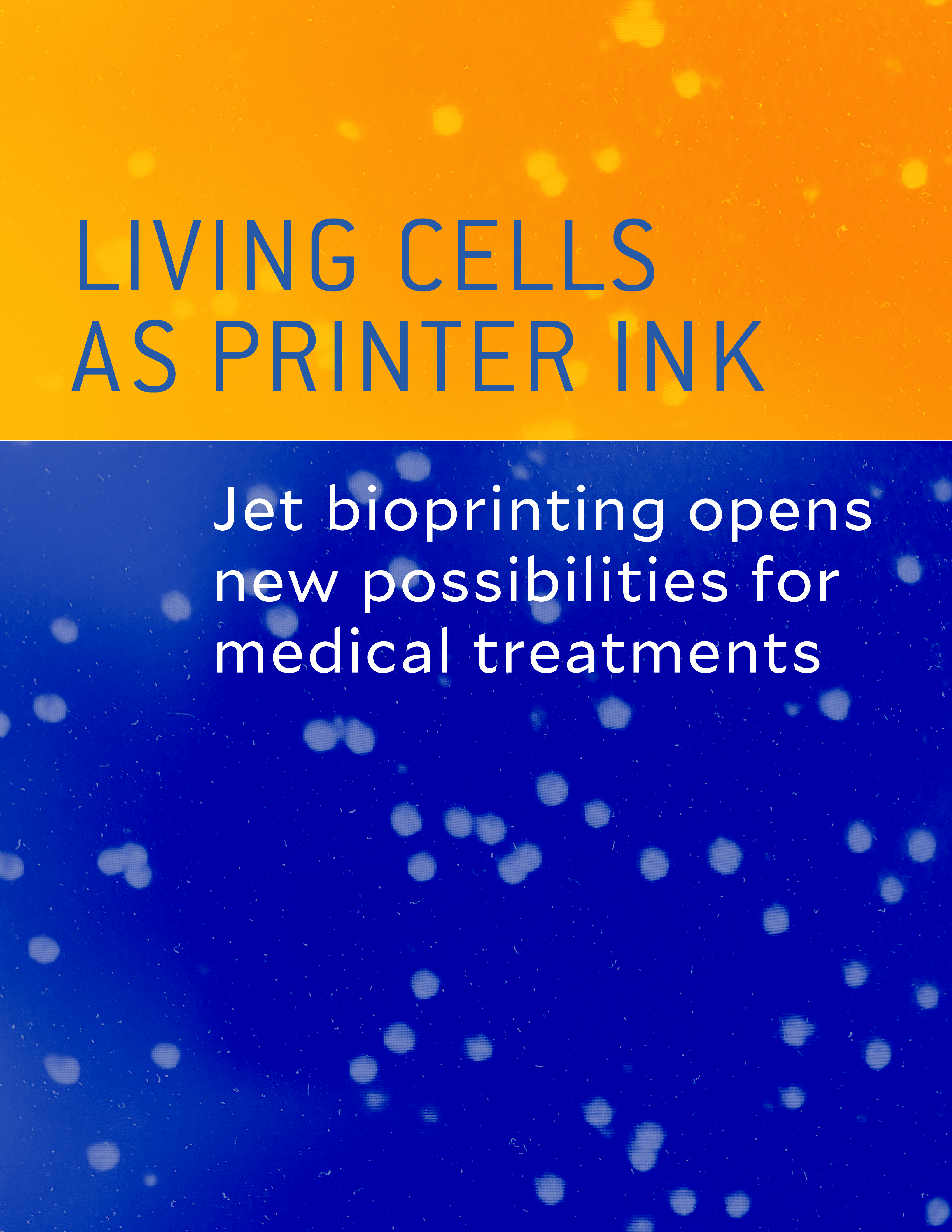
# LIVING CELLS AS PRINTER INK

## Jet bioprinting opens new possibilities for medical treatments



The Blended Reality Laboratory is an applied research hub for those exploring ideas that operate at the intersection of real and virtual worlds. Located at the Center for Collaborative Arts and Media (CCAM), the Blended Reality Laboratory draws upon CCAM's mission to connect the arts and sciences through experimental technology and collaborative research. The laboratory and its projects are underwritten through a hybrid structure of academic, industry, and applied research support.

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Learn more about the Blended Reality Project at http://blendedreality.yale.edu

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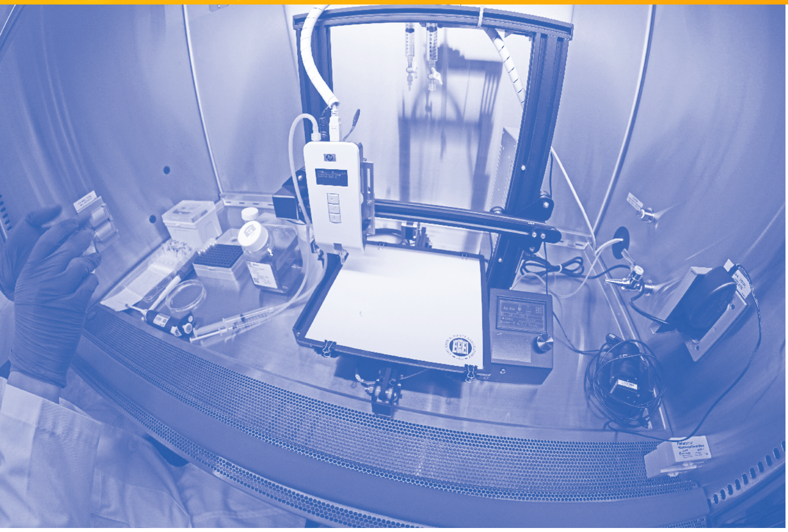


Can you use an HP inkjet print to print a new face? A heart? A kidney? When scientists discovered that it was possible to use commercial printers for bioprinting, and print living cells just as they can print homework assignments, the possibilities seemed endless.

"That's nice," says John Geibel, DSc, MD, professor emeritus of surgery at Yale University and director of the John B. Pierce Laboratory in New Haven, "but that's not reality. A kidney has over 11 different diameters in a single tubule, and contains millions of tubules, and 19 cell types."

Reality, according to Geibel, is using inkjet bioprinting to solve real-world medical challenges, helping people live better in the near future. Using HP inkjet technology, Geibel and a team at the Pierce Lab are investigating a way to sustainably print cells that can be transplanted into humans in vivo.

3D bioprinting replaces traditional ink with something called bioink, a liquid that contains cells suspended in polymers that can be extruded through a printer onto a substrate. That printed array of cells is then incubated, allowing the cells to develop into what ultimately becomes tissue that can be transplanted into a living animal. Geibel, prior to his connection with HP, was able to use an expulsion printer successfully print a section of aorta and transplant it into a living rat, who not only survived the surgery, but thrived.

Following that breakthrough, Covid-19 put a halt to animal studies at Yale, and his progress was stalled. Then Geibel met Randy Rode.

In December 2021, Geibel met Blended Reality's Randy Rode at a dinner at Yale's Jonathan Edward College, where they both serve as Fellows. Introduced by the Head of Jonathan Edwards, biomedical engineering pioneer Mark Salzman, PhD and Goizueta Foundation Professor of Biomedical and Chemical Engineering at Yale, Geibel described to Rode his contributions to bioprinting to date. Rode, along with Justin Berry, principal investigator of the Blended Reality Lab, connected Geibel with their partners at HP, who were seeking to explore using HP inkjet technology for bioprinting. With that touch of serendipity, Geibel went to work.

"I am excited about Dr. Geibel's work because it is one of the most advanced applications of 3D printing to print tissue using some of HP's core technology," said Louis Kim, an executive with HP's 3D printing organization. "We had been scouting a lot of research efforts in this area, and we found Geibel's work to be one of the most advanced, as well as one of the most achievable, approaches that we've seen, many of which have much longer horizons. Geibel's work is more practical, and nearer term."

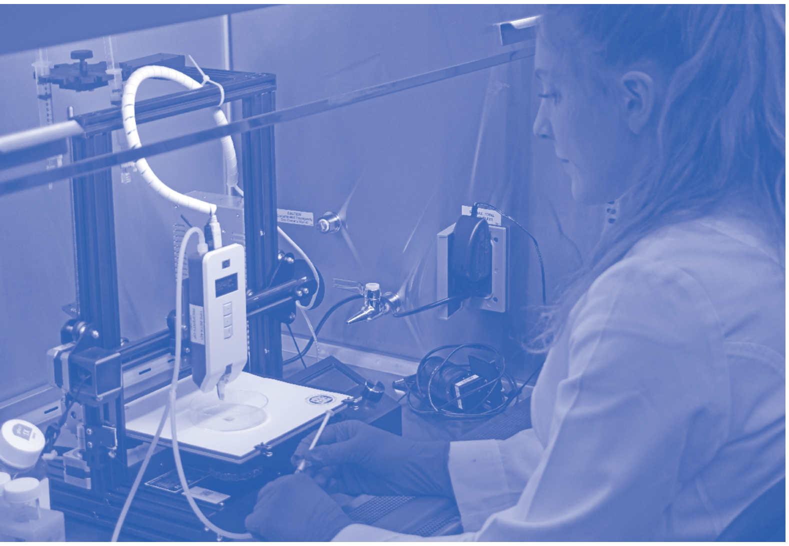
Late last year, Geibel received two ink jet printers from HP that were in beta, and well as two tips that attach to the printer, punctuated with pores that form a rectangle of 12 dots: two rows of six. The printers are the size of a cell phone (perhaps an older, larger model cell phone), small enough for Geibel to attach them to a traditional commercial 3D printer that can move the ink jet forward and back along three axes to create a variety of geometric shapes. At the Pierce Lab, an in-house team of engineers developed a platform using LabView software, allowing them to Interface with the printers and move them along three axes.

Geibel and his team set their sights on the first milestone in their work with inkjet bioprinting: creating a tubular structure, resembling a blood vessel, and demonstrating that it can be profused with liquid.

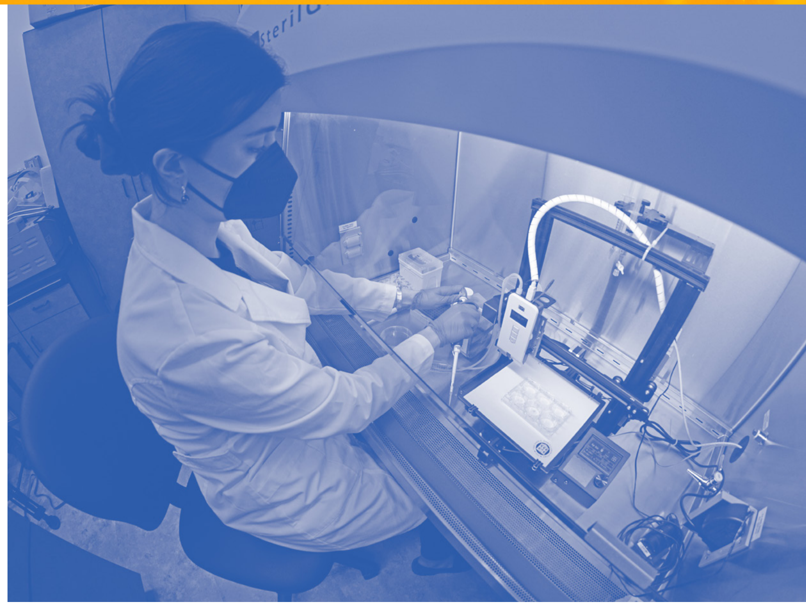
Annika Dell is a medical engineering master's student visiting from the University of Lubeck in Germany, and is working on her thesis at the Pierce Laboratory with Geibel. She spends her days nurturing these cells through the printing process, and performing experiments that are devised to advance the technology cell by cell.

The first step of the process was to ensure that the cells would survive the ink jet process. Geibel is using the ink jet to grow human kidney cells, which he chose for this portion of the research because they are resilient and a quick-growing cell line. The cell medium must be liquid so it will pass through tiny nozzles of the inkjet. Inkjet bioprinting is advantageous because the process distributes bioink in a digitally controlled pattern, with a high density of evenly-distributed cells, as compared to cells which are distributed by pipette.

"With the ink jet we are able to achieve perfect suspension, and control of the geometry," says Dell. The ink jet process requires heat that creates pressure to propel this bioink through the printer tip, and form a bubble and burst. Could the cells withstand this heat and pressure?



"The cells make it through alive very well through the printing process," says Dell.  More than three million cells can be printed onto a plate in a single jetting. The cells are loaded with a fluorescent marker that shows viability:



when they are alive, they glow. They are then placed in an incubator for two days.

"The cells begin finding each other and connecting within hours," says Dell. On a welcoming substrate, they begin the reach out appendages to touch others, connect, grow, and multiply.

"The inkjet is faster and more precise," than other bioprinting processes, says Geibel. It creates a uniform density of cells – much more so than a pipette would be able to produce, and more quickly. Within a few days, a sheet of cells forms, and can be lifted off the substrate, forming the building material for new human tissue. Step one of the bioprinting process is complete.

"Printing on a flat surface is fine, but unusual from a biological standpoint," says Geibel. Now, he and his team are at work finding a way to take a flat sheet of cells, and form it into a tube.

To form that tube, Geibel is currently collaborating with another lab at Yale, where Associate Professor of Biomedical Engineering Anjelica Gonzalez, PhD, is developing support matrices of polysaccharides, or sugar. Formed into tubes, these matrices act as scaffold for cells that can be printed directly onto the matrix. They would grow there, and then the matrix would then dissolve, and be consumed by the cells for food.  Currently Geibel and Gonzalez are examining the viability of this method of constructing tubes. If that proves unsuccessful, Geibel has a plan B: rolling cells printed on a sheet of hydrogel into a tube, and closing it with surgical glue.

One of the challenges of inkjet bioprinting is that the bioink must be almost water-like in order to pass through the printer. Geibel is investigating ways to stiffen the cell material, so that it can be manipulated into a rounded form like a tube. One method he is working on is to print on a surface of hydrogel, which contains high levels of hyaluronic acid, which puffs up cells and creates almost a pillow-like structure. However, at higher temperatures, it does not hold. Another option is alginate, a seaweed extract, but too much can cause the structure to become brittle. “Brittle blood vessels are essentially arteriosclerosis,” says Geibel.

Geibel is experimenting with using two printers to create sheets of bioink between two layers of hydrogel. This “hydrogel sandwich,” as he described it, is printed directly on a cooled piece of metal, which helps the layers hold their form.

Once Geibel is able to use the ink jet-printed cells to make viable tubes, the next question is: what cells to jet? Keratinocytes, or skin cells, are a promising option, says Geibel. “There is great potential here,” says Geibel. An inkjet print could be applied in real time to wound sites to offer an alternative to closing them “better than suture, staples, or glue,” he says. “If you can control bleeding by spraying on stem cells, you will never get a scar. Keratinocytes contain a calcium receptor that Geibel himself discovered, that if stimulated can accelerate wound healing. “That would be the logical next step, to show proof of principle,” he says.

“I can envision the jet printer for skin injury, such as a burn injury or stab wound, without a skin graft. I can see this type of printer being loaded with stem cells, a load up the jet, take off the burned skin, hold it and potentially spray cells back and forth, like you are spray painting a chair.”

From there, the challenge would be scaling up, and creating larger reservoirs of cells to use in printing, but at this point, this can be cost prohibitive. Still, the solution is out there: stem cells could possibly be used, says Geibel, because fewer of them are necessary to begin multiplying at a fast rate. Other potential uses he could see would be in plastic surgery, intestinal repair, or even in the space program, for treating wounds in astronauts well beyond the reach of an emergency room.

While the imagination can run wild thinking of all the possible applications for printed sheets of human cells, Geibel is more cautious in building this new technology. “I don’t want 3D bioprinting to turn into, like some technologies, a flash in the pan,” he says. To move the needle with sufficient data to transition into animal studies will take years. “The goal is reproducible processes,” Geibel says, so ultimately bioprinting will be available to people anywhere, anytime they need it.