Lab-grown organoids hold promise for patient treatments

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Zev Gartner is growing breast organoids with precise ratios of normal and tumor cells (shown at left) to understand how cell-cell interactions contribute to tumor growth. Credit: Photo by Elisabeth Fall, Cell Image by Gartner Lab

Ophir Klein is growing teeth, which is just slightly less odd than what Jeffrey Bush is growing – tissues that make up the face. Jason Pomerantz is growing muscle; Sarah Knox is growing salivary glands; and Edward Hsiao is printing 3-D bone using a machine that looks about

as complex as a clock radio.

Together, these members of the UC San Francisco faculty are cultivating organs of the craniofacial complex – the skull and face – which too often go terribly wrong during fetal development. Deformities of these bones or soft tissues, the most common of birth defects, can cut life short by blocking the airway or circulation. Or they can disfigure a face so profoundly that a child struggles to see, hear, or talk. Perhaps most painful of all, such deformities render children physically other, potentially leading to a lifetime of corrective surgeries and social isolation.

As director of the UCSF Program in Craniofacial Biology, Klein orchestrates a multisite research endeavor to translate basic science findings in tissue regeneration into improved treatments for these kids. Using stem cells from patients with craniofacial deformities, Klein, Bush, Pomerantz, Knox, Hsiao, and their colleagues are growing tiny functioning segments of organs, called organoids, to figure out exactly when and how in fetal development such design flaws occur.

They are among scientists across UCSF who are cultivating cellular systems such as miniature brains and breasts from patient cells. They serve as dioramas of disease – models derived from human cells – either displacing or complementing the mouse models that have served science well, though inexactly, for many years. The effort is one of the most obvious and viable payoffs to date from stem cell science. With these organoids, physicians and scientists can not only trace the pathways of normal and abnormal development, but also test drugs and other treatments for their effectiveness in humans. Organoids are also one tiny step toward the ultimate goal of generating complete organs, as a way to circumvent rejection issues and save the lives of those who now die waiting for transplants.

As the reservoirs of human development, stem cells take it upon themselves to tirelessly renew and differentiate into the myriad cell types required to build out a body from an embryo. In creating an organoid, typical construction metaphors do not apply. There are no building blocks to nail, stack, or solder and no job-site supervisor barking orders. "That's not how biology works," says Zev Gartner, PhD, an associate professor of pharmaceutical chemistry.

"It is a self-organizing process," he explains, a process that starts in the womb with embryonic stem cells (ESCs) or, in the case of organoids, induced pluripotent stem cells (iPSCs). iPSCs are mature cells that are stripped back to their earliest stage of development using a process devised by UCSF Professor of Anatomy Shinya Yamanaka, MD, PhD, who won a Nobel Prize for discovering the process. To make organoids, iPSCs are put through a series of solutions, then added to a gel that mimics the squishy 3-D cellular matrix of the embryo. The gel provides the right conditions for them to get to work.

"Take an organ like the lung. Its basic functional units are a tube and a sac, and outside that sac are capillaries that allow gas exchange. Hundreds of millions of tubes and sacs make a lung," explains Gartner. "You can make the little sacs and the tubes in a dish as an organoid model. But we don't know how to drive the self-organization of those units into much more complex, elaborate, highly ramified structures." The fundamental limitation of organoids is that they lack the vasculature that brings nutrient-laden blood to fuel the evolution of the larger structure.

Gartner notes that people who work with stem cells tend to focus on either regenerative medicine or disease modeling. Those interested in disease make models of tissues so that they can understand how diseases work, while those interested in regenerative medicine try to make models of healthy tissue that could be transplanted. Gartner straddles

both camps. He grows breast organoids. "The mammary gland is great because we can simultaneously think about these two phenomena as two sides of the same coin," he says. "One is regenerative medicine through self-organization, and the other is understanding the progression of breast cancer through a breakdown in self-organization."

So there's potentially a triple payoff in stem cell science: By deducing how a breast forms itself, Gartner might figure out how to grow the entire organ. By tracing how cancer throws a wrench in the works, he may be able to target ways to stop that process. And by growing a human organ in a dish, he avoids making cross-species assumptions or putting animals or humans at risk in testing potential drugs to cure breast cancer, greatly accelerating the push toward a cure.

Regenerate

On Klein's team, Jeffrey Bush, PhD, an assistant professor of cell and tissue biology, looks at organoids through the lens of disease.

The organoids he grows model craniofrontonasal syndrome – a birth defect that is caused by a mutation in a single gene and that dramatically impacts the shape of the face and head. He knows from studies reproducing craniofrontonasal syndrome in mice that the first place something goes wrong is in a cell type called the neuroectoderm. To create an organoid to study this, he obtained skin cells from Pomerantz, an associate professor of surgery, who has patients with the syndrome who were willing to donate tissue samples. Such collaborations between basic scientists and clinicians are key to bringing research out of the lab and into patient care.

"We studied this simple system to see how this mutation affected the organization of these cells," says Bush. His group has filmed cells as they rush about to self-organize when they're mixed together. In those films,

he explains, "you can see that the mutated cells, which are dyed red, segregate from the normal cells, which are green – they are like oil and water." In other words, the mutated cells completely disrupt the behavior of all the cells. By contrast, in the films of cells without the mutation, all the cells circulate easily among one another, like fish in an aquarium. This understanding has allowed Bush to begin to think about a drug that blocks this separation. He has several promising candidates that his team will test in pregnant mice. "Right now," he says, "there isn't a single drug that we can use for any kind of structural birth defects. If we could show that a medication blocks the effects of this mutation, it would serve as proof of principle that something besides surgery can be done. But we would have to know that it was safe for mother and child and that we could catch it early enough."

Reconstruct

Jason Pomerantz, MD, a plastic surgeon, falls into the regeneration camp. His clinical work is typified by a recent eight-hour operation on a 17-year-old boy with Crouzon syndrome, a severely disfiguring condition affecting every organ in the craniofacial structure – muscle, bone, and skin. "My patient is excited for the outcome, but not about the process," says Pomerantz, surgical director of the UCSF Craniofacial Center. For three months, the patient will wear a large metal frame on his head with wires that will pull the bones in his face forward. Prior to the surgery, the boy's face was nearly concave, collapsed inward at the nose.

Yet bone is not all Pomerantz needs to work with to restructure a face. The subtle bends, creases, and curves of expression that make a face one's own are the work of tiny muscles. "Right now we can move a big muscle – say, from the thigh to the face – so that people can smile," he says. "But we can't reconstruct the fine ones that enable people to move their eyebrows up or move the eyeballs around. That requires little

muscles. This is where we can make headway with stem cell biology.

"We have actually made a humanized organ in an animal," he continues, pointing to a picture of a mouse on his wall. Pomerantz is now considering incubating small human muscles in animals for use in his patients' faces. In a recent project, he inserted stem cells from human muscles into a mouse whose own muscle stem cells had been incapacitated. He then perturbed the muscle to stimulate regeneration. As the muscle healed, the cells created new muscle tissue, which the mouse's nerves innervated to make a functioning muscle. It's exactly the size of the muscles Pomerantz needs for full articulation of expression and function in a human face or hand.

Create

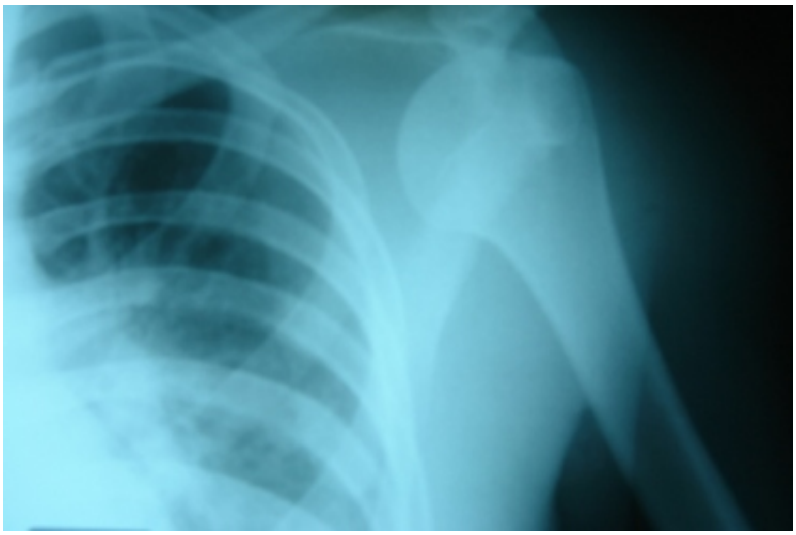
Muscles are part of a vast and intricate system strewn throughout the body. Teeth, on the other hand, are islands unto themselves. "Teeth intrigue me from a regeneration perspective," says Ophir Klein, MD, PhD, chair of the Division of Craniofacial Anomalies, the Hillblom Professor of Craniofacial Anomalies, the Epstein Professor of Human Genetics, and a resident alumnus. "They are discrete organs – all the parts are there." More intriguing still is the fact that many rodents have the ability to grow their front teeth continuously. Elephants and walruses also have ever-growing tusks, and even some primates – lemurs – can regrow their teeth.

A tooth can be regenerated in parts. Stem cells can be used to grow the root, and then a crown can be added to complete the tooth. To generate a whole organ at once, Klein's colleagues are planning to partner with bioengineers who can produce a biocompatible material that could serve as a framing device to jump-start the creation of dentin, one of the hard components of a tooth. If they start with the right cells, then the scaffolding will give the cells the shape information they need to create

the right design. But even that isn't Klein's endgame. "In my lab, we're interested in figuring out why humans can't regrow teeth," he says. "In studying species that can, we hope to unlock the regenerative potential in our own cells that might be turned off."

Klein's work to generate teeth is inspired by his patients with ectodermal dysplasia, a congenital disorder characterized by lack of sweat glands, hair, or teeth. Being able to generate the roots of teeth would be remarkable for these patients, since the rest can be done with a crown. Right now, they must be fitted with dentures.

Klein is also taking another tack to help these patients. "We completed a clinical trial of a drug that basically goosed up the development of the organs when they weren't forming properly," he says. The drug – a protein developed by Swiss collaborators of Klein's, based on studies of embryonic mice, who develop these organs in early- to mid-gestation – was given to infants with the disorder right after birth. The trial was unsuccessful. Now, scientists in Germany are running a trial of the same drug, giving it instead to mothers carrying babies with this genetic disorder. The scientists will try to gauge what the best timing is for delivering the drug.



"What's great about this drug is that it doesn't seem to have any effects on any other organs besides teeth, hair, and sweat glands," says Klein. "Drugs for other conditions are far riskier, because they affect pathways that are important in the development of many organs."

Maintain

Sarah Knox, PhD, an assistant professor of cell and tissue biology, is using stem cells to figure out how to regenerate salivary glands compromised by radiation treatments for head and neck cancers or by craniofacial deformities. Her focus is on how the environment contributes to the activation and maintenance of the gland. The salivary gland, like all organs, is continuously replenishing the supply of cells and tissues it needs to function. Knox's research shows that the gland takes directional cues from nearby nerve cells not only to remain functional, but also to continuously replace itself. Her organoids are made of cells from a patient and nerve cells (ganglia) from a fetal mouse. "We are trying to explore the relationship between the stem cells and the nerves," she says. "How do the nerves know the tissue is there? How do the nerves provide instruction and feedback? Individual cells die off and new cells have to replace them. Organoids are giving us insight as to where those new cells are coming from and how we keep repopulating [them] all our lives."

As head of the UCSF Program in Craniofacial Biology – which is based in the School of Dentistry and the Division of Genetics in the School of Medicine – Klein stands at one of science's most compelling crossroads: regenerative medicine and genetics. Far in the future, both fields have

potential that seem like science fiction today. We live in a world where people die waiting for organ transplants. What if we could pull these organoids from their petri dish and supply them with the fuel they need to become full-blown organs? Such a feat would necessitate either a host embryo – perhaps from a pig, because pigs have organs the size of human organs – or some other biological foundation. Some scientists are hoping to jump-start organ development with "scaffolding," or cells engineered to speed the developmental process. Others are zeroing in on the genome, particularly in kids with craniofacial anomalies caused by just one mutation, like craniofrontonasal syndrome; for example, a tool called CRISPR could allow scientists to splice that gene out and replace it with a normal gene. But the tool has yet to be used in humans, let alone a human fetus.

Ethical questions pepper either route. At their best, stem cells regenerate tissues; at their worst, they go rogue and grow into a tumor. "Yet with gene editing tools like CRISPR, you literally have the potential to change the species," says Klein. And in both scenarios, the cells can act with unforeseen off-target effects. Klein and his colleagues are in continual discussion about the repercussions of their work with the director of UCSF Bioethics, Barbara Koenig, RN, PhD '88. "Gene therapy is an example of an exciting new treatment that cured one serious pediatric illness – severe combined immunodeficiency syndrome (SCID) – but the genes unwittingly led to the development of leukemia," explains Koenig. "Genetic and stem cell interventions must be painstakingly studied before application. And, once they are ready, who will regulate them? There are many questions yet to be answered. The challenges are most extreme when we talk about modifying an egg or sperm cell, where the changes are passed on to the next generation."

So Klein and his colleagues proceed with caution, curiosity, and awe. "The next decade will be an incredibly exciting time," says Klein. "With continual advances in human genetics and developmental and cell

biology, we hope to be able to make drugs and use genetic tools to appreciably change the lives of our patients."

The Bone Printer

Bone grows like a runaway train in Edward Hsiao's patients with fibrodysplasia ossificans progressiva (FOP). The slightest bump or injury can set off a spurt of bone growth that can fuse their vertebrae, lock their joints, or even freeze up their rib cages, leaving them unable to breathe.

No one, to date, has successfully engineered bone. Hsiao, MD, PhD, is hoping to spark the process with the help of a 3-D printer from Organovo, a firm that specializes in bioprinting technology. From iPSCs, he can make many of the essential ingredients of bone, including mesenchymal stem cells, endothelial cells, and macrophages. "We are putting cells into the equivalent of an ink. Then we will print the structures with the ink, let the ink dissolve, and leave the cells," explains Hsiao. "The hope is that the cells can then recapitulate the normal developmental process."

If the approach is successful, Hsiao hopes to use the resulting models to test drugs and other treatments to halt or prevent bone deformities. Down the line, his progress also stands to transform bone and joint replacements. Through his work with FOP, he's uncovered one mechanism that drives rapid bone growth. "In these patients, we know that mature bone formation can happen in as quickly as two weeks, so it is possible to grow bone in an adult. We need to understand how to modulate that," says Hsiao. "Someday, my dream would be to be able to identify the cells we need, give someone a drug that induces the right genes and recruits the right cells to the correct site, and have the cells rebuild the joint from scratch."