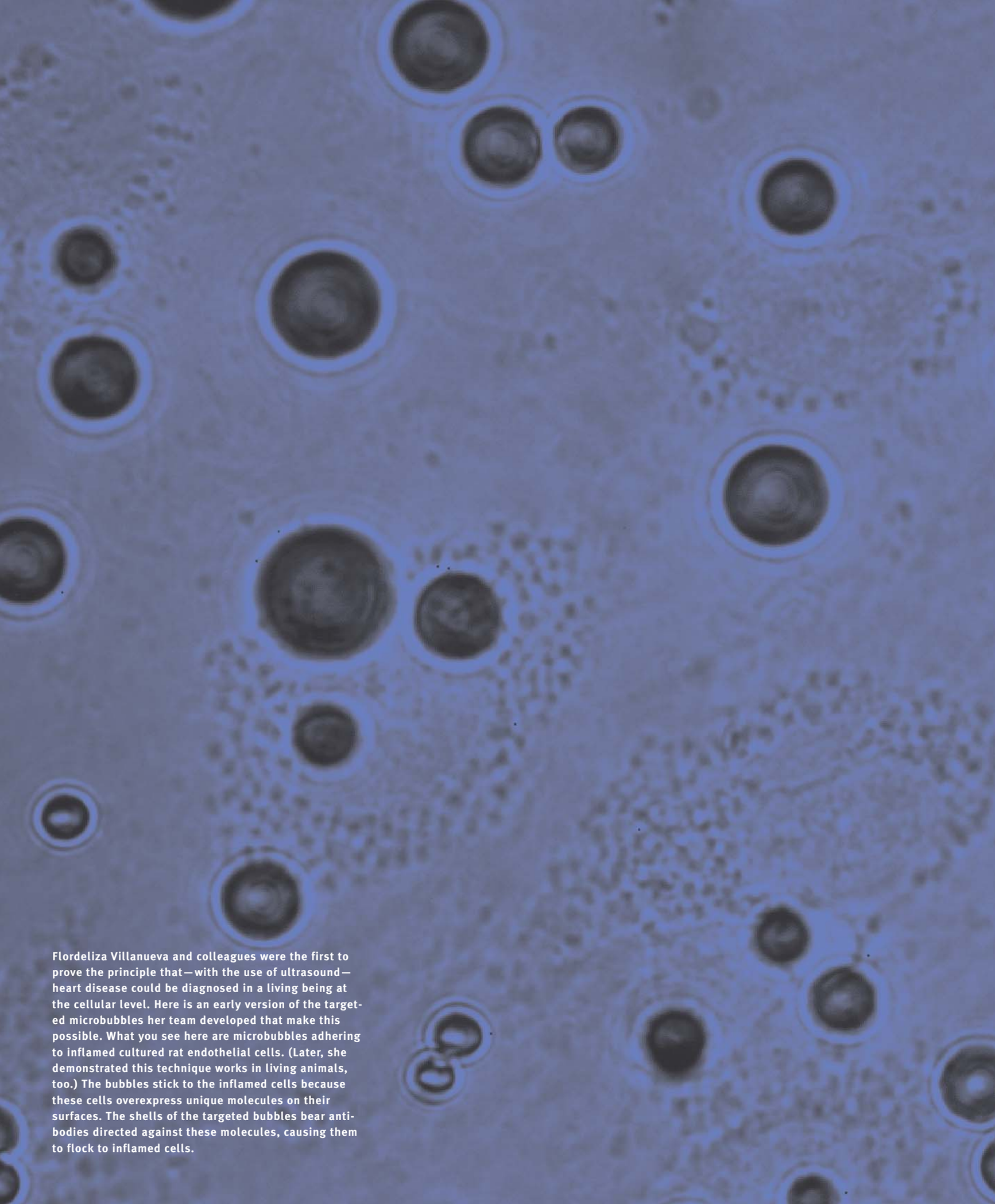


Flordeliza Villanueva and colleagues were the first to prove the principle that—with the use of ultrasound—heart disease could be diagnosed in a living being at the cellular level. Here is an early version of the targeted microbubbles her team developed that make this possible. What you see here are microbubbles adhering to inflamed cultured rat endothelial cells. (Later, she demonstrated this technique works in living animals, too.) The bubbles stick to the inflamed cells because these cells overexpress unique molecules on their surfaces. The shells of the targeted bubbles bear antibodies directed against these molecules, causing them to flock to inflamed cells.



THESE LITTLE ORBS MAY TELL US WHO HAS CARDIOVASCULAR DISEASE BEFORE SYMPTOMS EVEN DEVELOP

IMAGES | FLORDELIZA VILLANUEVA
TEXT | MEGHAN HOLOHAN

TINY BUBBLES

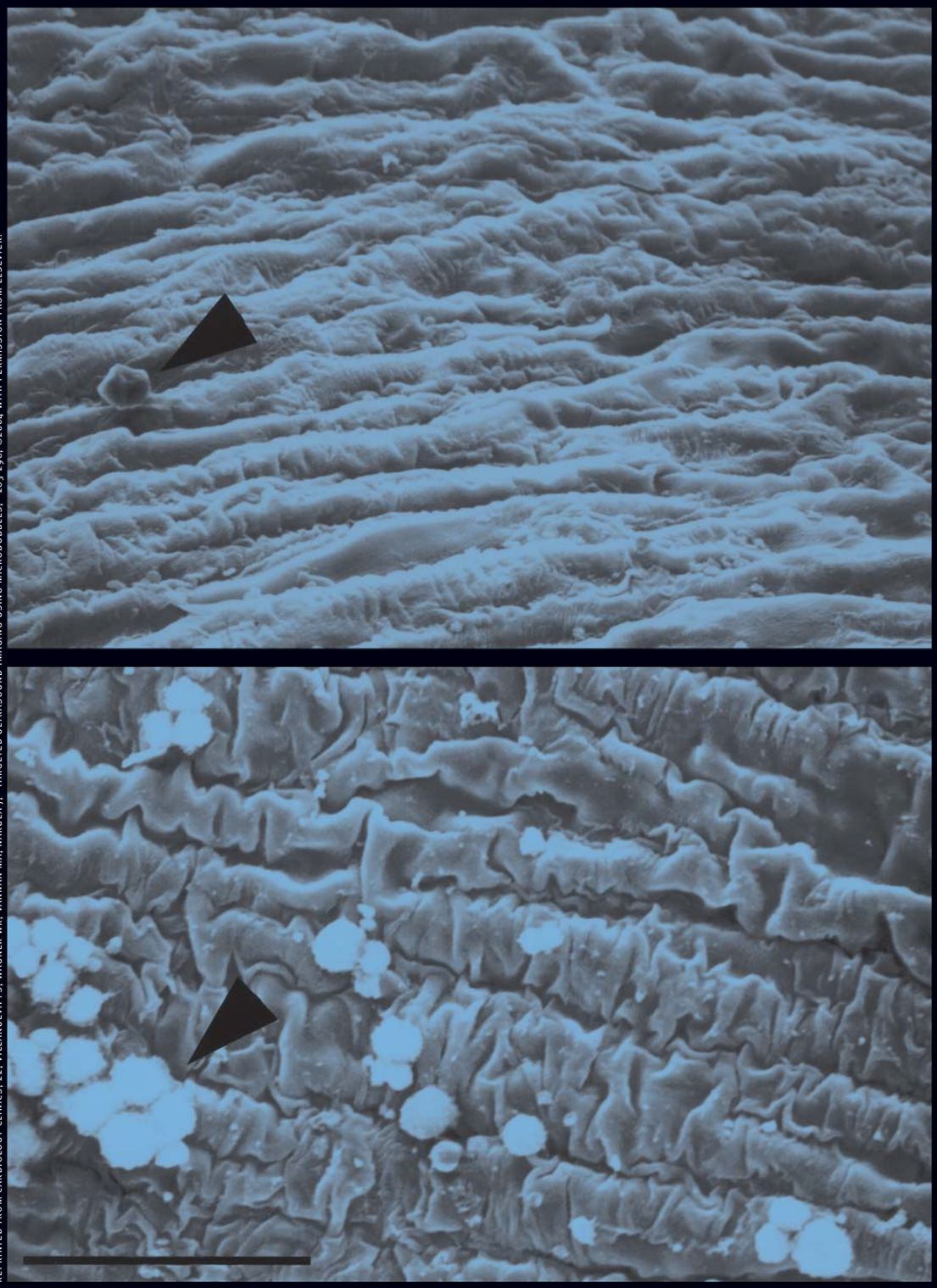
The stranger on the phone wanted to see Flordeliza Villanueva's research.

Gary Brandenburger explained himself: He was living at Family House in Pittsburgh (this was in 1995), while his wife, Linda Brandenburger, waited for a heart-lung transplant. Although she was very ill, Linda, a psychiatric nurse who specialized in counseling people facing medical crises, was keeping busy in the ICU, helping fellow patients and their families. Brandenburger, however, far from their home in St. Louis and his biomedical engineering work at a pharmaceutical company, was looking for something useful to do. A coworker recommended he visit Pitt cardiologist Villanueva.

Villanueva welcomed Brandenburger to her lab. As a cardiology fellow in the late '80s at the University of Virginia, she had studied noninvasive methods of imaging heart disease. It was there that she'd first investigated microbubbles, inert gaseous bubbles smaller than red blood cells. Brandenburger was an engineer for a company that designed the only commercially available microbubbles. These tiny bubbles zoomed through arteries and veins without causing blockage. When used with echocardiography, the sound waves bounced off the bubbles, causing the orbs to glow and reveal otherwise hidden processes like blood flow. Toward the end of her fellowship in Virginia, Villanueva had noticed that the bubbles sometimes clung to injured vascular tissue; this intrigued her.

When she came to Pitt, Villanueva continued to work with the commercial microbubbles, but she wished they consistently stuck to molecules appearing on cell surfaces in the early stages of heart disease. That way, she could produce pictures of disease during its genesis or shortly thereafter. *Imagine*, she thought to herself, *if I could get the bubbles to work, cardiologists could detect early coronary heart disease in people in their 20s—decades before symptoms even developed.* (In cases of atherosclerotic cardiovascular disease, physicians have no way of knowing there's a problem until a patient endures symptoms like chest pain, shortness of breath, fatigue—or until it's too late.) And using such bubbles, physicians would be able to identify early heart disease noninvasively. Villanueva was exploring this idea in the mid-1990s, when no one was producing images of disease on a molecular level with ultrasound, certainly not in living people.

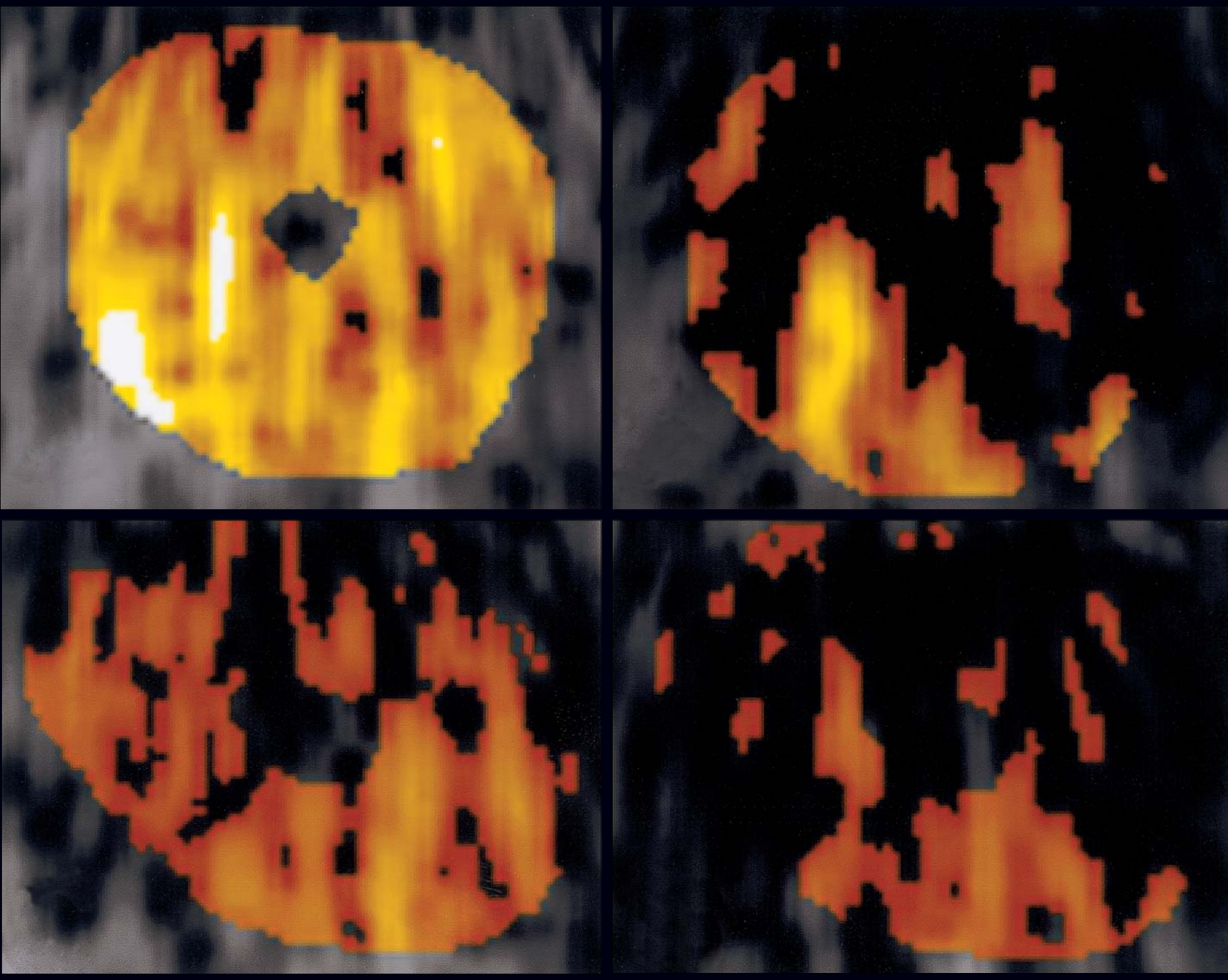
The proposition fascinated Brandenburger, too. After watching Villanueva's research group at work, he said, "Hey, do you guys want to create your own bubbles?"



In the bottom image, microbubbles hang onto a spot on a rabbit aorta damaged by a balloon. The bubbles don't cling to an aorta that hasn't been damaged, as shown in the top image.

REPRINTED FROM CARDIOLOGY CLINICS, 22, VILLANUEVA FS, WAGNER WR, VANNAN MA, NARULA J, "TARGETED ULTRASOUND IMAGING USING MICROBUBBLES," 283-298, ©2004 WITH PERMISSION FROM ELSEVIER.

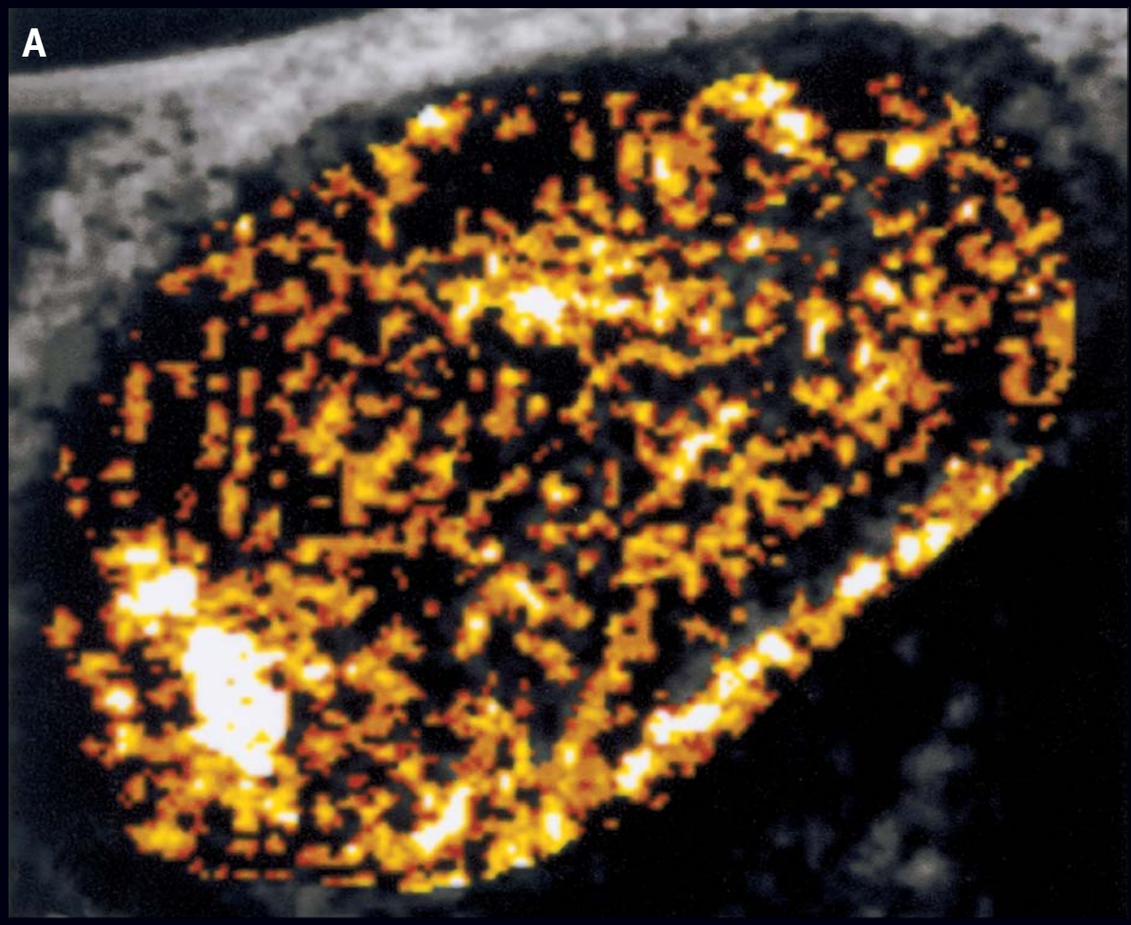
PAGE 25 AND PAGE 22: WELLER GE, LU E, CSIKARI MM, KLIBANOV AL, FISCHER D, WAGNER WR, VILLANUEVA FS, "ULTRASOUND IMAGING OF ACUTE CARDIAC TRANSPLANT REJECTION WITH MICROBUBBLES, TARGETED TO INTERCELLULAR ADHESION MOLECULE-1," CIRCULATION,108, 2, 218-224.

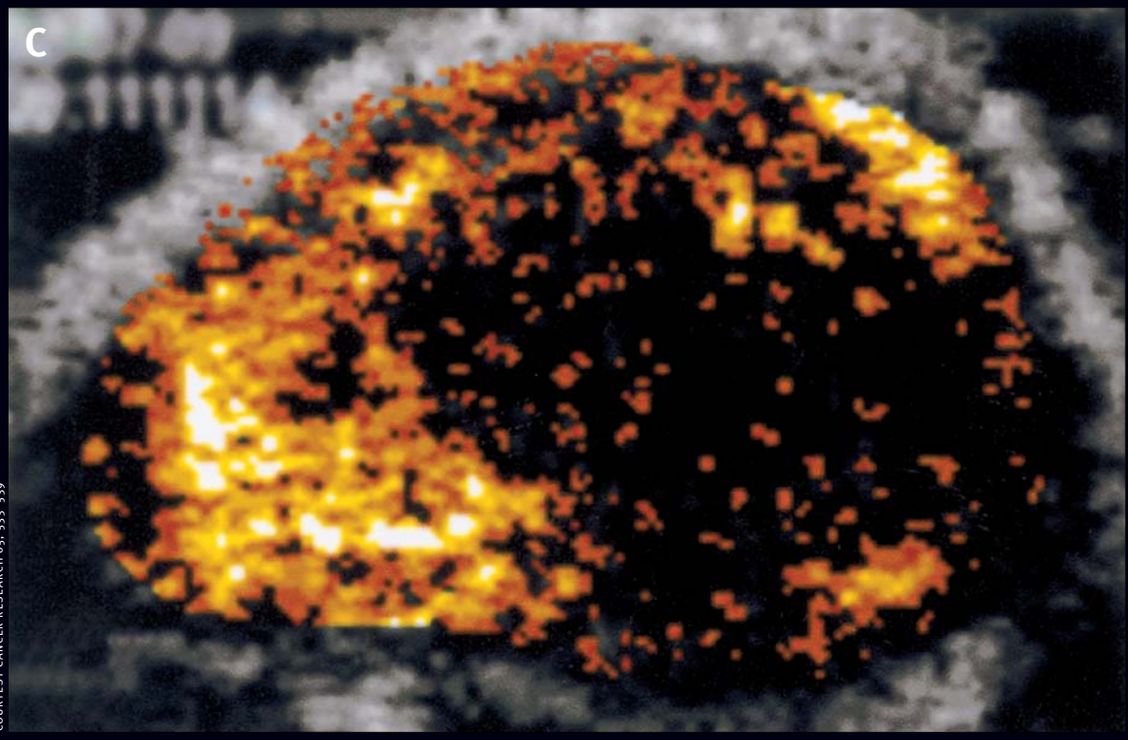


Currently, physicians take more than a dozen biopsies in the first year after surgery to determine a heart transplant recipient's chance of rejection. If the standard of care one day became targeted-microbubble imaging, patients would not be required to go through frequent, unpleasant biopsies. Gregory Weller (MD/PhD '05), as a grad student in Villanueva's lab, investigated this possibility. The top left ultrasound image shines brightly because Villanueva's targeted microbubbles cling to cells rejected after transplant. (Both top images are of rejecting hearts; the bottom images aren't rejecting. Both left images are made with targeted bubbles; the images on the right are made with bubbles that aren't targeted.)

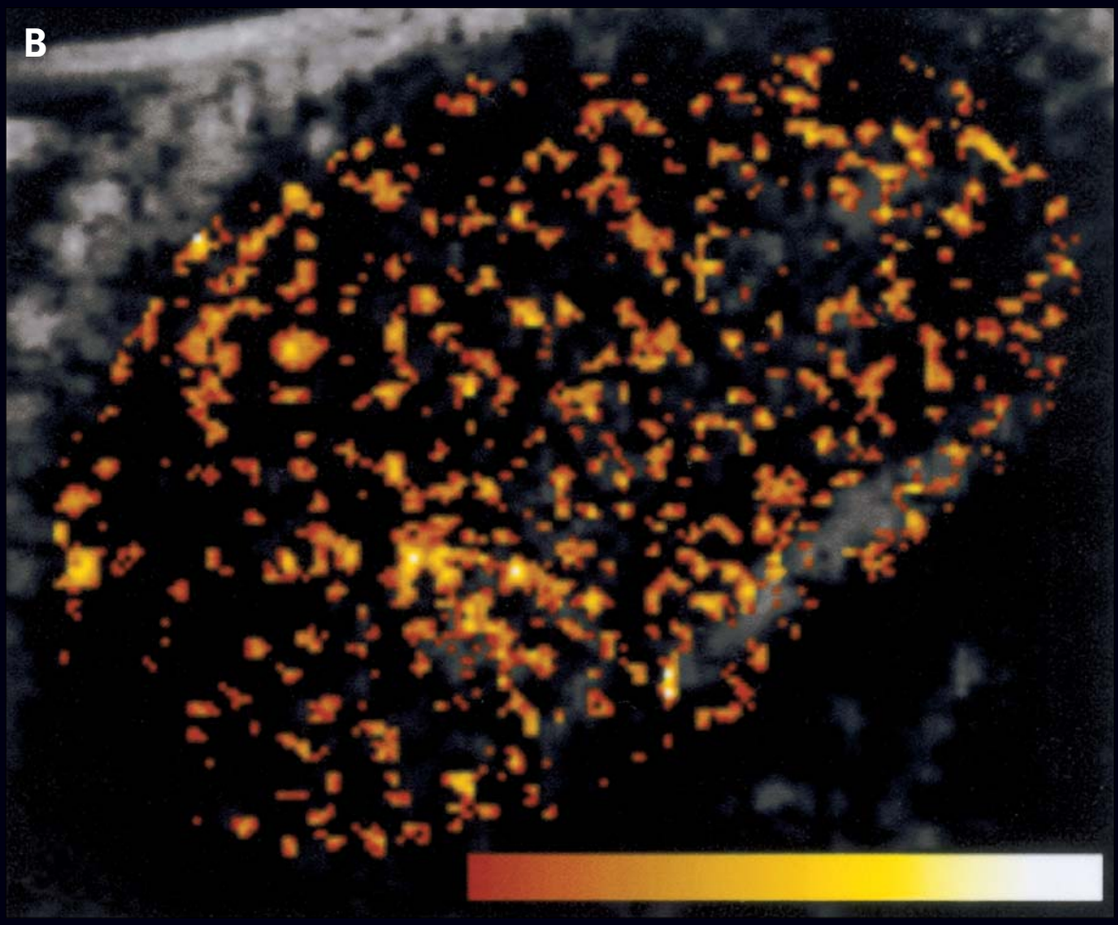
After months of trial and error, Villanueva (who's now an associate professor of medicine at Pitt), William Wagner (now an associate professor of bioengineering, of chemical and petroleum engineering, and of surgery at Pitt), and Brandenburger's colleague in St. Louis, Sasha Klibanov, created a bubble filled with an inert gas. The gas was encased in a lipid shell. Within the shell, the researchers had embedded a protein—an antibody that binds to molecules that appear on the lining of blood vessels in the early stages of disease. In other words, they'd created a bubble that consistently attached to damaged tissue, which otherwise would be hard to tell was damaged.

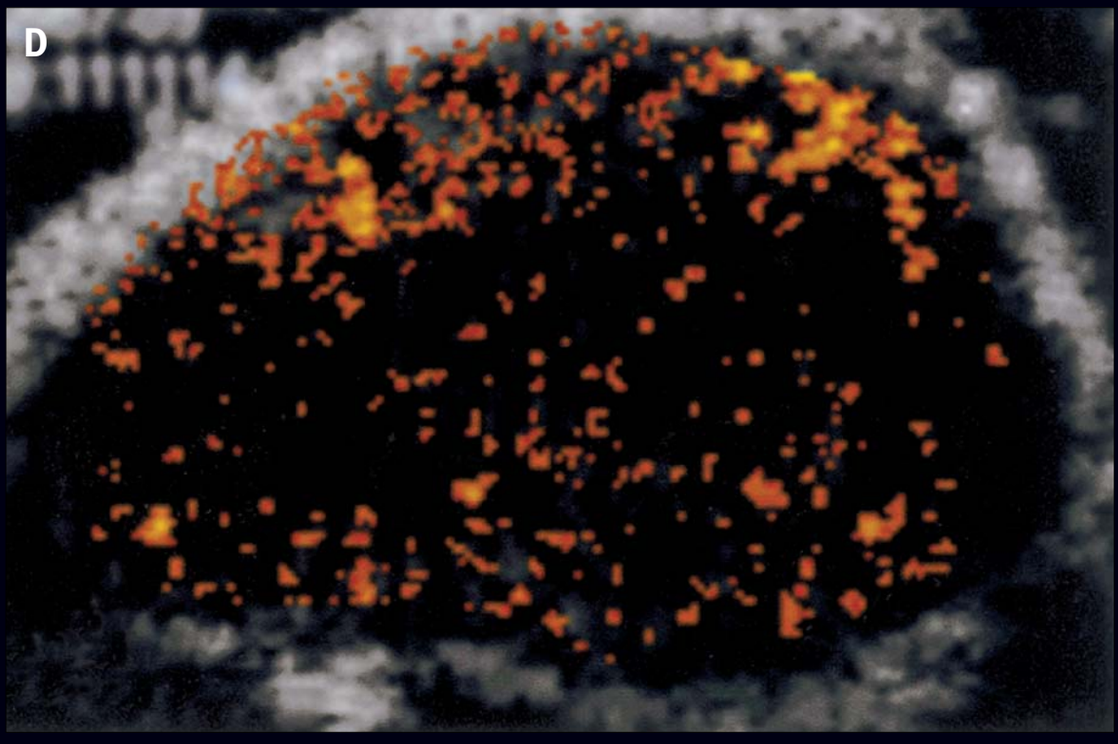
Brandenburger returned to his lab in St. Louis in 1996 after his wife received her new heart and lungs. In 2001, he came back to Pittsburgh with Linda—her body had rejected her lungs. Villanueva stood by his side as they watched Linda take her last breath. Their friendship not only led to the first targeted microbubbles (which are now being refined in animal models), but also to the founding of a charitable organization in Linda Brandenburger's name. Today, Villanueva believes these microbubbles will change how doctors detect other diseases and conditions as well, including transplant rejection. ■





COURTESY CANCER RESEARCH 65, 533–539





After the initial success with targeted microbubbles, Villanueva and University of Pittsburgh Cancer Institute colleagues have come to believe that the bubbles could improve noninvasive imaging techniques for many conditions, not just for cardiovascular disease and transplant rejection. They created microbubbles with a tripeptide embedded in their shells (blood vessels in cancers overexpress molecules that bind the tripeptide). In A, targeted microbubbles stick within a sarcoma engineered in a mouse model, and, when ultrasound is used, the microbubbles light up, generating a bright picture of the tumor. Image B is what a nontargeted-microbubble ultrasound looks like in the same tumor. In C, the targeted microbubbles illuminate a human prostate tumor growing in a mouse. The ultrasound in D is dark because nontargeted microbubbles are used in this same tumor.