Researchers tackle major obstacle to stem-cell heart repair

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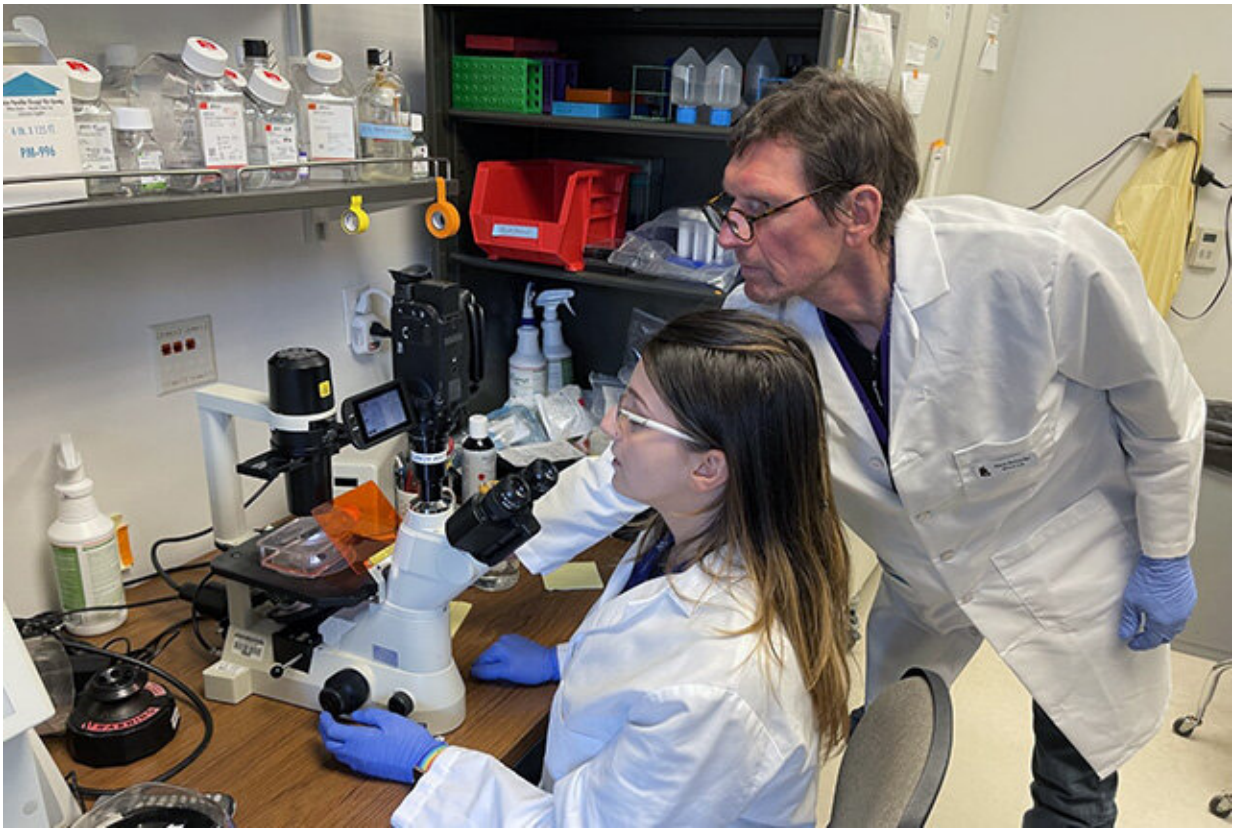

Postdoctoral fellow Silvia Marchiano and research scientist Hans Reinecke look at cardiac stem cells in Chuck Murry's lab at the UW Medicine Institute for Stem Cell and Regenerative Medicine Research in Seattle. Credit: Michael McCarthy

Researchers at the University of Washington School of Medicine in

Seattle have engineered stem cells that do not generate dangerous arrhythmias, a complication that has to date thwarted efforts to develop stem-cell therapies for injured hearts.

"We have found what we have to tackle to make these cells safe," said Silvia Marchiano, a postdoctoral fellow in the laboratory of Chuck Murry at the UW Medicine Institute for Stem Cell and Regenerative Medicine. Marchiano is the lead author of a paper describing the findings published Thursday, April 6, in the journal *Cell Stem Cell*. The work was done in collaboration with the Seattle company Sana Biotechnology.

In previous studies, Murry's team used heart muscle cells created from stem cells to repair heart muscle damage caused by myocardial infarction. This type of heart attack occurs when blood flow to the heart muscle is blocked, thereby causing heart cells to die. Heart cells do not regenerate, so the affected muscle is replaced by scar tissue. This weakens the heart and impairs its ability to pump blood. Severe damage can lead to heart failure and death.

To create their therapeutic heart cells, the Seattle researchers used pluripotent stem cells. Unlike adult stem cells, which have specialized to become specific cell types, pluripotent stem cells can become any type of cell in the body.

From 2012 to 2018 the Seattle team successfully injected pluripotent stem cells into damaged heart walls to create new muscle to replace that lost during an infarction. In animal studies, they showed that the grafted cells would integrate with the heart muscle, beat in synchrony with the other heart cells and improve the heart's contractility. These findings demonstrated that stem cell therapy could potentially be used to rescue damaged hearts.

But there was one major complication. During the early weeks of engraftment, the hearts tended to beat at a dangerously high rate. Unless a way could be found to prevent or suppress this problem, stem cells could not become a safe treatment for myocardial infarction and heart failure.

"Our goal is to create working contractile cells that would not try to set their own pace," Murry said.

In the mature heart, the heart rate is regulated by specialized cells called pacemaker cells. These cells generate electric signals at regular intervals that induce the other heart cells to contract.

In pacemaker cells, the voltage cycles back and forth from negative (hyperpolarized) to positive (depolarized). Murry compares it to a metronome with positive ions swooshing in and out of the cell through these channels. The rate at which this cycle of repolarization and depolarization occurs determines the heart rate.

In early embryonic hearts, however, this system, in which relatively few cells have become specialized pacemaker cells while the rest have become quiescent contractile cells, has not developed. All the cells are pacemakers. Murry and his colleagues suspected that the engrafted stem cells were behaving like early embryonic cells chaotically generating signals and causing the dangerous heart rhythms.

To sort out what was causing these cells to behave this way, the researchers used a technique called RNA-sequencing to find out which ion channels were being made at different times as the cells matured. The sequencing revealed that some types of ion channels appear early in development and then disappear as the cell matures while other types of ion channels appear later in development. Like an unfolding mystery, this gave the researchers their list of suspects.

To determine which ion channels were the culprits carrying the arrhythmia-causing current, the scientists used CRISPR-based genome editing to systematically knock out depolarizing genes or to activate repolarizing genes. This proved surprisingly complex. They had hypothesized that there would be a single ion channel causing the arrhythmia, but none of the single-gene edits eliminated the rapid heart rhythms. The researchers then undertook a painstaking process of "playing the combinations" by performing double and triple gene edits. Vexingly, none of these edits eliminated the arrhythmia, and some seemed to make it worse.

Finally, the scientists created a stem cell line in which three depolarizing genes were knocked out and one repolarizing gene was activated. That did the trick. Cardiac muscle cells generated from these stem cells were electrically quiescent, like adult heart muscle, but they contracted when given an electrical signal to mimic a natural pacemaker. The researchers termed these cells "MEDUSA" (for modifying electrophysiological DNA to understand and suppress arrhythmias). The MEDUSA cardiomyocytes engraft in the heart, mature into adult cells, electrically integrate into heart muscle, and beat in sync with natural pacemaking, all without generating dangerous heart rates. This, Murry says, is the sine qua non for heart regeneration.

Murry cautions that additional testing with the engineered cells will need to be done, but, he adds, "I think we've overcome the biggest roadblock to regenerating the human heart."

More information: Chuck Murry, Gene editing to prevent ventricular arrhythmias associated with cardiomyocyte cell therapy, *Cell Stem Cell* (2023). DOI: 10.1016/j.stem.2023.03.010. www.cell.com/cell-stem-cell/fu … 1934-5909(23)00081-4

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