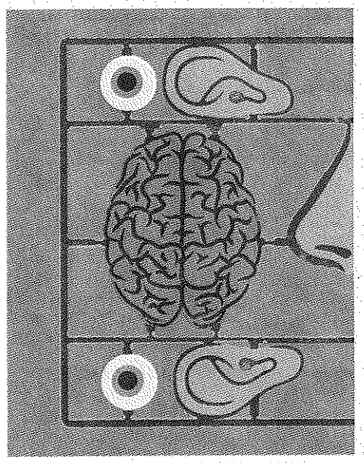
COULD WE ONE DAY REPLACE ALL OF THE TISSUES IN THE HUMAN BODY THROUGH ENGINEERING?



"In 1995 Joseph Vacanti and I wrote for this magazine about advances in artificial pancreas technology, plastic-based tissues such as artificial skin, and electronics that might permit blind people to see [see "Artificial Organs," by Robert Langer and Joseph P. Vacanti; SCIENTIFIC AMERICAN, September 1995]. All of these are coming to pass, either as real products or in clinical trials. Over the next few centuries it is quite possible that nearly every tissue in the body may be able to be replaced by such approaches. Creating or regenerating tissues such as those found in the brain, which is extremely complex and poorly understood, will take an enormous amount of research. The hope is, however, that research in this area will happen quickly enough to help with brain diseases such as Parkinson's and Alzheimer's."

ROBERT LANGER
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mice—pink, wrinkled little rodents that resemble scrotums with eyes and feet. They are nude in the sense that they were bred to have compromised immune systems that accommodate cells transplanted from other species—for instance, human spermatogonial stem cells with mutations—to allow researchers to better understand the biology of male infertility.

If, as Church says, "everything is going to be done in animals first," the road to human germ-line modification runs through rooms like these. CRISPR makes the task more efficient ("It is so freaking easy!" Orwig says), but scientists have been able to alter the genes of sperm-making cells for more than two decades, beginning in 1994, when University of Pennsylvania biologist Ralph Brinster (Orwig's mentor) did the pioneering experiments in mice.

Male infertility has many causes, including obstructive "plumbing" issues, glitches in the incredibly complex process of sperm creation, and underachieving sperm. But in many cases, males simply can't make sperm at all; the condition, known as nonobstructive azoospermia, affects roughly 350,000 men in the U.S., according to Orwig. Several genes have been associated with the failure to produce sperm, including *tex11* and *sohlh1,* and those cases form the backdrop of the experiment Orwig is eager to do.

What Orwig wants to do is take infertile mice, which have a dysfunctional version of one of these genes, remove the sperm-forming stem cells from their testes and correct the defect in those cells by using the new gene-editing techniques. Once the altered stem cells are grown to sufficient numbers in the test tube and screened for precisely the correct alteration, they can be transplanted back into the testes of the animals. And at least in animal experiments of this kind, there is no need for any fancy molecular tests—if the gene editing is successful, Orwig will know within a couple of months because the infertile males will unambiguously demonstrate their ability to become fathers.

"We've been transplanting stem cells for 25 years in almost every species—mice, rats, hamsters, sheep, goats, pigs, dogs and monkeys," Orwig says. "That's a pretty broad swath of evolution, and in all this time, in all these animals, as far as we know, nothing bad has happened." That is why Orwig is optimistic he can demonstrate that editing the genes of stem cells in mice can reverse infertility.

This may seem like an innocuous animal experiment, but to edit a sperm-forming stem cell is to permanently modify the germ line because the resulting sperm cells pass the correction along to the next generation. A potential treatment for male infertility would cross the red line. And although Orwig has no plans to do the obvious human follow-up in his Pittsburgh lab, a successful preclinical demonstration in mice and primates would provide the impetus for an attempt in the private sector—which is where Church believes the final steps will unfold. "Sperm-editing efforts will be privately funded," he says, "just like other therapies."

Developing such a clinical treatment would face technical hurdles, of course. For one thing, scientists would have to find a way to maintain human spermatogonial stem cells long enough to select the right ones for transplantation—still not a trivial task. But these male stem cells offer much less of a moving target than embryos, which are dynamic and change rapidly. The Chinese researchers who have attempted gene editing in embryos with CRISPR, for example, have reported both "untoward mutations" and "mosaicism," meaning that some cells in the embryos show successful editing, whereas others do not. Moreover, the DNA of gene-edited stem cells can be screened before an embryo is produced.

That is what makes Orwig's potential mouse experiment so politically inconvenient. Because of prohibitions enacted by Congress in the 1990s, the National Institutes of Health cannot fund any research that involves the destruction of human embryos. A human version of Orwig's proposed mouse experiment might sidestep that prohibition, but it would probably fall under a new obstacle that the House of Representatives introduced two weeks after the December summit on gene editing. In a two-sentence passage buried in the 2,009-page omnibus spending bill of 2015, Congress inserted language forbidding the FDA to consider any medical intervention relying on the use of gene-edited embryos; the wording does not explicitly prohibit editing germ cells, but Stanford University law professor Henry Greely believes "the FDA would take the position that those sperm were more than minimally manipulated human cells that would require FDA approval as a drug or biological product." The regulatory piece, he thinks, could add a decade or two to Church's timeline.

That does not mean Orwig's mouse experiment would be against the law—just a gentle nudge down the slippery slope toward germ-line modification. The step across the red line could happen in private IVF clinics, which have a long (and blemished) history of pushing the envelope on new reproductive technologies. "It's such an easy technology to apply that it would only take somebody with a little chutzpah to get together with someone in an IVF clinic and, you know, take a shot at it," says George Daley, a stem cell biologist at Boston Children's Hospital. "This is coming down the pike, and people need to start thinking about it," he notes. "This is a potentially disruptive reproductive technology."

It probably will not happen in the U.S. unless the public—and political—perception of germ-line modification becomes more accommodating, but Orwig is quietly preparing for that day. "We're going to work real hard behind the scenes," he says, "until the worldview changes."

CROSSING BORDERS

THE "WORLDVIEW" on germ-line editing is complicated and contradictory. A majority of Americans do not like the idea of editing genes in either embryos or germ cells, according to a recent analysis of 17 public opinion polls published in the *New England Journal of Medicine.* Yet paradoxically, most people support gene editing in adults "aimed at preventing one's children from inheriting certain diseases." (Robert J. Blendon, lead author of the study, says any intervention on the adult side that is positive for the next generation, including germ cells, would have "considerable public support.") Moreover, the *NEJM* study also pointed out that many of these public opinion polls pose their questions using language that "might not be scientifically precise." In other words, although the National Academies meeting adjourned last December with a pledge to continue the public conversation about germ-line editing, it is not clear that the public even understands the terms of that conversation. And while public forums struggle to find an effective vocabulary, the science races ahead.

As we spoke in his office last spring, Orwig nodded at a scientific reprint sitting on his desk. "I really, really love this paper," he said. He was referring to research published this past February in the journal *Cell Stem Cell* by a group headed by Qi Zhou of the Chinese Academy of Sciences. The experiment basically provided a recipe for the in vitro creation of germ cells.

The researchers showed that they could create sperm-forming stem cells in a dish; with a technique currently used in IVF clinics, these cells could be injected into egg cells to create fertile male mice. Harvard's Daley says of this advance: "With the addition of CRISPR, you've got the brave new world."

When Aldous Huxley imagined his brave new world in 1932, the story unfolded under one totalitarian regime, with neither national boundaries nor local regulations. In today's world, germ-line editing in any one place means the germ line is edited everywhere. "Regulation is country-specific, but science crosses borders," Harvard Law's Cohen says. Even if there were laws against germ-line modification in the U.S., you would have to build a wall much higher than the one proposed by Donald Trump to keep American germ lines insulated from an eventual influx of modified DNA.

"If you play out the world to 100 years from now, if anybody does this anywhere, that's the end game," Cohen says. "Over time those people will mate and create offspring and will cross borders and enter our shores. And if the safety and efficacy are worked out, it's inevitable that you're going to have people walking around in the world, and they're going to be reproducing, and they will end up in this country, and those changes will enter the U.S. gene pool."

As I am concluding my visit with Orwig, he glances at the computer on his desk. A reporter has sent an e-mail seeking comment on yet another experiment cozying up to the red line: a group in China has just reported its attempt to edit human embryos (nonviable) to be resistant to HIV infection. "Eventually we'll learn a vocabulary that acknowledges that we're there," Orwig says. "But I feel we're already there."

MORE TO EXPLORE

The Pandora's Box Congress. Michael Rogers in *Rolling Stone;* June 19, 1975.
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Complete Meiosis from Embryonic Stem Cell-Derived Germ Cells in Vitro. Quan Zhou et al. in *Cell Stem Cell,* Vol. 18, No. 3, pages 330–340; March 3, 2016.

FROM OUR ARCHIVES

Editing the Mushroom. Stephen S. Hall; March 2016.

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