from human pluripotent stem cells is a logical next step and these studies are already on their way. A hurdle to overcome will be human stem cells that can develop to trophoblasts but this will be solved in the near future. The development of gastruloids from human embryonic stem cells has recently been successful. The ability to generate viable (early) embryos from stem cells would open a whole new field in terms of cell biology, especially to study events that occur around gastrulation. Human embryoids (blastoids and gastruloids) could help to elucidate events that occur at around implantation, a developmental period that due to inaccessibility is relatively unknown and the time in pregnancy that most miscarriages occur. Additionally, human embryoids could also enhance our understanding of cell specification and could help to drive pluripotent cells to form specific tissues that currently cannot be generated. However, these studies do raise new questions of ethics and law. Currently, human (preimplantation) embryos can only be maintained in vitro for a maximum of 14 days in culture in most countries, the moment that gastrulation (formation of brain and heart) normally takes place in development. The closer these artificial structures from stem cells resemble real embryos, the more likely this 14-day rule will limit this research. Should live mouse offspring be born from a blastoid, then we must assume that a human blastoid (when they are eventually made) would have the capacity to form a new individual. The research would then be classified as "creating embryos for research," again prohibited by law in many countries, even if the starting pluripotent stem cells are actually human iPS cells.

If embryo-like structures, embryoids, could be generated from human skin cells turned into iPS cells, in principle this would be similar to human cloning. But are these structures embryos? Again, critical in this discussion might be the potential to develop into a human being, or having the potential to develop

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beyond a certain stage, such as the primitive streak stage. Even if the embryos are not transplanted to the womb but only used for research, the capacity to form a fetus could be interpreted to mean that the moral status of these structures is the same as that of a human embryo or fetus and that the same ethical and regulatory boundaries apply.

Box 16.5 Human stem cell models to study viral infections like COVID-19.

Organoids from adults stem cells or human (induced) pluripotent stem cells (hiPSC) can reveal mechanisms of pathogenesis. hiPSC-based organoid technology in particular allows modeling of key aspects of human fetal development including that of liver, brain, and heart. During the 2015 Zika virus (ZIKV) epidemic, a strong association between ZIKV infections and severe congenital abnormalities was observed, most notably microcephaly (or underdeveloped/small brain), but postnatal infections did not affect the brain. A series of studies using human cerebral organoids ("minibrains") derived from hiPSC provided proof of causation: ZIKV can replicate in the developing brain and preferentially infects and kills neural precursors, leading to reduced cortical expansion and microcephaly, just as found in newborns where the mother had been infected. When hPSC-derived neural progenitor cells were used to screen for anti-ZIKV drugs, emricasan was identified as protecting the neural cells and two other compounds identified as inhibiting ZIKV replication.

This type of study is illustrative of what stem cell research can offer to virologists and clinicians in the study of infectious disease. Organoids can also be used to document species-specific differences in susceptibility. Avian H7N2 and swine H1N1 influenza viruses, for example, mainly infect birds and pigs, respectively, and are difficult to replicate in culture yet some influenza viruses—such as the pandemic 2009 H1N1 (H1N1pdm) strain—can rapidly spread through human populations. There is no robust in vitro model for assessing the infectivity of emerging flu viruses in humans except short-term bronchus explant cultures from surgical resection material. It turned out, however, that airway organoids could be infected with human and avian strains of influenza A virus and that they yielded results comparable with those in primary lung explants. As airway organoids can be expanded over years and can be frozen and stored, organoids are clearly useful to assess the pandemic threat of animal influenza viruses.

SARS-CoV-2 has caused the COVID-19 pandemic in 2020. There was clearly an urgent need for physiological models to study SARS-CoV-2 infection using human disease-relevant cells. COVID-19 pathophysiology includes respiratory failure but involves other organ systems including gut, liver, heart, and pancreas. Organoids based on human stem cells rapidly showed they have the properties needed to understand more about how the virus entered the cells, how it

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induced such a profound and sometimes fatal inflammatory response, and why tissues showed severe long-term damage like fibrosis and necrosis. Diabetics, for example, are highly sensitive to COVD19 infection and have a high mortality risk. Several studies have shown that human pancreatic beta cells and liver organoids are highly permissive to SARS-CoV-2 infection, just as observed in adult primary human islets and adult hepatocyte and cholangiocyte organoids. SARS-CoV-2 infection was also shown to cause striking expression of chemokines, as also seen in primary human COVID-19 pulmonary autopsies.

Many cell types can be included in organoids and ACE2, the putative receptor of SARS-CoV-2, is heterogeneously expressed in different cell types. However, some cells do not express ACE2 and it was a puzzle as to how the virus actually entered cells. Further research showed that there are at least two other receptors for SARS-CoV-2 (called neuropilin and CD147) and that these may be distributed differently in the body. Further, stem cell studies showed that androgen hormones could increase the expression of ACE2, perhaps explaining the greater susceptibility of men than women to COVID-19.

Taken together, these studies have shown hPSC-derived cells/organoids provide valuable models for understanding the cellular responses of human tissues to SARS-CoV-2 infection and for disease modeling of COVID-19. COVID19 is not the first pandemic causing so much morbidity and mortality and it is unlikely to be the last. The availability of human models based on stem cells may increase "research readiness" in combating these kinds diseases in the future.

A final note

Whilst regretting much of the hype and false hope surrounding stem cells and acknowledging the challenges ahead, many scientists share the conviction that human stem cell technology and greater understanding of stem cell biology will in the coming decades fulfill much of its promise and revolutionize medicine as we know it today, improving patient care without past precedent.