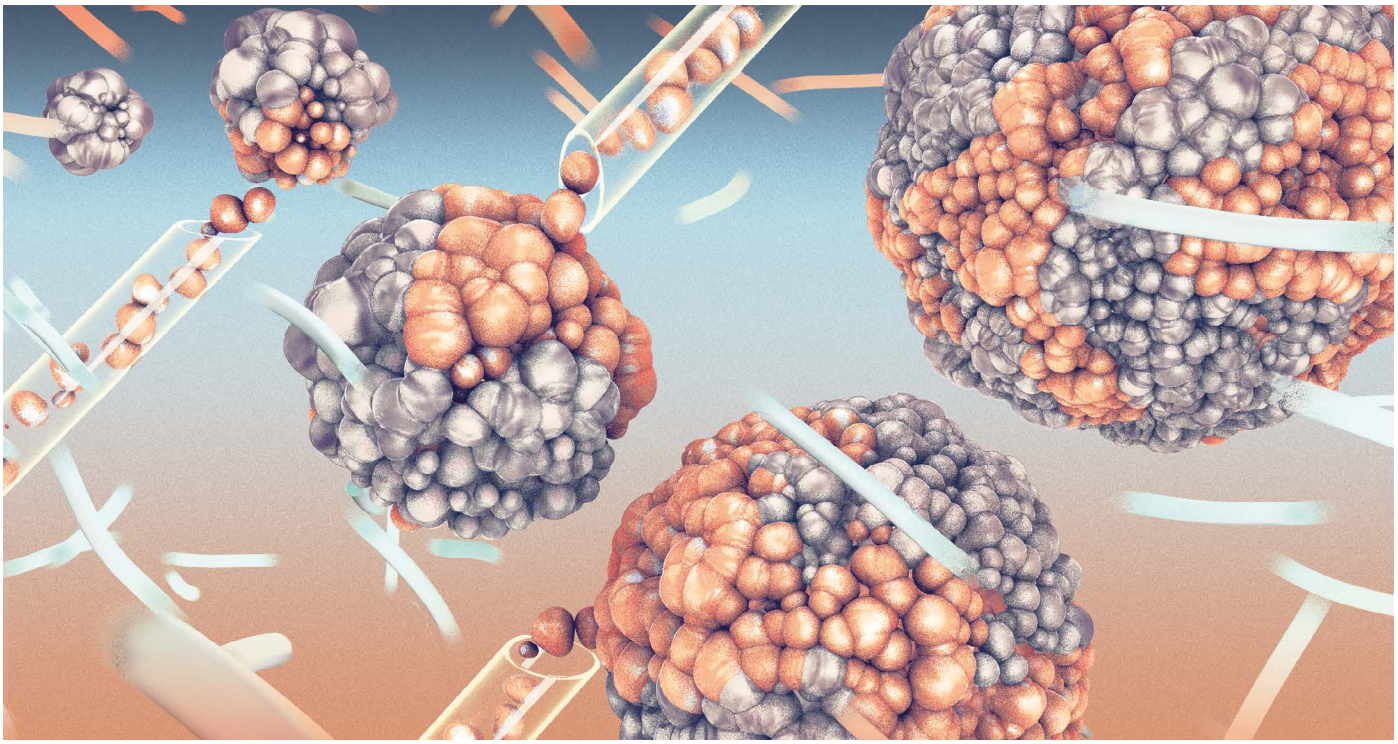

ILLUSTRATION BY MARKOS KAY

The chimaera challenge

Despite progress in growing human organs in animals, technical obstacles and ethical questions mean that they are still some way from being ready for transplant patients. **By Liam Drew**

It's 2036, and you have kidney failure. Until recently, this condition meant months or years of gruelling dialysis, while you hoped that a suitable donor would emerge to provide you with replacement kidneys. Today, thanks to a new technology, you're going to grow your own.

A technician collects a small sample of your blood or skin and takes it to a laboratory. There, the cells it contains are separated out, cultured and treated with various drugs. The procedure transforms the cells into induced pluripotent stem (iPS) cells, which, like the cells of an early embryo, are capable of generating any of the body's tissues.

Next, the technician selects a pig embryo that has been engineered to lack a gene required to grow kidneys, and injects your iPS cells into it. This embryo is implanted in a surrogate sow, where it develops into a young pig that has two kidneys consisting of your human cells. Eventually, these kidneys are transplanted into your body, massively extending your life expectancy.

This hypothetical pig is a chimaera: an animal composed of cells derived from more than one fertilized egg. The name comes from the part-lion, part-goat, part-serpent chimaera of ancient mythology. And, right now, a small number of scientists are working to make the above kidney scenario a reality.

Among them is Hiromitsu Nakauchi, a cell biologist at Stanford University in California. Trained as an immunologist, Nakauchi's research was initially on how to prevent transplants from being rejected by recipients' immune systems. But a simple realization made him change direction. "The major problem is not the immunological rejections," he says, "but the shortage of donor organs."

The World Health Organization estimates that only about 10% of the global need for organ transplants is now being met. In the United States, around 20 people die every day waiting for transplants. This situation is a main motivation — and the moral foundation — for pursuing chimaera research.

Nakauchi's early chimaera work was met with consternation. "I was a crazy scientist ten years ago," he says. Today, he thinks that the biomedical community broadly supports his efforts to produce clinically useful organs and tissues by melding human and animal cells. Nevertheless, as chimaera technologies advance, ethical questions continue to swirl — as indicated by responses to the report this April of the production of monkey–human chimeric embryos.

"The unique risk is the blending and blurring of the lines between human and animals," says Nita Farahany, an ethicist at Duke University in Durham, North Carolina. "We need to be looking at where the research is heading to ensure that it's conducted in a way that's ethically permissible — and that it doesn't exceed lines that we as a society wouldn't want to cross."

Making chimaeras

Scientists have grafted tissues from one species into another for centuries to improve biological understanding. Many people walking around today are able to do so only because

their hearts contain valves taken from sheep or cows. But interspecies stem-cell transplants are a new frontier. In some instances, these cells proliferate and integrate with the host species to yield what could be described as new types of animal.

Growing organs of one species inside another involves a technique called blastocyst complementation. A blastocyst is the tiny ball of cells formed by the first rounds of cell division after fertilization. From these pluripotent cells, every cell type of the mature organism emerges. If pluripotent cells from another member of the same species are added to a blastocyst, a creature develops that is made from a mixture of the two cell populations.

When stem cells from a different species are added to a blastocyst, how much they contribute to the mature animal seems to depend on the evolutionary distance between host and donor. Two closely related mouse species and two cow species have been shown to form chimaeras readily. Other species that also combine are sheep and goats, and rats and mice, albeit less well. In the case of rats and mice, donor cells typically contribute about 20% of adult cells.

Donor cells can also end up in diverse places in the resultant chimeric animals — they often mingle with host cells across many tissues, but tend to be more prevalent in some regions than others. When Nakauchi entered the field in the mid-2000s, he attempted to direct donor cells to specific tissues. In 2010, after finally recruiting two students willing to undertake the uncertain project, his laboratory produced a landmark paper describing the development of mice that had pancreases composed of rat cells[1].

The researchers' crucial intervention was to delete a host-mouse gene that is required to develop a pancreas. The pluripotent cells of a blastocyst generate the body's many tissue types by dividing and differentiating — through the expression of distinct genes — into various lineages of cells that each form particular tissues. In mammals, the pancreas is created from a lineage that uniquely expresses the gene *Pdx1*, so when Nakauchi's lab deleted this gene from mouse embryos, the mouse cells could no longer become pancreas cells.

Nakauchi's hope was that this would clear the way for donor rat cells to step in — and that's exactly what happened. A pancreas of rat cells (albeit with mouse-derived vasculature) grew to the normal dimensions of a mouse pancreas.

In 2017, Nakauchi showed that the reverse process also worked, growing mouse pancreases in rats[2]. Moreover, if pancreatic islets from these rat-grown mouse organs were transplanted into mice that had diabetes, they corrected the impaired blood-sugar control.

Nakauchi and others have since deleted genes that are needed for the development of kidneys, lungs, eyes, the liver and other organs in mice, and have shown that rat cells will also fill those niches. Achieving this outcome with pig hosts and human donor cells, however, will require more than this single tactic.

Human elements

In 2013, Nakauchi showed that deleting the *Pdx1* gene from pig embryos and supplementing them with stem cells from donor pigs produced pancreases made entirely of donor cells[3]. However, introducing human iPS cells into pig embryos has so far produced only very low levels of chimaera formation.

Refinement of the type of human iPS cell used has yielded small gains. But most researchers think that pigs and humans are so distantly related that there is a fundamental barrier to chimaera formation. The barrier is probably due to differences in the timing of development and in the signalling pathways that distantly related species use.

Some of the most successful efforts to overcome this barrier have so far come from a lab at the University of Minnesota in Minneapolis, where Mary Garry and her husband Dan Garry, a transplant cardiologist, are working on growing pigs that have human muscle, and pigs with human vasculature. The latter aim is crucial, because a human transplant recipient might reject an organ composed of their own cells if it contained porcine blood vessels.

Having deleted the respective genes that pig embryos need to develop these tissues, the Garrys injected human iPS cells that had been genetically modified in one of two ways to improve their survival. In one case, a tumour-suppressor gene that normally inhibits cell division was deleted; in the other, a gene that halts programmed cell death was overexpressed.

After gestation in surrogate pigs for 17–27 days, the chimeric embryos contained the beginnings of haematological and muscle tissues formed entirely of human cells.

Mary Garry acknowledges that these genetic alterations are not the solution to growing transplantable human organs in pigs, because the modifications could present major cancer risks for recipients. "The goal was really to see if we could enhance chimerism by taking kind of a sledgehammer approach," she says. The lab is now analysing the surviving human cells in the hope of developing subtler approaches to enhancing chimaera formation.

One potential alternative to modifying the human cells, which both Nakauchi and the Garrys are exploring, would be to genetically modify host cells in ways that put them at a growth disadvantage compared with donor cells. Another is to dig more deeply into the cell biology and signalling pathways that are at play when donor human cells do survive in the embryo of another species, to devise strategies for keeping them alive in animal species with which human cells form chimaeras less readily.

This was the rationale for the development of monkey–human embryos reported in April[4]. The international team of scientists that developed these chimaeras argued that if successful chimerism is more likely with more closely related host and donor species, then human cells should survive better in monkey embryos than in pig embryos.

The researchers introduced human stem cells into macaque monkey embryos, and maintained these embryos in culture. They said that some embryos lived for up to 19 days, and that, in a small minority, human cells persisted throughout. This allowed them to check the gene-expression profiles of the human cells, to see how the genes responded to the monkey host environment.

Although this research conformed to rigorous ethical reviews at the host institutions, many commentators nonetheless expressed discomfort and questioned its acceptability. "It generated a huge amount of hope and anxiety," says Alfonso Martinez Arias, a stem-cell biologist at Pompeu Fabra University in Barcelona, Spain, who does not work on interspecies chimaeras. However, he is unsure that either reaction was warranted. Martinez Arias is sceptical about how healthy the cultured embryos were and the extent to which healthy human cells were growing in them.

Two of the study's lead authors were asked to comment for this story, but both declined the invitation.

An open dialogue

Researchers who are trying to use chimerism to produce a new supply of transplantable organs think that the shortage of organ donors more than justifies their work. "People talk about the ethics of doing the science," Mary Garry says, "but I would also argue that we should consider the ethics of not doing this science."

Martinez Arias thinks that this need could be met solely by genetically modifying pigs to produce porcine organs that could be safely transplanted into people. But advocates of chimerism stress the advantages of fully 'humanized' organs — especially if the organs are made from a recipient's own cells. Although data are sparse, one survey suggests that a small majority of the US public supports

Dan and Mary Garry have grown human muscle cells in pig embryos.

MIKE MADISON

the development of human organs in animals for transplantation purposes[5].

As the technology advances, further applications will present themselves. Some uses of chimaeras might be swiftly adopted, such as screening new drugs for hepatotoxic side effects in mice with humanized livers. Other uses, however, will require further discussion of the ethics involved.

One proposed application that might prove problematic would be using iPS cells from people with schizophrenia, for example, to grow cortical neurons in monkey brains[6]. Although this could be a useful tool for studying the condition, growing human neurons in another primate's cortex carries the risk of that creature gaining some human-like cognition or self-awareness. Some people might see that as a reason not to conduct such work.

The possibility of this kind of cognitive humanization is perhaps the most troubling aspect of chimaera research. But there is also concern over chimeric animals producing human gametes. For instance, if such animals could make human sperm and eggs, there is the risk of a human embryo being conceived in an animal uterus — although there is currently a blanket ban on chimeric animals breeding. Farahany also notes that we might be morally troubled if animals were to be generated that simply more closely resembled humans — through having human-like skin or faces, for example.

Currently, ethical oversight falls mainly on local institutional review bodies. Their decisions on whether an experiment is permissible are typically informed by guidelines produced by the International Society for Stem Cell Research (ISSCR), based in Skokie, Illinois, which were updated in May for the second time since 2007. The US National Institutes of Health, meanwhile, has been essentially silent on the topic since it stopped funding any research involving embryonic human–animal stem-cell chimaeras in 2016.

Insoo Hyun, chair of the ISSCR ethics committee and a bioethicist at Harvard University in Cambridge, Massachusetts, and Case Western University in Cleveland, Ohio, says the guidelines try to provide practical advice for researchers and regulators to decide whether currently viable experiments should be permitted. When seeking approval for an individual experiment, Hyun says, researchers cannot justify it by simply stating a noble, long-term goal such as developing transplantable kidneys or curing schizophrenia. Instead, ethicists must assess the plausible gains of that specific experiment.

Using this approach, experiments involving human–monkey chimeric embryos (maintained outside the uterus for a short time) are acceptable if the researchers can make a case that such experiments will provide genuine insights into interspecies cell signalling, which could help to overcome obstacles to growing human tissues in livestock.

Farahany agrees that studies should be assessed on their own merits, but stresses the importance of considering how the field might best move towards its ultimate goals. "I think you always need to have an eye for the future," she says. "Ethics isn't just reactive — it has to be proactive." Rather than appraising the acceptability of experiments as they arise, she wants ethicists to help researchers to avoid going down a path that would lead to developments that society finds objectionable. Only by considering where a research field is heading, she says, can ethicists help to plot an acceptable path forwards.

Researchers seeking to use chimaeras to produce transplantable organs are taking various approaches to navigate this tricky ethical terrain — especially where the brain is concerned. Hyun argues that, in rodents or livestock animals developed to host humanized organs such as the kidneys or pancreas, it is highly unlikely that a small number of errant neurons would give rise to anything close to human cognition. When the Garrys screened all the 27-day-old pig–human chimeric embryos they produced, they saw no evidence of human neurons (or gametes). This finding helped them to gain clearance from institutional review boards to gestate the embryos for 90 days (of a pig's natural 113–115-day gestation period). Nevertheless, Nakauchi has gone even further in the quest to alleviate concerns about humanized brains — he is working with human iPS cells that have been genetically engineered to lack a gene required for cells to become neurons.

Nakauchi and the Garrys have also chosen to avoid generating monkey–human chimaeras. The Garrys are studying the ungulate–primate barrier by combining pig blastocysts with macaque iPS cells, and Nakauchi's group is studying cells from chimpanzees or various monkey species in macaque embryos.

"We have to do everything we can to make this as safe and acceptable to society as possible. I think that's our obligation as scientists," Mary Garry says. And she views public engagement as an essential part of that process. "Unless we have an open dialogue about it," she says, "we're not going to understand what the concerns are."

Farahany agrees. "There ought to be democratic deliberation about what we're okay with and what we're not okay with," she says. With the Garrys hoping to bring pig–human chimaeras to term in the coming years, with Nakauchi pressing for approval to do the same for mouse–human chimaeras, and with each of them eyeing clinical trials in around five years, the time for that dialogue is now.

Liam Drew is a freelance writer based near London.

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