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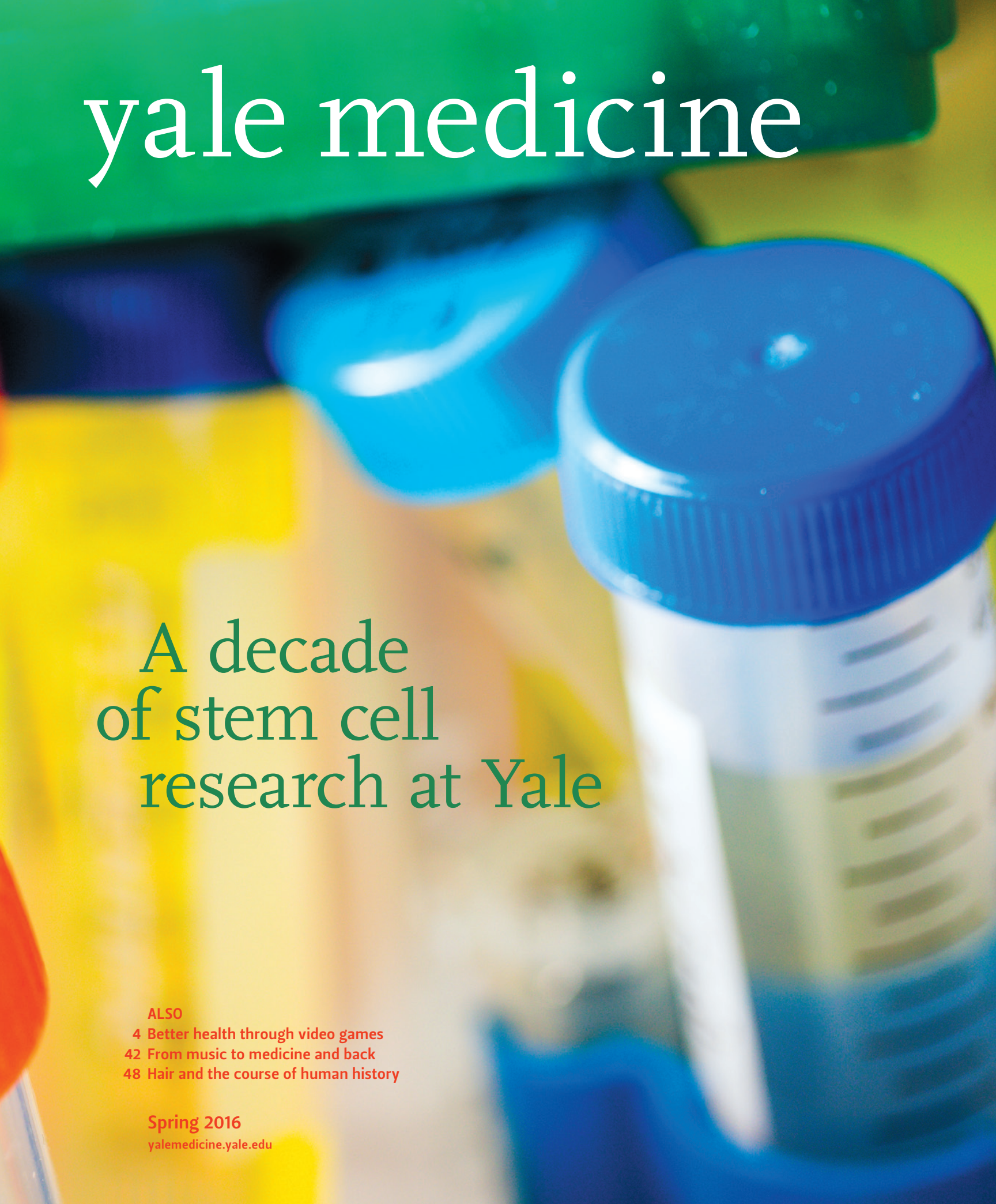

## A decade of stem cell research at Yale

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As an anesthesiology resident in Boston, Laura Niklason watched as cardiac surgeons sought usable veins for bypass surgery. That's when she began working on creating new blood vessels in the lab. Now, her work in tissue engineering has moved on to the lung and trachea.

# Organ in a bottle

Laura Niklason works to engineer organs as replacements for those that fail.

BY ASHLEY P. TAYLOR
PHOTOGRAPHS BY ROBERT LISAK

On the windowsill of Laura Niklason's office is an unusual knickknack: a clear glass jar like a jug turned on its side with two cork-shaped stoppers along its top, thin protrusions on both ends, and, inside, a paper sailboat. A ship in a bottle. The boat, stuck in there, Niklason says, by a stir-crazy grad student late one night in the lab, is no miniature yacht. But inside bottles like these, called bioreactors, Niklason, Ph.D., M.D., the Nicholas Greene Professor of Anesthesiology and Biomedical Engineering, builds structures that trump the trickiest marine art project: blood vessels, windpipes, and lungs.

Niklason first had the idea to build organs in the lab when, during her time as an anesthesiology resident in Boston, she watched cardiac surgeons struggle to find transplantable veins for bypass operations: "We ought to be able to grow a new one," Niklason remembers thinking. She also has a doctorate in biophysics, and was a postdoc in the lab of chemical engineer and biomedical engineering guru Robert Langer, Ph.D., at the Massachusetts Institute of Technology. It was there that she grew her first blood vessels.

She continued her vascular engineering research at Duke University and founded a company, Humacyte, to build replacement tissues for clinical use. It is now testing engineered vessels for use in hemodialysis patients. Since coming to Yale in 2006, Niklason has expanded her tissue engineering work to the lung and trachea. She has also been collaborating with the core labs of the Yale Stem Cell Center, where she has a secondary appointment. Stem cell technology touches every area of her research. Eventually, she hopes to use stem cells, which can differentiate into many tissue types, as the cellular materials for all of her engineered organs.

"She's an amazing inventor," says Haifan Lin, Ph.D., the Eugene Higgins Professor of Cell Biology; professor of genetics; and of obstetrics, gynecology, and reproductive sciences; and director of the Yale Stem Cell Center. "She's a modern Renaissance woman."

## A BIOREACTOR

Niklason's initial framework for engineering a replacement artery was to take blood vessel cells from a patient or animal, culture them on a tube-shaped biodegradable scaffold, and then implant the engineered vessels into the same patient or animal. To avoid immune rejection, the idea was to "make an engineered artery just for that patient or just for that animal." In the Langer lab, her attempts to grow an artery this way failed for two years—until she started pumping nutrient-rich liquid (called medium) through the vessel as it developed within the bioreactor, causing it to expand and contract as blood vessels do in the body. To set up a blood-vessel bioreactor like the one in Niklason's office, a researcher takes a silicone tube, sews a scaffold of biodegradable mesh around it, and threads it between the glass container's protruding ends. The researcher then pipettes a solution of cells onto that scaffold. As the cells grow around the tube, medium courses through it, strengthening the developing vessel. Other tubes for providing nutrients and gas exchange enter the bioreactor through the stoppers on top, and the whole thing is filled with medium and kept in an incubator to control the temperature and atmosphere. Making a blood vessel this way takes about two months. Near the end of the growth period, the silicone tubing is removed, and the vessel is suitable for implantation into the body. In 1999, Niklason reported in *Science* that by using this method, she had successfully implanted pigs with engineered arteries made from their own cells.

At Duke, Niklason tried the same approach using vascular cells isolated from elderly patients, but ran into problems. Because the patients' cells were old, they had limited lifespans—they stopped dividing and became difficult to grow. Additionally, the tissues they spawned were not as sturdy as those derived from younger cells. Niklason tried using gene therapy to trigger a lifespan-increasing enzyme, with limited success. "The cells lived longer, but they didn't make tissues that were mechanically more robust."

Once at Yale, Niklason tried a new approach: differentiating stem cells into smooth muscle cells (SMCs), a major component of blood vessels, and using them to create arteries. These newly differentiated human cells, unlike those from elderly patients, would be young enough to work. In 2008, her lab reported that it had created an engineered artery by differentiating bone marrow-derived stem cells into SMCs and then coaxing the cells to form a blood vessel as before.

Now her team is building arteries and other tissues with induced pluripotent stem cells (iPSCs). These cells are created by taking such differentiated cells as skin cells and treating them with certain factors that convert them into stem cells. Since a skin biopsy is an easier and less painful procedure than bone-marrow isolation, this method has clinical advantages. Eventually, Niklason hopes, a doctor could take cells from a skin biopsy, create patient-specific iPSCs, and then differentiate those iPSCs into the tissue types needed to make personalized replacement organs.

The technology is a few steps behind this vision, however. "We can differentiate the cells from stem cells; we can make smooth muscle cells fairly well," says Niklason, "but not well enough such that they create arteries that are implantable and strong enough to function long term."

Laura Niklason

"Stem cell science has just exploded in terms of what we know over the last 20 years. The building blocks and the tools that we have at hand now are just so vastly different from what they were even 10 years ago."

Down another avenue of research, however, Niklason realized that personalized organs weren't always the best choice. Beyond the problem of old cells, which stem cell technologies could circumvent, there were the constraints of time. If you need a new blood vessel, Niklason says, "you probably don't have the luxury of sitting around three or four months and waiting for me to grow you a new artery. We started racking our brains about how to solve this problem in such a way that we could make a universal artery." Her solution was something called decellularization.

Our cells secrete proteins that form the scaffold on which they live and move—the extracellular matrix. Wash away the cells, and the scaffold remains; this washing step is decellularization. Niklason washed away the cells of her engineered blood vessels and implanted those decellularized arteries into animal models. The decellularized vessels, she found, worked as well as transplanted native blood vessels—and with no immune rejection. "I can take this tissue, and I can implant it into any human, and it won't be rejected by the body because we've washed away the stuff that came from somebody else." Furthermore, the vessels didn't stay decellularized forever. Taking cues from the extracellular matrix, "cells from the patient infiltrate into this tissue and turn it into a living tissue over time."

The hemodialysis graft that Niklason's company is currently testing is one such decellularized blood vessel. Hemodialysis grafts create a conduit between a vein and an artery in the arm into which large-bore needles can be inserted to transport blood to and from the dialysis machine. Such grafts are made with either a patient's own vein from elsewhere in the body or from plastic, and they often fail. Phase III trials comparing Humacyte's decellularized grafts to their plastic equivalents are slated to begin this year. "My hope is that we'll be able to grow thousands of these tissues that can then go out to hospitals and be implanted into any patient who needs a replacement blood vessel of this type," Niklason says.

Niklason is also using decellularization to engineer tracheae (windpipes). Unlike blood vessels, which are flexible, the trachea—supported by rings of cartilage surrounding the airway—is designed to resist collapse. In Niklason's engineered windpipes, which she has tested in rats and primates, that function is served by a metal stent on which the tracheal cells are grown. "We've got a stent-tissue composite," she says.

The cells that Niklason cultures in order to make decellularized blood vessels or tracheae for patients come from organ donors, but Niklason would like to use stem cells instead. She envisions a "bank" of stem cells that could be differentiated into various cell types as needed. "If we had an infinite cell bank, that would be wonderful in terms of the reproducibility of the vessels that we make," says Niklason.

## MORE COMPLEX TISSUES

The decellularization strategy worked with blood vessels and tracheae, Niklason says, because many of their functions come from their physical architecture, which remains when you strip away the cells. That is not the case with such complex tissues as the kidney, heart,

and lung, Niklason says. "Their function is derived from the cells that are there. ... So if you strip the cells away you have no organ function."

It is for engineering and transplanting one such complex organ, lungs, into rats that Niklason is perhaps best known. For that project, reported in a 2010 *Science* paper, she also used the technique of decellularization, but this time for a different purpose. Instead of trying to engineer the many branching airways and air sacs of the lung, she decided to take a donor lung, wash away the cells, and use it as a scaffold on which to regrow the recipient's own lung cells in a bioreactor that both pumped fluid through the lung's vascular system and used another pump to make the lung "breathe." Then, the recipients were rodents, but the goal is, of course, to build customized lungs for patients.

To reach that goal, the Niklason lab is counting on iPSCs. The lung contains many "flavors of epithelium," she says, and growing enough of those various cell types from lung tissue biopsies is difficult. Instead, she and her team are developing tools to differentiate them from iPSCs.

It helps that her lab is right above the stem cell core, the labs that specialize in producing human embryonic stem cells and iPSCs. "We work with them constantly," says Niklason. As Niklason's engineering approaches turn more and more toward iPSCs, that collaboration is bound to continue.

"Stem cell science has just exploded in terms of what we know over the last 20 years," says Niklason. "The building blocks and the tools that we have at hand now are just so vastly different from what they were even 10 years ago." Then, scientists had just figured out how to create iPSCs from mouse tissue. Now, Niklason is using them to develop personalized engineered organs. "It's a very exciting time to be working in this area, and I feel very fortunate." */yale medicine*

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