Cotton candy machines may hold key for making artificial organs

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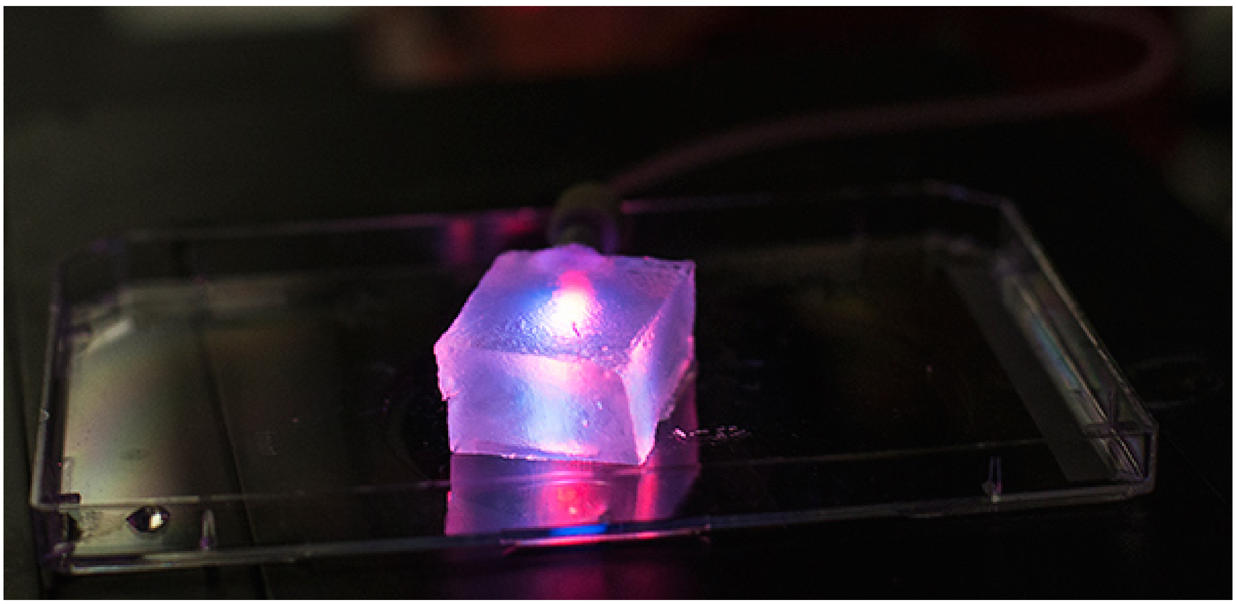


Three-dimensional slab of gelatin that contains a microvascular network. Credit: Bellan Lab / Vanderbilt

Cotton candy machines may hold the key for making life-sized artificial livers, kidneys, bones and other essential organs.

For several years, Leon Bellan, assistant professor of mechanical engineering at Vanderbilt University, has been tinkering with cotton candy machines, getting them to spin out networks of tiny threads comparable in size, density and complexity to the patterns formed by capillaries - the tiny, thin-walled vessels that deliver oxygen and nutrients to cells and carry away waste. His goal has been to make fiber networks that can be used as templates to produce the capillary systems required to create full-scale artificial organs."

In an article published online on Feb. 4 by the *Advanced Healthcare Materials* journal, Bellan and colleagues report that they have succeeded in using this unorthodox technique to produce a three-dimensional artificial capillary system that can keep living cells viable and functional for more than a week, which is a dramatic improvement over current methods.

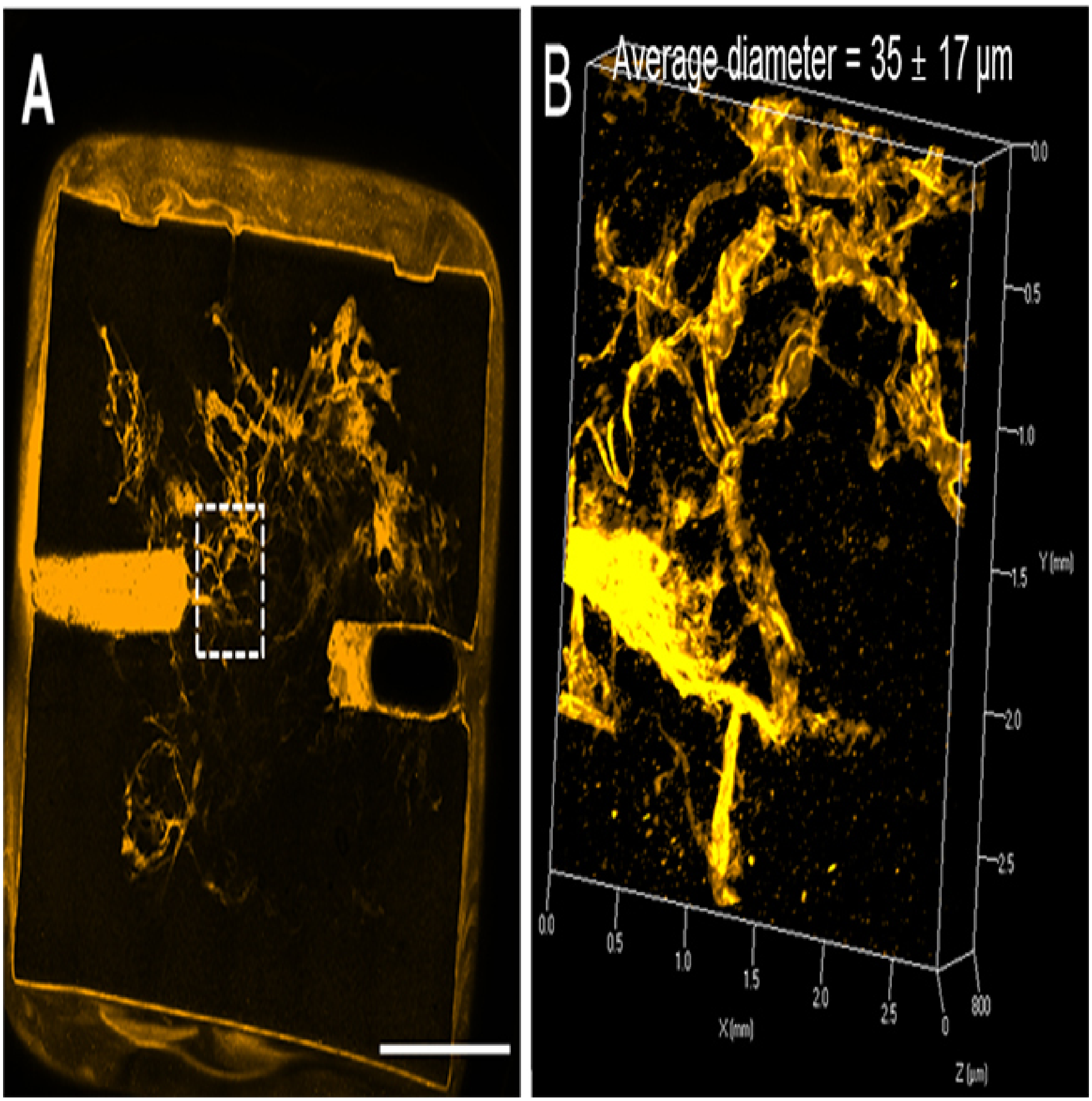
"Some people in the field think this approach is a little crazy," said Bellan, "But now we've shown we can use this simple technique to make microfluidic networks that mimic the three-dimensional capillary system in the human body in a cell-friendly fashion. Generally, it's not that difficult to make two-dimensional networks, but adding the third dimension is much harder; with this approach, we can make our system as three-dimensional as we like."

Many tissue engineering researchers, including Bellan, are currently focusing their efforts on a class of materials similar to hair gel - water-based gels, called hydrogels - and using these materials as scaffolds to support cells within three-dimensional artificial organs.

Hydrogels are attractive because their properties can be tuned to closely mimic those of the natural extracellular matrix that surrounds cells in the body. Unlike solid polymer scaffolds, hydrogels support diffusion of necessary soluble compounds; however, oxygen, nutrients and wastes can only diffuse a limited distance through the gel. As a result, cells must be very close (within the width of human hair) to a source of nutrients and oxygen and a sink for the wastes they produce, otherwise they starve or suffocate.

So, to engineer tissues that have the thickness of real organs and keep cells alive throughout the entire scaffold, the researchers must build in a network of channels that allow fluids to flow through the system, mimicking the natural capillary system.

There are two basic methods that researchers use to create artificial capillary systems: bottom-up and top-down.



Microvascular network perfused with liquid. Figure B is magnification of the area in Figure A outlined in white. Credit: Bellan Lab / Vanderbilt

In the bottom-up process, scientists culture cells in a thin slab of gel, and after some time they spontaneously begin creating capillaries. Although this approach has the advantage of simplicity, it has one fundamental problem: It can take weeks for the cells to create such a network. So it isn't possible to stack the cells too high or the ones in the center begin dying off before the crucial capillary network forms.

As a result, Bellan is using a top-down approach. He reports that his cotton-candy spinning method can produce channels ranging from three to 55 microns, with a mean diameter of 35 microns. "So far the other top-down approaches have only managed to create networks with microchannels larger than 100 microns, about ten times the size of capillaries," he said. In addition, many of these other techniques are not able to form networks as complex as the cotton candy approach.

Bellan's focus on this unique use of a cotton candy machine dates back to graduate school. At the time, he was doing research on electrospinning, a process of making nanofibers using strong electric fields. He went to a lecture on tissue engineering where the speaker discussed the need to create an artificial vascular system to support cells in thick engineered tissue. He realized that electrospinning can make networks somewhat resembling capillaries, but at a much smaller scale.

"The analogies everyone uses to describe electrospun fibers are that they look like silly string, or Cheese Whiz, or cotton candy," said Bellan. "So I decided to give the cotton candy machine a try. I went to Target and bought a cotton candy machine for about $40. It turned out that it formed threads that were about one tenth the diameter of a human hair - roughly the same size as capillaries - so they could be used to make channel structures in other materials."

However, getting from that point to creating artificial capillaries that work was not a simple matter. If you create a network of fibers using sugar, when you pour a hydrogel on it, the sugar dissolves away because the hydrogel is mostly water.

This illustrates what Bellan describes as the "Catch-22" in creating such sacrificial structures. "First, the material has to be insoluble in water when you make the mold so it doesn't dissolve when you pour the gel. Then it must dissolve in water to create the microchannels because cells will only grow in aqueous environments," he explained.

The researchers experimented with a number of different materials before they discovered one that worked. The key material is PNIPAM, Poly(N-isopropylacrylamide), a polymer with the unusual property of being insoluble at temperatures above 32 degrees Celsius and soluble below that temperature. In addition, the material has been used in other medical applications and has proven to be rather cell-friendly.

The researchers first spin out a network of PNIPAM threads using a machine closely resembling a cotton candy machine. Then they mix up a solution of gelatin in water (a liquid at 37 degrees) and add human cells, like adding grapes to jello. Adding an enzyme commonly used in the food industry (transglutaminase, nicknamed "meat glue") causes the gelatin to irreversibly gel. This warm mixture is poured over the PNIPAM structure and allowed to gel in an incubator at 37 degrees. Finally, the gel containing cells and fibers is removed from the incubator and allowed to cool to room temperature, at which point the embedded fibers dissolve, leaving behind an intricate network of microscale channels. The researchers then attach pumps to the network and begin perfusing them with cell culture media containing necessary chemicals and oxygen.

"Our experiments show that, after seven days, 90 percent of the cells in a scaffold with perfused microchannels remained alive and functional compared to only 60 to 70 percent in scaffolds that were not perfused or did not have microchannels," Bellan reported.

Now that Bellan and his team have shown that this technique works, they will be fine-tuning it to match the characteristics of the small vessel networks in different types of tissues, and exploring a variety of cell types.

"Our goal is to create a basic 'toolbox' that will allow other researchers to use this simple, low-cost approach to create the artificial vasculature needed to sustain artificial livers, kidneys, bone and other organs," Bellan said.

Historical note: The cotton candy machine was invented by a Nashville dentist in 1897 and made its debut at the 1904 World's Fair in St. Louis.

More information: Jung Bok Lee et al. Development of 3D Microvascular Networks Within Gelatin Hydrogels Using Thermoresponsive Sacrificial Microfibers, *Advanced Healthcare Materials* (2016). DOI: 10.1002/adhm.201500792

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https://phys.org/news/2016-02-cotton-candy-machines-key-artificial.html