Tooth root pulp becomes rich source of stem cells

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Dr. Karl Kingsley and Dr. James Mah. Credit: Josh Hawkins/UNLV Creative Services

Stem cells. Few research discoveries hold as much promise of single-handedly expanding medical treatment options as they do. Miraculously able to act as transformers—either re-creating or morphing into a variety of cell types found within the organisms they originate from—stem cells offer humanity hope for new, more effective therapies against a number of chronic and terminal diseases. And finding them is surprisingly easy.

"Stem cells can be extracted from nearly any living tissue," said Dr. James Mah, director of UNLV's advanced education program in orthodontics, doctor of dental surgery, and dental researcher. "In fact, stem cells can even be found in tissues of the deceased."

But in spite of all their potential, there's a catch: "The biggest challenges with stem cells are gathering enough of them to work with and keeping them viable until they are needed," Dr. Mah said.

He and UNLV biomedical sciences professor Karl Kingsley—along with a handful of undergraduate, graduate, and postdoctoral dental students—decided to take on this challenge, cutting their teeth in stem cell research by exploring those pearly whites in new ways. In the process, they developed a new method for extracting large numbers of stem cells they could then preserve from a surprisingly abundant source: wisdom teeth.

"More and more adults—approximately 5 million throughout the country—have their wisdom teeth, or third molars, removed," Kingsley said. "Extracting teeth is relatively common among patients undergoing orthodontic treatments. And the majority of those teeth are healthy, containing viable tooth root pulp that offers opportunities for reproducing cells that have been damaged or destroyed by injuries or disease."

A tough nut to crack

Tooth root pulp is home to two types of prized stem cells. The first, pluripotent stem cells, have the ability to become any cell in the organism from which they're drawn. The second, multipotent stem cells, transform into specific types of cells within that organism.

Knowing where to find these cells was one thing. Recovering them, the researchers knew, would be another.

Common methods for extracting root pulp involve drilling into, removing the top of, or shattering the tooth. Each method has its detriments, Dr. Mah said, all of which lead to a low stem-cell recovery rate: damaging heat from drilling, corrosive elements in the water teeth are rinsed in, contaminating enamel particulates, and more. So the researchers sought to discover how to extract pulp in a manner that consistently produced a higher yield.

"Initially, the answer seemed simple: crack the tooth in half like a nut and remove the pulp," Dr. Mah said.

Unfortunately, teeth have irregular surfaces and non-uniform shapes, so cracking teeth usually produces the same shattering effect as a hammer, thereby reducing the number of viable stem cells.

Happy Ghag, then a dental student working with Dr. Mah and Kingsley on the project, thought he might have solution to the dilemma. He approached Mohamed Trabia (UNLV Howard R. Hughes College of Engineering's associate dean for research, graduate studies, and computing) and Brendan O'Toole (Mendenhall Innovation Program director and mechanical engineering researcher) to discuss fracture analysis.

"Happy had reviewed fracture mechanics literature and decided on a technique that scored the tooth to enable a clean break, similar to the process for custom-cut glass," O'Toole said. After a few discussions, some of Engineering's personnel helped Ghag fabricate the device.

The completed instrument, which the research team facetiously dubbed the "Tooth Cracker 5000," uses a clamp to hold a tooth in position for a cutting tool to score the surface and a blade to crack it. The result: a perfectly halved tooth, with immediate access to undamaged and uncontaminated root pulp.

For O'Toole, this was just another successful collaboration between the two units, as Mechanical Engineering had been interacting with the School of Dental Medicine's orthodontic program for a few years.

"Orthodontics, by definition, is a bioengineering topic," O'Toole said. "They design and place mechanisms in people's mouths that help move teeth into optimum position. The interaction between our departments makes a lot of sense."

With the Tooth Cracker 5000 complete, Dr. Mah and Kingsley tested the fracture rate of 25 teeth, achieving a 100 percent rate of success. The fracture idea and design prototype had worked perfectly.

Excavating for success

Now that the researchers had cracked the challenge of accessing the root pulp, it was on to determining how many viable stem cells they could recover from the fractured teeth. Average pulp recovery rates employing common extraction methods (i.e., shattering, drilling, etc.) come in at around 20 percent, Dr. Mah noted.

It was time to test the mettle of their new fracture method. Dr. Mah and Kingsley dyed 31 fractured teeth pulp samples to highlight any viable stem cells the teeth contained. Dead cells would turn blue when exposed to the dye. Living cells would appear clear.

They looked under the microscope. Eighty percent of their extracted cells remained clear after the dye was introduced.

"Saying the test results were promising is a gross understatement," Dr. Mah said. "We realized we'd invented an extraction process that produced four times the recovery success rate for viable stem cells. The potential application is enormous."

Replicating for a rainy day

After mastering fracturing and extraction, it was time for the team to determine what kind of stem cells could be harvested and how best to store them.

Normal cells within the body typically die after 10 replications or passages, whereas stem cells can replicate indefinitely, Kingsley indicated. To isolate the stem cells from the rest of the root pulp, the researchers harvested cells from the pulp and cultured them on a petri dish. Once the cells covered the dish, they split the culture in half and repeated the process between 10 and 20 times.

By the end of the culturing, all nonstem cells had expired. Kingsley captured the remaining stem cells and collected their ribonucleic acid (RNA), which is converted into proteins that become biomarkers his team could use to characterize each stem cell type and its respective rate of replication.

"Scientists around the world are trying to figure out what type of stem cells can be coaxed into becoming new cells or different tissue types," Kingsley said. "We already know some populations of dental pulp stem cells can be converted into neurons, which could become therapies for cognitive diseases such as Alzheimer's or Parkinson's."

Kingsley noted that teams of scientists around the world are working with animal models to test using stem cells to treat neurological conditions. Early indications, he said, are positive. Although there is still a need for additional tests, Kingsley indicated that the next logical step in this research would be to test stem cells in humans to treat any number of chronic illnesses people face.

"There are potential applications of stem cells for multiple diseases, including cancer, arthritis, and lung disease," Kingsley said. "The next challenge is reliably collecting the stem cells early enough and storing them successfully so they can be used when needed."

Preserving the prize

According to multiple studies, the number of pluripotent stem cells found in teeth decrease dramatically after adults reach the age of 30, Kingsley said. However, people could donate stem cells found in their teeth much like they may donate their blood prior to a surgical procedure or preserve their umbilical cords. If people elected to have their wisdom teeth removed or were having a root canal performed, their stem cells could be harvested at that time and stored for future use.

Creating that possibility has led Dr. Mah and Kingsley to the next step in their research: the cryogenic process.

"There is no standard cryogenesis, or freezing process, for storing stem cells," Kingsley said. "There are multiple organizations that collect and freeze teeth for future studies and use, but there is no evidence about the long-term effects of cryopreservation. We can't answer yet just how long the cells will survive."

In 2011 dental student Allison Tomlin studied different populations of stem cells and their viability after being thawed. Every year since, Kingsley and his team have thawed a portion of Tomlin's sample and evaluated the viability of remaining stem cells. Initial findings—which Kingsley, Tomlin, and R. Michael Sanders (clinical sciences professor in the dental school) published in their *Biomaterials and Biomechanics in Bioengineering* article "The Effects of Cryopreservation on Human Dental Pulp-derived Mesenchymal Stem Cells"—indicate that rapidly dividing cells have higher rates of viability year after year compared to slower dividing cells. If these results remain constant, the stem cells could be sorted before the freezing process based on when they might be needed.

"The work Dr. Kingsley and I are doing is part of a paradigm shift," Dr. Mah said. "Our fracturing process could hasten the collection and cryogenesis process, thereby preserving a high stem-cell count that furthers research into how using these cells can aid healing and potentially cure diseases."

More information: Allison Tomlin et al. The effects of cryopreservation on human dental pulp-derived mesenchymal stem cells, *Biomaterials and Biomechanics in Bioengineering* (2017). DOI: 10.12989/bme.2016.3.2.105

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