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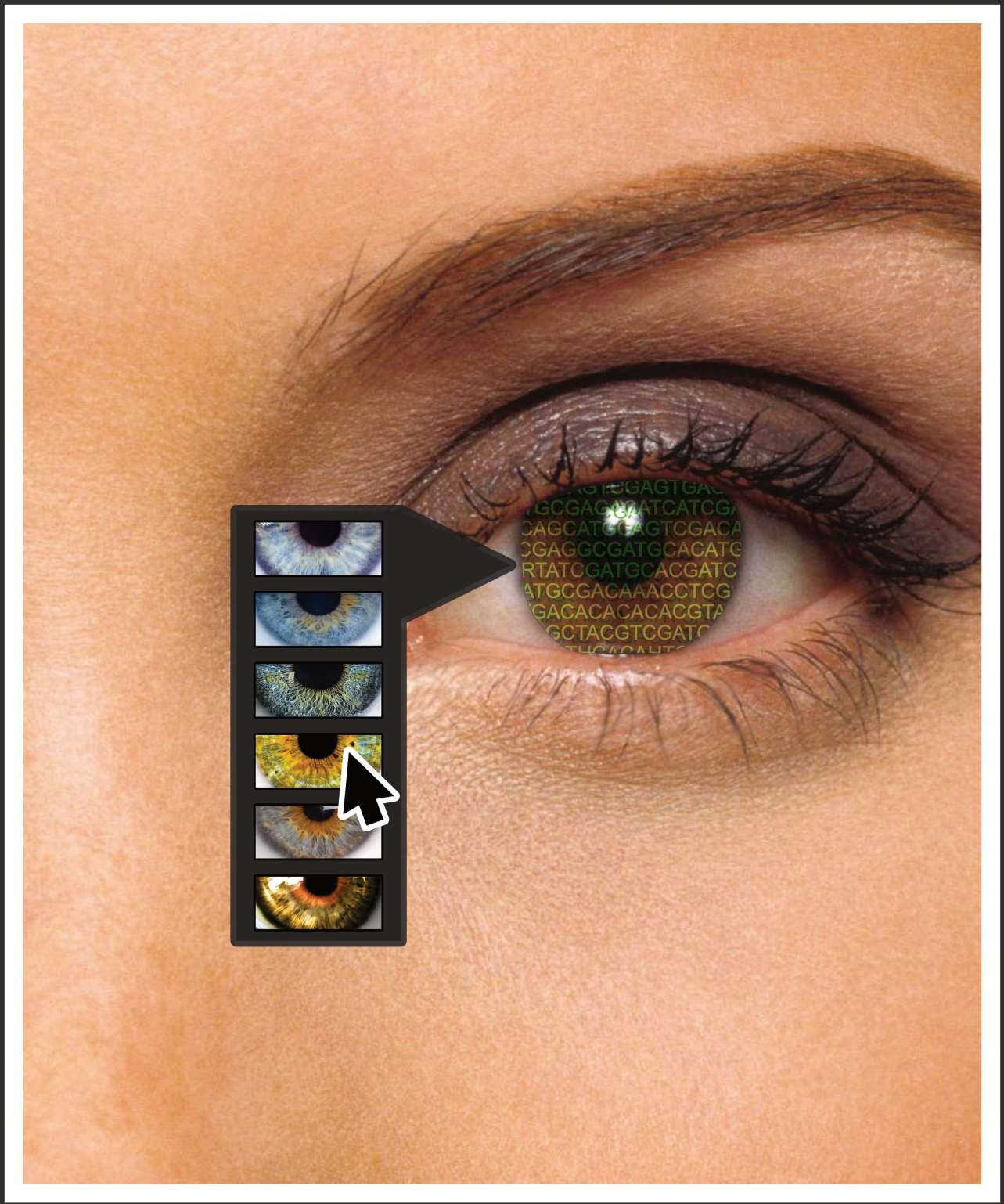

CRISPR:
Mankind's Hand in Fate

Denatured Journal | Issue 1

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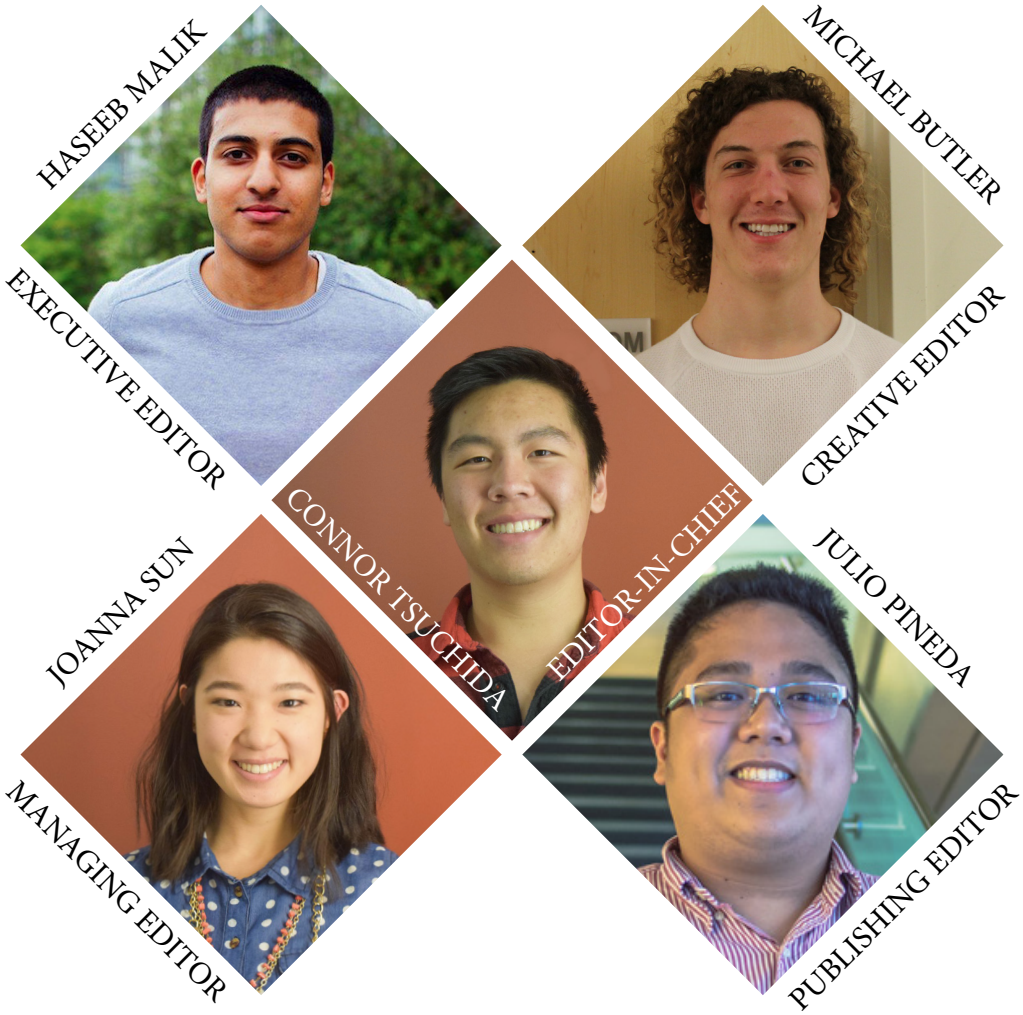

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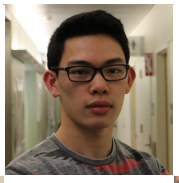

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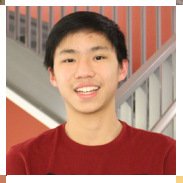

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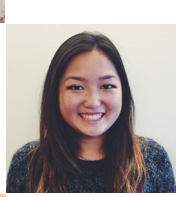

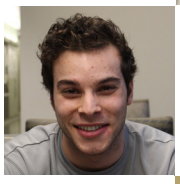

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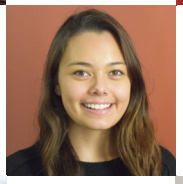

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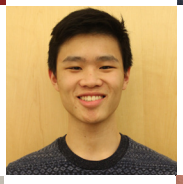

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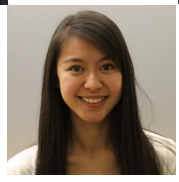

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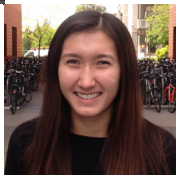

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ISSUE NOTES

FROM THE EDITOR

To say this journey has been a challenge is an understatement. To say I am thankful for each individual who has toiled hours writing, designing, brainstorming, and editing is an even bigger understatement. I have been incredibly humbled throughout this creative process and feel extremely indebted to everyone who made this vision a reality.

To be completely honest, I had no idea what I was doing when I started Denatured. In fact, I don't think any of us officers knew what we were getting into, the frustration we would face, and the ultimate product we would create. All we knew was that we had a vision. A passion. A drive to share the fields of biotechnology and medicine we so passionately study and research. While the University of Washington has an immense number of resources and opportunities avaliable for anyone to explore, we found a gap that needed to be filled. No where on campus could we find easily accessible, engaging, and informative writing on biotechnology and medicine. And so the idea for Denatured was born.

Over the past year, I witnessed that spark ignite, grow, and eventually catch fire into a registered student organization seeking to bring their passions to anyone willing to listen. Many of the usual challenges came with the territory of writing an inagural issue. Everything was new. We made mistakes. I made mistakes. Still, numerous people weathered the storm with me captaining this year long voyage, and I am insurmountably thankful for those who did. We had our challenges, but in the end we produced something I am extremely and sincerely proud of. I hope you enjoy reading this journal as much as I enjoyed creating it, and I hope it unravels some of the complex and revolutionary research being done in biotechnology and medicine.

Connor Tsuchida

THE ART

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Breaking Barriers: Michael Butler & Connor Tsuchida
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SPECIAL THANKS

We would like to extend our gratitude to the following people for their assistance, mentorship, and belief in our mission and journal.

Dr. Dianne Hendricks
Dr. Suzie Pun
Dr. Elizabeth Nance
Dr. Alyssa Taylor
Kelli Jayn Nichols
Holly Williams
Dr. Karen Thickman

Dr. Suzie Pun is the Robert F. Rushmer Professor of Bioengineering and Adjunct Professor of Chemical Engineering at the University of Washington. She obtained her PhD in Chemical Engineering from the California Institute of Technology, and her bachelor's degree from Stanford University also in Chemical Engineering.[1] Following her graduate accomplishments in developing directed polymer-based drug delivery vehicles, Pun worked as a Senior Scientist at Insert Therapeutics, founded by her advisor to commercialize Pun's work. She came to UW as an assistant professor three years later in 2003. Pun is a renowned researcher and instructor, with numerous awards for her research and teaching including the prestigious Presidential Early Career Award for Scientists and Engineers (PECASE) and recognition in MIT Technology's "Top 100 Young Innovators" in 2002.[2] Pun's more notable research includes PolySTAT, an injectable polymer that strengthens clotting with a single injection, and Targeted Axonal Import (TAxI), a peptide that can transport a protein into motor neurons for treating ALS. Denatured Journal sat down with Pun to learn more about her life path and what advice she has for current students.

Getting to Know Dr. Suzie Pun

Joanna Sun, Leo Lansky, Larry To, Natasha Paranjapye

Q. Why did you choose to pursue degrees in Chemical Engineering?

A. Bioengineering did not exist as a major at Stanford when I was in college. Stanford Engineering had a program that covered all music tuition charges for engineering majors. At the time, I played the piano and organ and was deciding between Chemical Engineering and Chemistry as a major due to an inspiring chemistry teacher I had in high school, so I went ahead and declared Chemical Engineering in freshman year. I figured that I could always switch majors, but ended up really enjoying ChemE.

Q. You joined the UW Bioengineering department as a faculty member in 2003, and have been here for thirteen years now. Why did you choose UW BIOE in the first place?

A. UW BIOE is incredibly collaborative and with a lot of biomaterials experts like Allan Hoffman, Buddy Ratner, and Patrick Stayton. Also, the Med School is right here for us to do medically oriented research.

Q. Did you always know you wanted to do academia?

A. No, I didn't know for a while. I began to consider academia while working at Insert Therapeutics. Working in industry gave me the maturity and practice that I needed to build the confidence to enter academia. I love what I do now and even if I were to win the lottery tomorrow, I wouldn't leave.

Q. Do you have any advice for students considering academia as a future path?

A. Work in a lab and get to know other people in your field. Exposure is important and allows one to learn from mistakes. Read broadly and be curious. I believe that some of the most important characteristics of a successful researcher are fearlessness, strong work ethic, good communication and scientific integrity. I advise my own kids to go into a career that is "the intersection of what you like and are good at".

Q. You've had a lot of incredible research projects; where do ideas for research come from and how do you decide on what project to go forward with?

A. In the early days, I had lots of time to dedicate purely to brainstorming but I no longer have that luxury. Now, new ideas for our research are usually generated during meetings with students or faculty members from different fields. I also try to keep up with literature and seek ideas from other areas. I look for projects that are built on our previous expertise of lab members and collaborators and, of course, contain potential impact and will make it clinically, like PolySTAT.

Q. What's the next step for PolySTAT?

A. We are raising funds and writing grants for more in vivo testing, so that we can transfer the technology clinically. Eventually we hope to get enough data to move toward clinical trials.

Q. Is there a research project that you are excited about, but hasn't yet gained nation-wide recognition like PolySTAT has?

We have been working on TAxI, a peptide that delivers material to the spinal cord. This originated from a pet project that I came in as an assistant professor and wanted to do. The idea is to hitchhike on motor proteins to shuttle drug delivery vehicles around the cell.

Q. What were some major challenges you faced developing TAxI?

A. Originally, we were trying to rationally-design a peptide for hitchhiking our delivery vehicles to motor proteins, but we worked on it for four years without success. One of my graduate students decided to go for a library screen (a molecular data bank for protein design) and then a postdoc, Drew Sellers, used phage display directly in the animal to try to find suitable peptides, which worked.

Q. What is the progress on this project so far and where do you see it going?

A. Since we have showed that we can get protein into the spinal cord, our next goal will be testing on whether we can deliver a therapeutic protein. Another goal is to get a broader delivery to the CNS (central nervous system). To treat degenerative diseases, you would ideally want to get the drug throughout the CNS. The current method is intramuscular injection that could only deliver drug to a localized area of the spinal cord. We are finding other ways of administration or types of peptide that provide broader delivery.

Q. Outside of research, how do you like to spend your free time?

A. Before I had children, I liked listening to music, reading, and running to stay healthy. But now, looking after my children (twins) takes priority over my other hobbies. To maintain a work-life balance, we have to know our priorities. As we get more things to do, we say no to more things.

Q. Where are you hoping to take your research in the distant future?

A. I would love to see my research going forward and making it clinically. I think that macromolecule-based drug delivery can revolutionize treatment. It's a whole new class of drug that has been relatively under-explored because of past delivery issues.

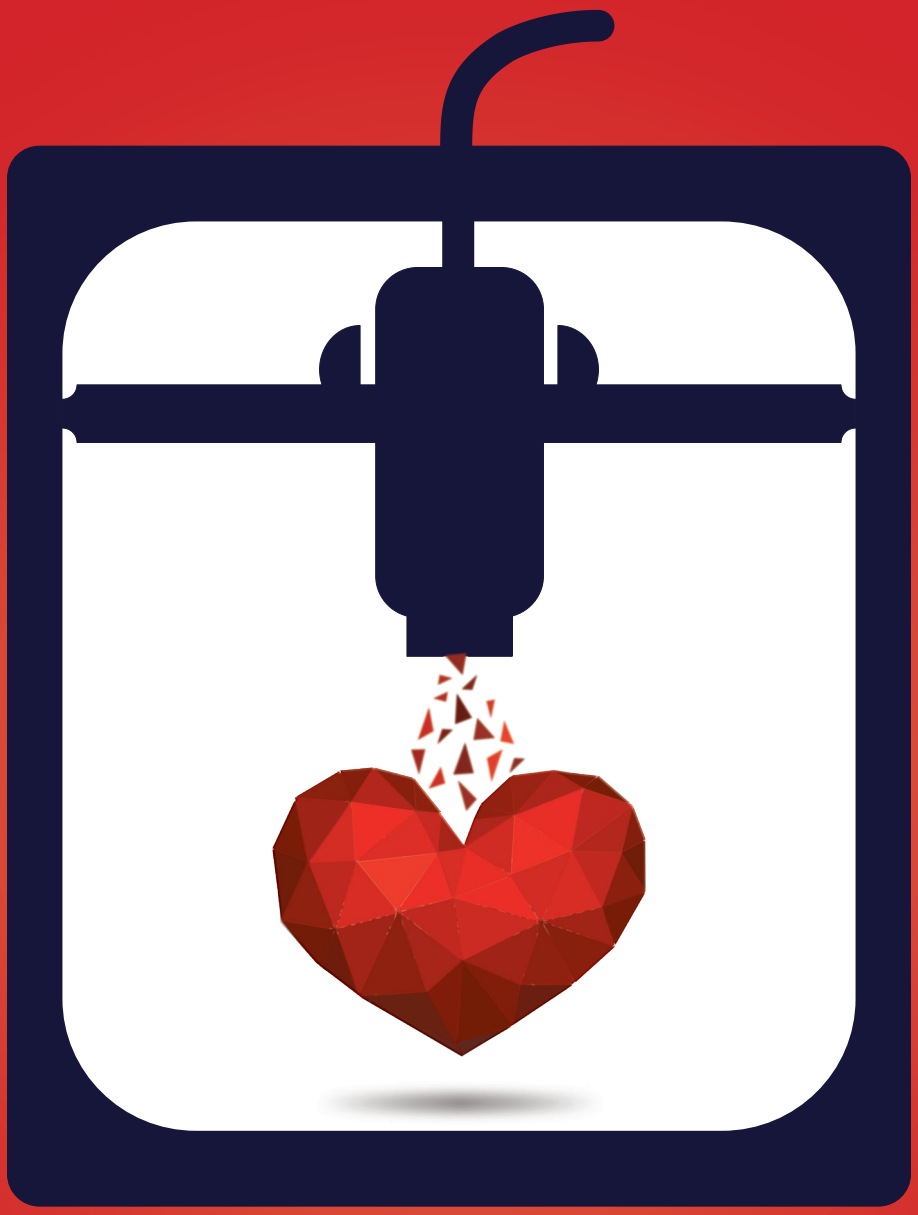
Name:
PolySTAT

Function:
Engineered blood clotting polymer

Name:
TAxI

Function:
Small peptide to shuttle proteins into the Central Nervous System

Want To Learn More?
Check out the Pun Lab website!



PRINTING THE THIRD DIMENSION OF MEDICINE

Connor Tsuchida, Annapurni Sriram, Brianna McIntosh,
Erin Ichinotsubo, Alexander Novokhodko

Entering the Third Dimension

What desperate patients currently wait months to years for, could soon be made in days. What use to be adapted for the body, can now be made of the body. Aside from prototyping and manufacturing, 3D printing, has found its niche in biotechnology and medicine. Whether printed to enhance, support, or replace components of the human anatomy, 3D printing has brought medicine to a place once reserved for science fiction.

The history of 3D printing is as rapid as the manufacturing process itself. In just three decades, the technology has evolved from layering plastic prototypes to printing functioning organs, capturing the imagination of hobbyists and medical researchers alike.

In 1986, U.S. Patent 4575330 was issued to Charles Hull for "An Apparatus for Production of Three-Dimensional Objects for Stereolithography." [1] Hull's first printer utilized photopolymers: materials that transform from a liquid to a solid under UV light.[2] In a vat of liquid photopolymer, Hull's machine laser cured two-dimensional cross sections from liquid to solid, layering the sections to create a three-dimensional object.

FDM, along with stereolithography, was envisioned to revolutionize the manufacturing industry, not the biomedical field. Pioneers foresaw their machines replacing manufacturing lines, not saving lives. Even Charles Hull, when asked what surprised him most about his invention, said: "To me, some of the medical applications. I didn't anticipate that…[4]" Still, much to the surprise of 3D printing pioneers, biomedical researchers have adapted 3D printing to solve some of medicine's largest problems.

Recreating Lost Parts

Leopard geckos grow back whole tails and zebrafish can regenerate massive portions of a damaged heart, yet compared to the rest of the animal kingdom, humans poorly regenerate. While broken bones and cuts heal, lost limbs or failing organs are gone for good.
Worst-case scenario: the organ is vital and death is imminent. This is the case for the 122,000 people nationally who are currently waiting for organ donations as their vital organs fail.[5]
Best-case scenario: the loss of limb or organ only reduces the victim's quality of life.

This is the case for the 1.7 million people living with limb loss in the United States alone.[6]
Either scenario, the piece of human anatomy is gone forever without any chance of growing back to restore function… at least not naturally.

> ***"To me, some of the medical applications. I didn't anticipate that…"***

Just five years later, Scott Crump and his company Stratasys developed fused deposition modeling (FDM), a now equally popular method for 3D printing.[3] FDM works like a traditional hot glue gun, but replace the operator's hand with a precise computer and the arts and crafts for solid three-dimensional objects. Rather than using UV light to cure material in layers like stereolithography, FDM heats thermoplastics to a semi-liquid state and extrudes a two-dimensional cross section onto a stage. The printer then continues this process, adding layer upon layer of quickly curing thermoplastic until the object is created.

A possible solution to these problems lies at the intersection of 3D printing and medicine. Rather than printing airplane parts, biomedical engineers sought to print prosthetics, implants, tissues, and even functioning organs. While huge limitations in the regenerative abilities of the human anatomy challenge medical scientists today, 3D printing presents an enthralling possible solution.

Printing For The Body

3D printing is revolutionizing the way that biotechnology interfaces with the human body. While much of the human anatomy does not regenerate, researchers and engineers have tapped into the seemingly endless potential of 3D printing to create replacements for failing human anatomy.

While not living tissue, these replacement parts offer similar structure and function to greatly enhance quality of life. From prosthetics to surgical implants, 3D printing is ushering in a new realm of cost-effective, individualized, and innovative healthcare solutions.
In the case of Draje Josevski, an Australian man suffering from an advanced case of chordoma (a rare spinal cancer), 3D printed titanium vertebrae were a second chance at life.[7] Chordoma left his spine riddled with malignant tumors and showed no signs of stopping – without new vertebrae his prognosis was poor.

Still, medical pioneer Dr. Ralph Mobbs was able to implant custom titanium printed vertebrae to replace the cancer-riddled spine. The surgery to implant the 3D printed vertebrae – the first of its kind – took 15 hours and required Dr. Mobbs to detach Josevski's head from his neck, implant the printed vertebrae, and then reattach the head and neck. The successful surgery and new vertebrae allowed Josevski to live a normal cancer-free life.

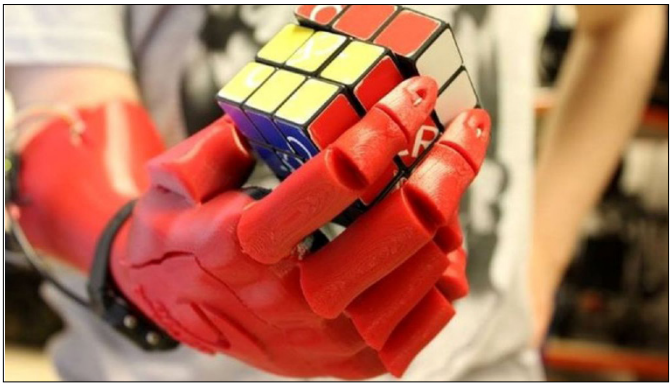
Similarly, another first-of-its-kind surgery at the University Medical Center (UMC) in Utrecht, Netherlands successfully saved a life by utilizing rapid and customizable 3D printing.[8]

A young woman with Van Buchem Disease, a fatal disease where a thickening skull significantly impacts brain function, was brought to UMC Utrecht after experiencing vision loss.[9] To treat her, Drs. Bon Verweij and Marvick Muradin successfully replaced the patient's abnormal skull with a 3D printed model in a 23-hour surgery. After the 3D printed skull was implanted, the patient was able to gain normalcy in her life, and even miraculously regained her vision.

In addition to 3D printed surgical implants helping people from within the body, 3D printed prosthetics is a burgeoning field within medicine that utilizes 3D printing to replace outer portions of the body.

Open Bionics, an English startup company, is equipping hand amputees with comfortable prostheses. The prostheses look very similar to hands and use myoelectric sensors to allow the wearer to control the prosthetic. The customizability that comes with 3D printing is extremely important in creating individualized prosthetics for people of all shapes and sizes.

In addition, these 3D printed prostheses offer a more affordable option to conventional prostheses: the Open Bionics hand prosthetic cost around $2,900 compared to the conventional prosthetic cost of around $50,000.[10]



Open Bionics 3D printed prosthetic hands could be an affordable and customizable option for patients with limb loss.

Printing *THE* Body

While plastic or metal replacements can save and enhance lives, the question lingers: can we do better?

The fundamental components of the human body are cells, which layer to form tissues. Rather than plastic or metal replacements, researchers sought to make cellular replacements. Using living cells, researchers hoped to create replacement anatomy as close to the original biological structure as possible.
The basic strategy involves 3D printing a scaf-

fold to assist with cell growth. Much like how vines require a wall to grow up, cells require a solid structure to grow into and around. By 3D printing this scaffold structure in the desired geometry, and then covering this with cells, a customized tissue can be grown.

Scientists at the Wake Forest Research Institute in North Carolina have used these techniques in human trials.[11] The team 3D printed an artificial biological bladder for actual human cells. Cells from the patient's failing bladder were removed and placed on a bioprinted bladder-shaped scaffold, which encouraged growth into the proper organ geometry. By employing the patient's own bladder cells to regrow an artificial organ of the same structure and function, the medical solution was uniquely personalized.

Similarly, researchers at Heriot Watt University in Edinburgh, Scotland adapted the technology to create a 3D printer that can print the cells themselves.[11] Rather than just printing the scaffold base and growing cells on top, their 3D bioprinter is delicate enough to print cells as well, integrating them into a more complex cell structure.

Whether the cells are seeded on top of a printed scaffold or printed by the bioprinter, utilizing patient specific cells could greatly reduce the chances of organ rejection. Organ rejection is a major problem faced when a patient's immune system does not recognize the "foreign" transplanted organ and mounts an immune attack. Since 3D printed organs would be made of the patient's own cells, not the cells of an organ donor, this problem would be avoided entirely.

Where Can I Get One?

Given the amazing potential of 3D printed organs, why aren't they accessible yet? Why are 3D printed hearts not available and why is there still an organ transplant waiting list?

There are two types of challenges facing 3D printed organs. First, there are technological limitations to the complicated process of 3D bioprinting organs. Second, even for the organs we can print, there are regulatory and financial barriers to commercializing the technology to a large scale.

While research on 3D bioprinting of organs has exploded in recent years, scientists and technology still can only print relatively simple organs. Bladders and tracheas consist of one or two cell types and have a relatively simple physical structure. On the other hand, many patients are currently waiting for heart, kidney, and liver transplants. These organs have many more cell types, are much more complex structurally, and have uniquely difficult features to replicate. Printing these organs is, for now, regarded as unfeasible.

To address this difficulty, researchers have found a way to "recycle" damaged organs like hearts, kidneys, and livers that are ineligible for transplant. The old cells can be removed from the organ structure (decellularized) leaving just the structural matrix behind. The patient's own cells can then be reinserted into the decellularized structure, and the new organ can be grown. While this does not involve customized 3D printing of organs, it does allow medical researchers to "regenerate" complex human organs.

Another technical challenge is delivering oxygen to cells within the artificial organ. Cells require oxygen to live, and in the body blood vessels create a well-developed network to supply every cell with this essential element. Without the vascular network to transport oxygen, tissues and organs – including 3D printed ones – suffocate and die. Currently researchers face significant challenges with printing vasculature, which in turn hinders the creation of printed organs.[12]

Finally, we come to the regulatory and financial challenges. Gaining FDA approval for such a radically new technology is challenging. In order to guarantee the safety of the technology, massive clinical trials and testing need to occur over a multi-year span. While researchers have been able to print amazing biological organs, it will likely be years – if not decades – before these printed organs will be deemed safe for the general public.

Tengion, a company founded by regenerative medicine pioneer Dr. Anthony Atala, holds the patent for bioengineered bladders in the US and is in the process of acquiring FDA approval. The expensive and lengthy regulatory process forced the company into bankruptcy in 2014, though it was bought back, allowing its research and efforts to obtain FDA approval to continue.[13]

What's Next?

Some time from now, 3D bioprinters could be found in every major hospital around the world. Emergency rooms would have one on stand by, ready to rapidly print any organ, device, or implant on command.

The advantages of this approach are speed and personalization. For patients waiting for a lung transplant, 27% died waiting for organ transplants in the first year.[14] 3D printing can reduce a year's wait to just hours, potentially saving these lives.

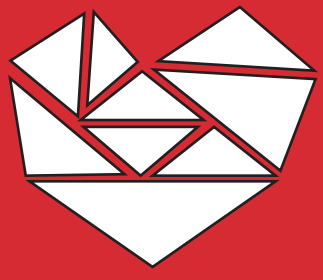
In addition, humans are incredibly unique, and vary widely from person to person. Patients waiting for a transplant often have a hard time finding a donor who has a tissue match. Prosthetics and implants also need to fit perfectly in order to avoid later complications with function and compatibility. 3D printing could eliminate this challenge by providing customized medical solutions. In order to fit unique individual specifications, 3D printing can create organs, implants, and prothetics precisely to the desired size and geometry.

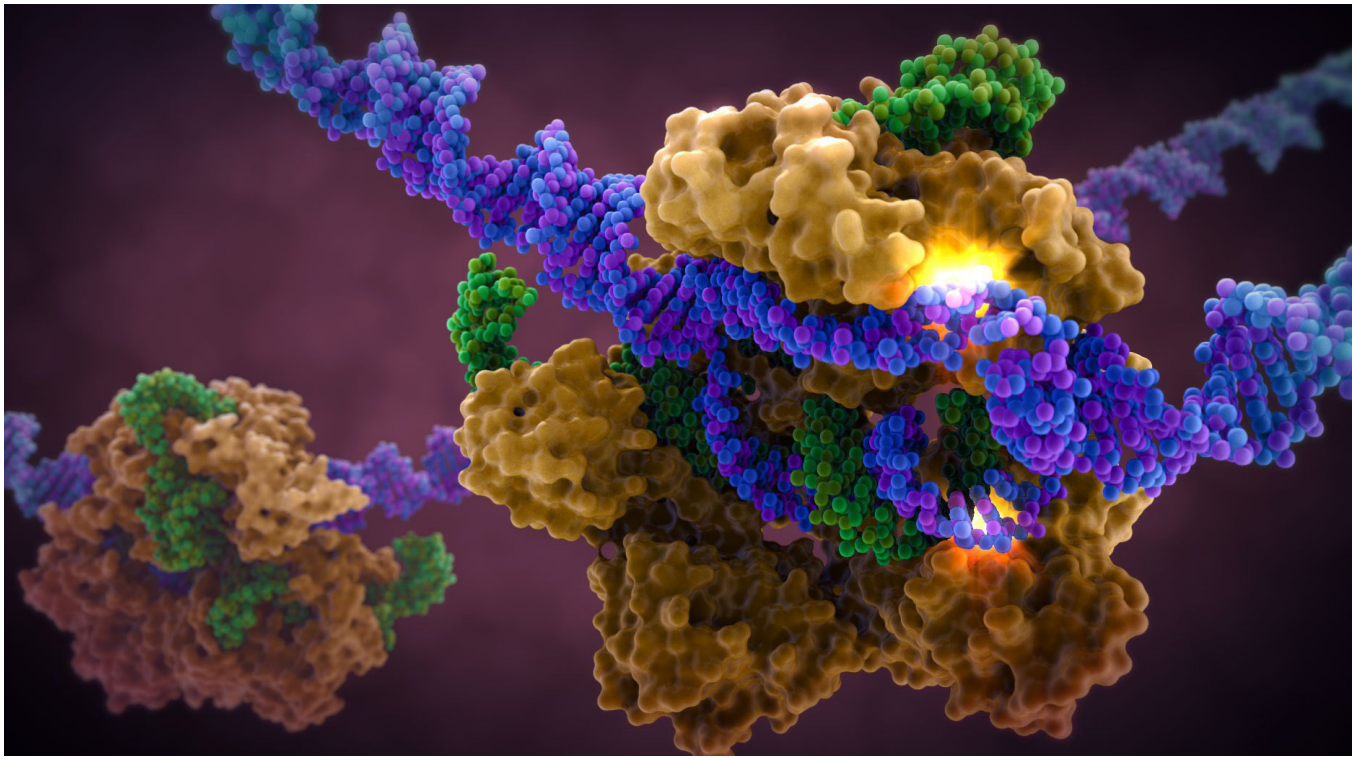
While amazing medical advances have been made thanks to 3D printing technology, formidable barriers have yet to be overcome, stalling the large-scale reproduction of these technologies.

> ***"3D printing can create organs, implants, and prothetics precisely to the desired size and geometry..."***

The complications recreating every functional characteristic of a human limb or remaking the complex environment of a human organ, paired with the monetary and regulatory challenges are grand.

Still, researchers are enthused by the amazing medical technology printed everyday. By continuing to perfect and discovery the capabilities of 3D bioprinting, researchers hope to solve physiological failure.





CRISPR: Mankind's Hand in Fate

Haseeb Malik, Julie Pham, Nina Reese, Kayla Hogan, Neil Gerstenmaier, Leonard Chen

A baby with his brown eyes, a baby with her curly hair. Every prospective parent has hopes for his or her children, long before they are even born. Is it any surprise that parents hope their child could have every advantage in life? Intelligence, charisma, an outgoing personality. These are all traits people hope their children will possess. However, many parents' hopes are even more basic than what the child will look like or what their personality will be; their only wish is for their child to be born healthy and happy.

When this hope is shaken, and the health of a child is called into question before that child ever takes a breath, a parent is faced with some of the hardest decisions of his or her life: either cling to hope or prepare for the worst case scenario. Even then, they may learn their hopes are false, that the life they envisioned for their child is not possible and they must reevaluate how they prioritize the things they once took for granted.

What if there was a way to prevent this heartache? A way to flip back the switch and restore these parent's hopes? What if you could select specific traits that your child would possess? This idea has fascinated society for generations; from novels such as Aldous Huxley's A Brave New World to movies such as GATTACA, our society has questioned time and again what would happen if we had the power to control our genes, and to some degree our fates. While this has been an interesting hypothetical, it has been pushed into the forefront of the scientific world with the advent of a study in China, led by Junjiu Huang, in which a group used a technique known as CRISPR/CAS9 to edit the genes of human embryos.[1] Although Huang's study has been discontinued because of issues with using CRISPR, this is the first time in history that CRISPR technology has been used to edit human embryos, a landmark in the journey towards gene editing.

When did something that seemed to belong solidly in science fiction become a tangible reality?

The Scientist Behind it All

Jennifer Doudna became a scientific celebrity when she discovered a method to edit an organism's DNA; however, her rise to fame began during her undergraduate education. She received a Bachelor's in Chemistry at Pomona College[2] before obtaining a Ph.D. in Biochemistry at Harvard, and did post-doctoral work at the University of Colorado. Since then, she has been on the faculty at Yale University and University of California, Berkeley, where she has pioneered groundbreaking research and earned notoriety.

Jillian Banfield of UC Berkeley recruited Dr. Doudna to collaborate on her research after Banfield found that the genomes of microbes living in a highly acidic environment are made up of repeated sequences. These clusters are called "clustered regularly interspaced short palindromic repeats," or CRISPR. They came from viruses that had infected the bacteria and were integrated into the bacteria's genome, which allowed the bacteria to recognize the virus in any future infections.
Dr. Doudna, along with Dr. Emmanuelle Charpentier from Umea University in Sweden, revealed how this was possible through the discovery of the Cas9 protein and guide RNA. These components are used to cut DNA at specific locations and allow DNA sequences to be deleted or added. Dr. Doudna then demonstrated the ability to use this natural process to alter a genome in a test tube. While genome editing methods already existed, the CRISPR/Cas9 method is simpler and more streamlined. CRISPR has the potential to cure genetic diseases or even create designer babies.

Not since the advent of the polymerase chain reaction (PCR) has the field of genomics been so stunningly influenced by a technology

Both the medical and ethical implications for the possible applications of CRISPR/Cas9 have put Dr. Doudna's work in the spotlight not only in the scientific realm but also in the mainstream media. Since these revolutionary findings, Dr. Doudna has been noted among Time Magazine's 100 most influential people in the world, and has been invited to events teeming with Hollywood stars. While she has achieved celebrity-status, Dr. Doudna does not seem to be cashing in her retirement early. She recently received $1.5 million from Paul Allen to continue her research on modifying the CRISPR/Cas9 system to attack RNA rather than DNA. All eyes will be on Dr. Doudna as she strives to contribute another medical breakthrough for the fight against genetic diseases.

Although Dr. Doudna was the first to start researching CRISPR, she was not granted the patent, leading to a prolonged legal battle on who actually owns CRISPR.

Or is She…?

Jennifer Doudna and her team used the CRISPR-Cas9 system to target particular genes of their choosing in 2012, filing a patent in March 2013. By the time she had filed the patent, this system was being used in human cells by another group, leading to the potential for use in gene therapies. CRISPR

gained popularity among multiple companies, one of which was led by Feng Zhang, a researcher at the Broad Institute and the Massachusetts Institute of Technology.

The United States Patent and Trademark Office (USPTO) currently has a first come, first serve system: the first person to file the patent will be granted the patent, regardless of whether they were the first to invent it or not. However, this was only put in place after both parties had filed a patent, making an older rule which stipulates that the first to conceive an idea or put the idea to work will be granted the patent. Zhang filed a patent under a special expedited process and he received the patent first, giving his research center commercial control over its development.[3] Doudna's original patent had yet to be reviewed.

In 2015, Doudna's team requested the USPTO to determine who developed CRISPR first. When a patent is contested, the process resembles a court case, in which evidence from both labs is reviewed to determine who the rightful patent holder is. Usually, patent disputes are settled through sharing rights to an invention, but this patent dispute does not seem as compromising as other disputes. Cases like these could take years, costing millions of dollars.[4] Even if a decision is reached, an appeal can be made, drawing out the process. Greg Aharonian, the director of the Center for Global Patent Control, can "see many hundreds of thousands of dollars being spent." [5] There have been previous cases of patent holders placing a fee for taking a license out on patented technology, even among academic researchers. However, the impact that this patent dispute has on those utilizing CRISPR has not yet been determined.[6]

The confusion of who owns the rights to the technology may be potentially hindering commercial efforts, delaying products and treatments. Others claim the patent fight is taking the attention away from the most important aspect: the science. The emphasis of the patent is on rights over the technology use and potential Nobel Prize awards, rather than looking at how new methods and applications can be developed with all of this time and money. Already, the number of publications on CRISPR is predicted to surpass 1,100.[5]

Due to CRISPR's capabilities and potential, the stakes are high with this patent dispute, with only one winner for the rights of the CRISPR system. With the growing attention this technology is receiving, it is easy to forget that CRISPR is not the first gene-editing tool to date.

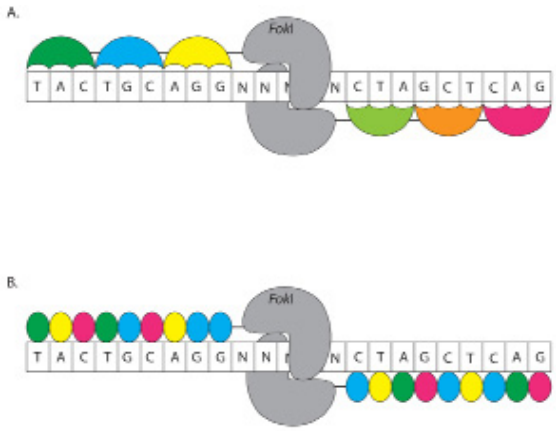


Illustration with binding proteins in color and nucleases in grey, showing (A) zinc finger nuclease and (B) TALENs.

B.C. — Before CRISPR

Nucleases have two major components: the first component is the binding protein that recognizes a specific region of the DNA and the second component is the nuclease, which "snips" the DNA and breaks it apart.[7] At this point, a scientist may be able to incorporate a new strand of DNA into the genome. CRISPR uses an enzyme called Cas9, which is a gene-editing nuclease, an enzyme capable of cleaving DNA into its two component strands. Although it now seems to be the nuclease of choice for gene-editing, researchers were initially looking at other options. The first two gene-editing nucleases being explored prior to the discovery of CRISPR are called zinc finger nucleases and TALENs.[8]

Zinc Finger Nucleases consist of Zinc Finger Proteins, which make up the binding region

of the nuclease; each protein binds to three specific nucleotides. If a Zinc Finger protein fails to bind correctly to its target nucleotide triplets, then the nuclease will be unable to snip the DNA. In this way, the DNA strand is like a barcode, and the Zinc Finger proteins are the scanner which can only react if it finds one barcode.

TALENs relies on a similar idea. In TALEN, the binding component of the nuclease is composed of Tal effector proteins, which each bind to one specific nucleotide. This makes it easier to construct TALENs over Zinc Finger nucleases because each tal-effector protein is paired with a specific nucleotide.

Zinc Finger nucleases have been challenging to construct at an affordable price while maintaining both high activity and low cytotoxicity.[9] Although TALENs has a low cytotoxicity risk, it has high mutation rates; in other words, it is prone to making mistakes when cutting and adding a DNA sequence to the genome.[3]

"CRISPR has the potential to outperform TALEN and Zinc Fingers in both accuracy and speed"

Furthermore, both Zinc Fingers and TALENs require a new nuclease for each location of interest in the genome, making the protocol tedious and inefficient. The process also requires an optimization step to optimize binding to the region of interest, which is time-consuming.

CRISPR has the potential to outperform TALEN and Zinc Fingers in both accuracy and speed, gaining the attention of scientists worldwide.

The Mechanism Behind All the Magic

The CRISPR-Cas9 enzyme complex, at its core, allows for specificity in cutting DNA sequences and opens up the genome for insertion of desired DNA sequences. It is found naturally in the bacterial immune system as a defense against retroviruses, phages, and viral genome vectors. The basic mechanism involves the CRISPR system using Cas9, the primary endonuclease, as "scissors," which are guided to the desired site through the use of guide RNA (gRNA).[10]

gRNAs are short RNA transcripts that have a binding site to Cas9 and a complementary binding site for the DNA being targeted. As long as the appropriate gRNA can be created, the Cas9 enzyme can find and target the DNA. Creating the most effective gRNA involves knowing the sequence of the target region on the genome in order to build a unique sequence complementary to the target. The Cas9 complex can then successfully bind to the target DNA and the magic can begin.

Using the complementary base pair binding granted by the gRNA, the Cas9 activates and cuts that sequence of DNA.[11] Once the cut has been made, the cell will try to repair this gap using DNA polymerase, but this procedure is error-prone and leads to many mutations. Instead of relying on this natural process, researchers can provide a template for the cell to use to create a new sequence, effectively editing the DNA.

This is the main motivation behind using CRISPR: to be able to edit sequences of DNA and potentially remove and repair deleterious alleles that cause disease. Additionally, the wonder of the CRISPR complex is that the gRNA can be modified while the Cas9 does not need to be modified to function with a new gRNA, so they can be used on more sequences of the genome, and multiple CRISPR complexes can be active simultaneously, increasing the efficiency of gene editing.

While Cas9 is being touted as the endonuclease that can do it all, researchers believe there is an alternative option in Cpf1.[12] Cpf1 is a smaller endonuclease than Cas9, making it more effective at entering and working inside cells. Additionally, Cpf1 gives researchers better control over the insertion of the DNA. This method, spearheaded by Feng Zhang of the Broad Institute in Cambridge, Massachusetts, is promising, but understanding whether it is truly applicable is still in the early stages.

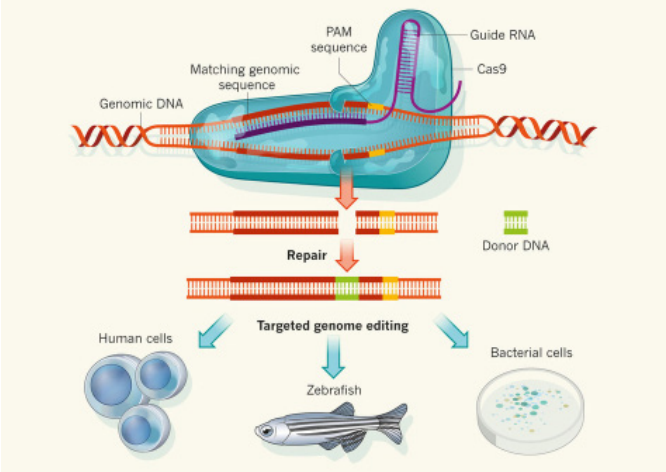
The Promises (and Perils) of CRISPR-Based Technology

Not since the advent of the polymerase chain reaction (PCR) has the field of genomics been so stunningly influenced by a technology as has been recently with CRISPR-mediated gene editing. The emerging applications and theoretical future of this class of techniques, founded upon select methodological patents merely from the last three years alone, are poised to bring about a wealth of industries valued in the billions of dollars. Broadly speaking, this unfolding science will empower research in three fields: medicine, biotechnology, and fundamental biology research.[13]

Perhaps the most immediate impacts and benefits of CRISPR technology on human society will come from the field of medicine. Increasingly, medicinal research, both in understanding diseases and improving drug efficacy, relies on our capacity for manipulation on the nanoscale of genetics.

Every aspect of biology is encoded in the genome which instructs a given cell to synthesize the biomolecules necessary for its particular role in an organism. The tremendous diversity of life on the planet flows from differences between species' genetic instructions. Moreover, the ever-challenged survival of a particular genome through reproduction forces an organism to adapt. In an era of little threat from the tusks and teeth of antiquity, our primary enemies are the ubiquitous microbe and the genes involved in heritable diseases and predispositions as well as aging. To meaningfully address these afflictions will require reliable and precise molecular tools, and CRISPR-mediated gene editing promises exactly this.

Using CRISPR, researchers can do more to probe the relationship between genes and disease. Because of CRISPR's unprecedentedly high target-specificity and in vivo (within a living system) efficacy, the current state of gene therapy stands to benefit immensely from this technology.[13] For example, a particular drug for cancer may be ineffective in 40% of the population of lab mice while markedly effective in the rest. One method for understanding this incompatibility would be to alter a single or set of genes of a cell with CRISPR and study its effect on drug action. This way, when certain gene alterations inhibit drug activity, they can be categorized and accounted for in future drug design. In drug research more generally, CRISPR can be used to save time and money by introducing genes that ensure, for example, that a lab mouse will develop a specific type of cancer for therapeutic trials.[14]

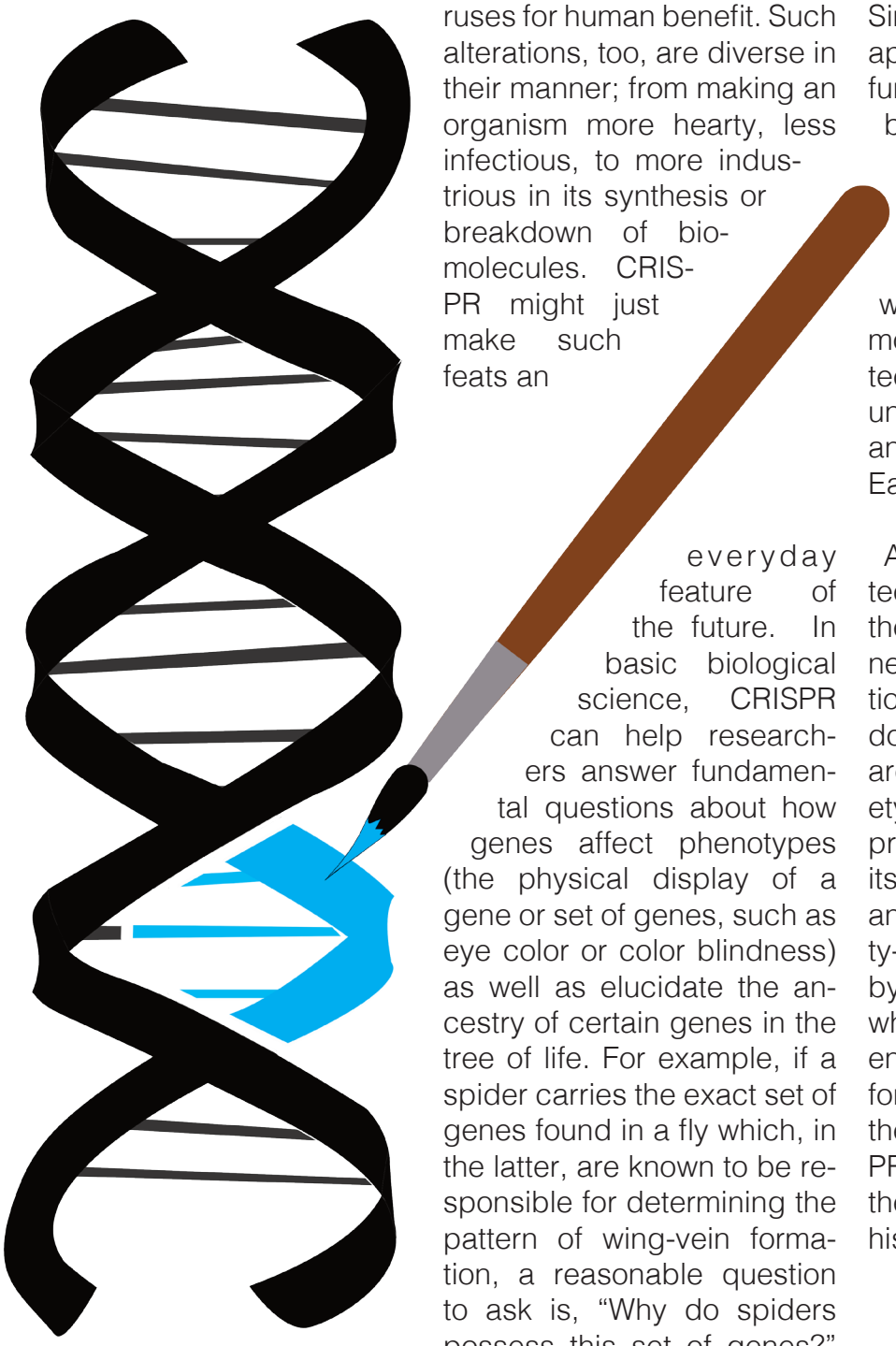


The many uses of CRISPR, revolutionizing research in the biomedical field.

Ostensibly, CRISPR can even be used to modify embryonic DNA to prevent developing mice, or even humans, from inheriting gene varieties known to be linked to certain health complications. Progress here, however, is likely to be slow considering the ethical conversations and yet-to-be-drafted guidelines necessary for such practice.

Although CRISPR is essentially just a way to cut and paste genes, innovative thinkers in the field of bioengineering have proposed using it to create so-called "gene drives." Generally, a given gene or segment of genes gets passed on by 50/50 chance from either one's mother or father. Still, some genes can manipulate cell division so as to ensure their own replication. This is referred to as biased-inheritance. A proposed gene drive involves linking select genes of chance with genes of certainty, thereby ensuring the continued replication of any sequence chosen by the scientist.[14]

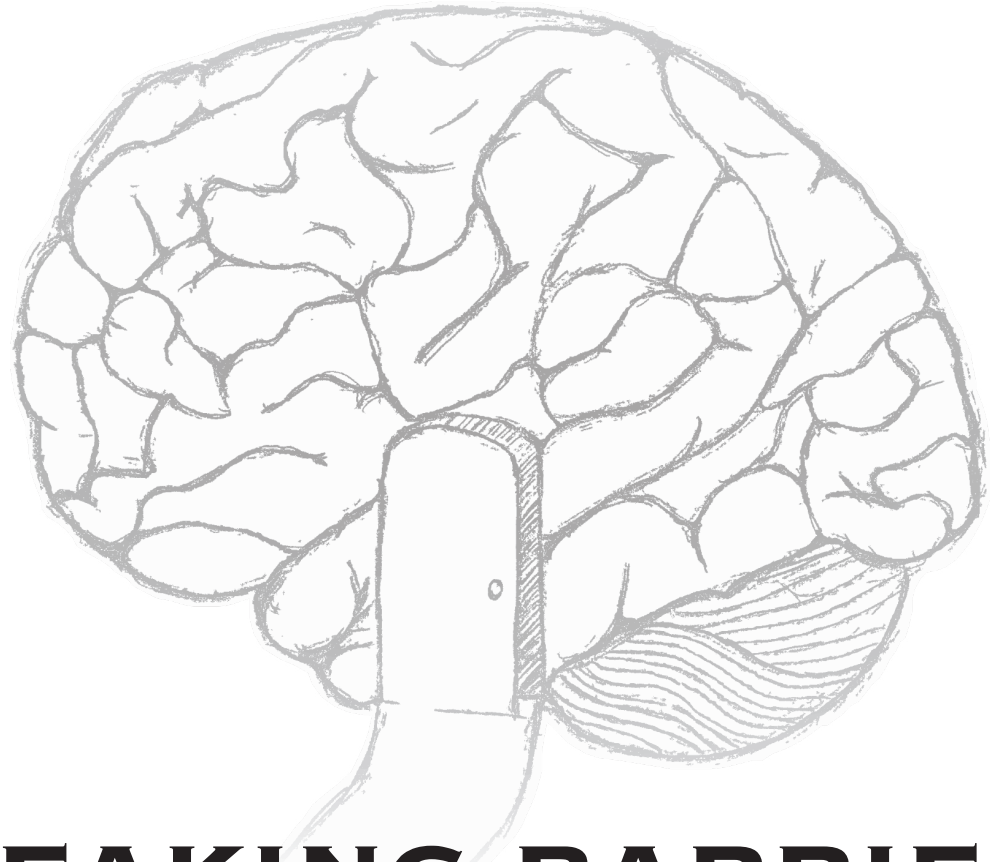
Such a tool could, for example, instigate population-wide fertility loss among mosquitos or simply render the organism immune to the plasmodium parasite responsible for malaria in humans.[14] A CRISPR-powered gene drive would make this existant method vastly more effective as well as economical. Similarly, CRISPR can help increase viral resistance of bacterial strains used in the food industry, such as Streptococcus thermophilus.[15]



Like utilizing a hill to roll a large snowball rather than building one by hand, CRISPR allows scientists to utilize exponential reproduction in the wild to spread the tailored genes. Gene drives thus offer the ability to reinvent whole populations of organisms, whether mosquitos, cash crops, bacteria, or even viruses for human benefit. Such alterations, too, are diverse in their manner; from making an organism more hearty, less infectious, to more industrious in its synthesis or breakdown of biomolecules. CRISPR might just make such feats an everyday feature of the future. In basic biological science, CRISPR can help researchers answer fundamental questions about how genes affect phenotypes (the physical display of a gene or set of genes, such as eye color or color blindness) as well as elucidate the ancestry of certain genes in the tree of life. For example, if a spider carries the exact set of genes found in a fly which, in the latter, are known to be responsible for determining the pattern of wing-vein formation, a reasonable question to ask is, "Why do spiders possess this set of genes?" CRISPR-mediated excision (removal) of this gene set from a spider might lead to physical deformity or instead, exhibit no impact. If the absence of genes were to prove inconsequential, it could be postulated that such genes are vestigial, or leftover, from an evolutionary ancestor of the spider, or perhaps from the last common ancestor of both the spider and the fly.[14]

Similar approaches can be applied to a wide range of fundamental questions in biological science. Evolutionary biology and taxonomy (the arranging of species in the tree of life) have come a long way without CRISPR, yet with more questions ahead, the technology will help us better understand the relationships and ancient history of life on Earth.

As CRISPR-mediated gene technologies begin to make their way into the bioengineer's toolbox, their applications and permutations will no doubt expand to unforetold areas of research and society. Though this technology promises pervasive benefits, its power calls for wisdom and forethought. The twenty-first century will be defined by the creativity and extent to which we use the tools of science to create a better future for the human species and the planet. In this way, CRISPR will contribute not just to the sciences, but to the very history of our species.



BREAKING BARRIERS

In The Brain, Between Science And Medicine, And In Life

Joanna Sun, Leo Lansky, Larry To, Natasha Paranjapye

The blood-brain-barrier (BBB) is a protective membrane layer covering the brain that maintains a safe environment for the brain, regulating what passes to and from it. It acts as a guard wall for the most important organ of the body, but becomes a problem for accessing the brain for therapeutic drugs. Developing therapeutics that directly reach the brain would take a huge step towards treating diseases of the central nervous system (CNS) such as Alzheimer's, Parkinson's, cerebral palsy, and brain tumors. Historically, researchers have faced problems with dose and efficacy. That is, until transformative findings led by Dr. Elizabeth Nance demonstrated the first successful completely bio-inert drug delivery platform capable of penetrating the BBB and moving in the brain microenvironment.[1] This safe and biodegradable polymeric nanoparticle-based platform was shown to not only penetrate the BBB, but locally and specifically travel to the desired therapeutic area in living rat brains. Published in August 2012, this work represents enormous progress in the potential for sustained, targeted, and regulated delivery of drugs into the brain and brought Dr. Nance worldwide attention, including recognition in a '30 under 30' list of young scientists changing the world by Forbes magazine.[2] Denatured Journal spent a morning meeting with Dr. Nance, now a Clare Boothe Luce Assistant Professor in Chemical Engineering and Adjunct Professor of Radiology at the University of Washington, to learn about her plans for her research and how her unique background and philosophy drives her passion for discovery.

Nance's approach innovates in drug delivery, uptake, and tunability

To develop their approach, Nance's team first characterized the transport rates, chemical reactions, and effective pore size of nanoparticle transport in human and rat brains. Nance's team discovered that 28% of spaces between cells in rat and human brains are greater than 100 nanometers (1 nm is 10-9 meters), enabling potential of larger drug delivery particles than previously assumed.[1] Diffusion distances and rates of polyethylene co-glycol (PEG)-coated nanoparticles of various sizes were evaluated in brain tissue. Polymeric nanoparticles have several advantages, including stability in the bloodstream, greater drug load, and controlled drug release over time.[1] Nance and her team found that 60 and 75 nm particles were the optimal size for delivery, and those even up to 114 nm in diameter penetrated successfully. Large particles offer vast flexibility in drug delivery design and have higher drug holding capacity than other approaches. Additional PEG coatings enhance nanoparticle penetration and circulation, and are expected to lead to greater drug distribution in the brain. It was found that even much smaller nanoparticles (40 nm) are essentially immobile in the brain if uncoated.

Tracking particle movement via confocal microscopy, Nance's team showed that not only do the nanoparticles travel to the desired area under safe pressures, but other areas are left untouched.[1] The beauty of their platform lies in the ability to tune delivery time as well as nanoparticle and microbubble properties for specific drug delivery purposes. The team also confirmed minimal tissue damage through the experimental protocols, concluding that the in vivo rat brain tissue maintains physiological functioning during the experimental time frame.

The bottom line? Nance's brain-penetrating nanoparticles allow localized drug delivery, can be patient- and disease-specific, and are deeper-penetrating than ever before. This research could mean targeted treatment for CNS disorders such as Parkinson's, Alzheimer's, depression, and epilepsy.

Diverse translational applications mean a bright future for this technology

Nance's approach to developing this technology was to focus on overarching principles governing the system and use these to adapt to specific complexities, such as honing in on common aspects of disease that influence movement. Previous researchers focused their energies on getting drugs to the brain but not on what came after cross-

"Sometimes in science we tend to look at things through a very singular perspective and we tend to do it with the tools we have on hand. But usually when you do that you are going to miss out on all the additional information that potentially factors into the outcome you're trying to achieve."

ing the blood-brain barrier, Nance explained. "If you're doing that, you're not leveraging the technology and maximizing it to the best of its ability. This doesn't serve diverse patient populations, because these are reasons why your technology doesn't work." This project arose from a previously-developed drug delivery platform in the lab of Justin Hanes based on overcoming mucosal barriers, the membrane protective layer that coats your lungs, gastrointestinal tract, and eyes, to name a few. This platform failed to work in the brain, and Nance adapted this technology and meticulously tailored it to its current use in the brain.

This brain-penetrating nanoparticle platform is currently being used in other research, not only in the brain but in other areas of the body for its power as a well-characterized, well-controlled, and bio-inert platform. Collaborators at the University of Virginia combined Nance's platform with MRI-guided focused ultrasound to effectively visualize drug movement in the brain, using contrast agents that enhance uptake by the BBB.[3] As far as next steps for her, Nance would like to maximize the platform to leverage and control transport in the brain, and get real-time quantitative information about diseases that are not yet well-understood, such as autism. Projects in the Nance lab are currently trying to find out how the autistic brain differs from other brains and how that affects the way therapeutics are delivered. In yet another application, Nance's lab uses this same technology to track and image the Zika virus (see page 22) in the brain to gain information on how to treat it based on the virus's mechanism of movement.

The future of nanotechnology is bright, no doubt in part thanks to Nance's contributions. Nance says that the field has a lot of potential, stating, "I believe nanotechnology will have the ability to provide real-time quantitative information about a disease state, will help reduce healthcare and patient costs, will provide effective and safe therapeutic interventions, and will help provide us with more in-depth understanding about our bodies and how they function."

Nance believes that in order to truly address the needs of patients and clinicians, engineers need to have the kind of "hands-on" medical understanding that clinicians have. As a graduate student with mastery of nanoparticle research, she saw her lack of understanding of the brain as an opportunity to come at the research from the angle of neurosurgeons and neuroscientists to understand their language and way of thinking. This go-getter attitude guided Nance's educational journey and manifests itself in other aspects of her life as well.

In her training, Nance synthesized motivation, education, and new knowledge

Nance was inspired to pursue this field of study because of a personal connection. An undiagnosed neurodegenerative disease runs in her family, and witnessing her family members struggle gave her a firsthand view

of the shortcomings of research in this field. Medicine could not diagnose the disease, science could not provide an explanation, and neither field could treat it.

Nance admitted that chemistry was not her best subject in high school, but her personal motivation combined with a passion for problem solving led her to pursue her undergraduate degree in Chemical Engineering, with minors in English and biotechnology. The major especially appealed to Nance because students learn broad fundamental principles that they can then apply towards applications that they are interested in. Going into her Ph.D., Nance knew that she wanted to pursue Chemical Engineering with some application to the brain.

From the moment she chose this field, Nance was sure she wanted to pursue a Ph.D. She described how it appealed to her independence, love of learning, and curiosity. She also enjoyed that the amount of "smart time", intentional and focused time, could directly impact the outcome of her efforts. While she knew that she wanted a Ph.D., it was not until months into her postdoctoral fellowship that she was set on pursuing academia. Nance's reservations stemmed from insecurities about whether or not she could come up with enough original ideas to lead a research lab and to get funding. However, she worked through these insecurities using advice from mentors, and by using a problem-solving approach. She says, "I treat insecurities and uncertainties as points of opportunity to gain more information.

"I always treat insecurities and uncertainties as points of opportunity to gather more information."

Nance's passion and excitement about the possibility to revolutionize the field and provide mentorship to students drove her to cast aside these insecurities and join academia. She started as a Clare Luce Booth Assistant Professor at UW in September 2015. Since then, she loves the field she chose to pursue. She was excited to talk to us about the many facets of her job- her research, her role as an instructor, and the mentorship she can offer a diverse body of students every day.

A philosophy of open-mindedness and bridging gaps

Nance's underlying philosophy is to maintain openness and honesty, both with herself and the students she mentors. She carries this philosophy throughout every aspect in her life, and hopes to be as open as possible about her process of choosing academia and the insecurities that she had. Through this approach, she hopes to make herself more accessible and useful to the students that she mentors.

Nance's openness to outside opportunities led her to pursue her post-doctoral fellowship at Johns Hopkins School of Medicine, working alongside neuroscientists. She believes that this experience was integral to her training as an engineer, saying, "People should be able to explore non-traditional paths. People should be able to step out of their expertise to get necessary information to allow that expertise to be applied in an efficient way". She found that getting a post-doctoral position as a chemical engineer in neuroscience was difficult, as people are usually expected to specialize and work in their own field. She hopes that one day, it will be easier for students to gain diverse experience in different fields. Her lab at UW aims to bridge those gaps, and her mentorship philosophy includes encouraging students to not shut the door on any learning opportunities by the idea of specializing in only one area. One day people in the scienc-

"People should be able to explore non-traditional paths. People should be able to step out of their expertise to get necessary information to allow that expertise to be applied in an efficient way."

es will be more focused on how we form connections between different fields, and medical outcomes will be improved as a result of this greater scope of knowledge. Nance hopes to touch the lives of students so she can impart her philosophy to those she mentors and make a widespread impact in the culture of the field.

Nance creates opportunities for real mentoring relationships

Nance's philosophy and approach make her a rather non-traditional chemical engineer. On top of that, she is one of few women in a male-dominated field. However, she is an ardent supporter of the mindset that she is a chemical engineer first, although she is still proud of the fact that she is a woman. As she said, "Science can be a very lonely road, especially if you feel like you have to work twice as hard for everything you want to achieve, and you want to do it differently than everyone else has done. It can be very difficult but also be very rewarding, and can allow you to have life experiences to share with students going through the same thing."

"Science can be a very lonely road, especially if you feel like you have to work twice as hard for everything you want to achieve, and you want to do it differently than everyone else has done. It can be very difficult but also be very rewarding, and can allow you to have life experiences to share with students going through the same thing."

This idea, and the realization that women in the chemical engineering department needed a way to connect with each other and share experiences, led Nance to found Women in Chemical Engineering at UW. She noticed how many people came to her seeking advice and perspective from a female faculty member, and decided to create a sustainable network of support for women in the field to find connections, advice, and leadership experience. After forming the initial framework for the group, the students have grown the organization, with undergraduates, graduate students, alumni, and faculty all involved.

Nance's underlying goal of connectedness in science has also become an overarching theme in her life-- she stressed that she does not see anything as being mutually exclusive. An avid reader, photographer, and animal-lover, Nance maintains a crucial work-life balance by forming connections between everything that she does. She described how even when relaxing with a book, she always thinks about how she can use it in interactions with students or in her job as a professor. Through this, she makes the most of the time that she is at work, while still making time to read and take care of her rescue boxers.

Nance hopes to revolutionize the field of chemical engineering through her non-traditional approach, and to bridge the gaps that still exist between science and medicine. She shows a remarkable passion for connecting with and helping students, having an open door policy, and showing enthusiasm for the wide diversity of stories that she gets to hear every day. But more importantly, she is willing to share her experiences to help guide these students towards the same success that she has attained.

CHECK OUT THE **NANCE LABORATORY** IN THE UW CHEMICAL ENGINEERING DEPARTMENT

Fighting Disease at the Front Lines

Vaccines for New Epidemics

Anastasia Nicolov, Jeffery Ni, Michael Butler

Each year, news reports herald the latest infectious disease scare. In the last few months, Zika virus has been on the forefront of the public's awareness, with concerns over the safety of Rio's Summer Olympics and calls from South American governments to postpone pregnancy until further notice in order to curtail side effects of the disease.[1] Within recent memory, Ebola virus, swine flu, and SARS have all posed global threats.

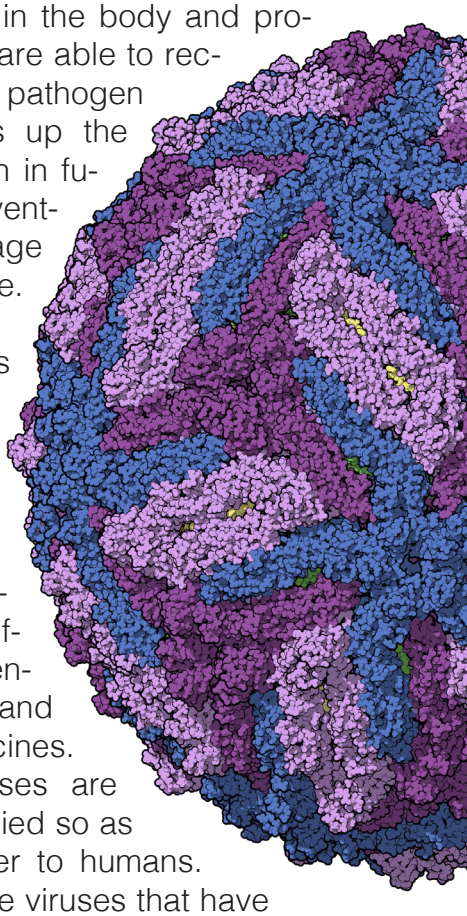
Without first-hand experience and expert understanding of these diseases, it is difficult to sort out truth from hype. However, one thing is certain: vaccines have and continue to protect vast numbers of people from the devastating effects of infectious diseases.

One of the greatest marvels of modern medicine, vaccines have in the last century led to the complete eradication of diseases like smallpox, as well as the near-elimination of polio, hepatitis A and B, and measles in the Western world.[2] Inoculation against chicken pox, whooping cough, and the seasonal flu save thousands of lives each year in the United States, and widespread vaccination has led to herd immunity, dramatically reducing disease risk for the most vulnerable members of the population – those who are medically unable to be vaccinated or who are immunosuppressed.

Despite the clear benefits of vaccines, few people have more than a superficial understanding of how they work or the enormous effort that goes into making them safe and effective. Here, we hope to give insight into the vaccine production process as well as recent innovations.

Basic Immunology

As the body's most intricate line of defense, the immune system is comprised of cells that recognize the difference between self and foreign entities in our body and target foreign or abnormal particles for elimination from the body.[3] While there are numerous cell types, lymphocytes (namely, B and T cells) are the most involved with targeted responses to specific diseases. Commonly referred to as white blood cells, these cells find and destroy disease particles, known as pathogens. Some of these cells contain a memory component; they are long-lasting in the body and produce antibodies that are able to recognize that particular pathogen again. This speeds up the process of elimination in future exposures, preventing extensive damage caused by the disease.



Essentially, vaccines stimulate this memory response without the presence of the disease-causing element.[3] Vaccines in widespread use generally come in two different types: live attenuated virus vaccines and inactivated virus vaccines. Live attenuated viruses are viruses that are modified so as not to present danger to humans. Inactivated viruses are viruses that have been killed through heat or a chemical reaction.

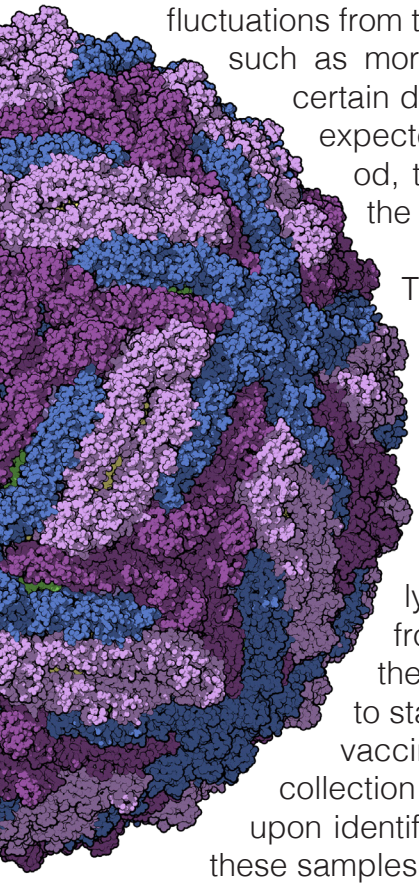
In summary, when a vaccine is injected into the body, it produces an immune response, but no illness occurs because the malignant components of the virus are not functioning.

Epidemiology and Virus Identification

Whenever a new illness is observed in the public, particularly one that spreads easily to others and causes severe symptoms, a high-

ly-trained group of disease experts and biological scientists descends on the affected region with the goal of creating a vaccine. The United States Centers for Disease Control (CDC) and the World Health Organization (WHO) are two organizations that play key roles in this process.

The term "epidemic" carries a weight in the public vernacular indicative of a great, impending danger. Although this may be true in some cases, the term is quite loosely defined. The CDC is responsible for determining what constitutes an epidemic in the United States.[4] It collects data on trends in disease statistics and constantly has a team of analysts gauging fluctuations from the norm. When statistics such as morbidity or incidence of a certain disease are above what is expected for an extended period, the CDC officially deems the event an epidemic.



The WHO has a similar role in disease identification on a global scale.[5] For viruses with constantly mutating strains like influenza, the WHO has built a network of laboratories that routinely collect blood samples from patients infected with the circulating strain in order to start development of a new vaccine. For new epidemics, collection of blood samples occurs upon identification of a threat. From these samples, viruses are isolated and purified in order to create a laboratory stock. From this, a new vaccine can be developed.

Vaccine Development

From the isolated strain, a modified "vaccine virus" must be created.[5] Methods for doing this vary depending on the disease, but in most cases, the virus is made less dangerous by identifying and mutating regions that cause severe reactions in humans. Modifications may also be made to improve growth efficiency in certain conditions to ease translation to large-scale manufacturing. For example, with the flu vaccine, the vaccine virus is better able to grow in chicken eggs, as this is the standard manufacturing protocol.

Next, the vaccine must be extensively tested in vitro (using living cells in a dish) and in vivo (in living animals) to ensure that it produces an immune response without a dangerous reaction.[5] This is easier said than done, as each disease has a different set of challenges. For example, rabies vaccines use inactivated viruses, so ensuring the viruses stay inactive is a top priority. Pneumococcal pneumonia has multiple strains, all of which need a different chemical conjugation method before they are blended into one vaccine. Hepatitis B vaccine viruses are difficult to produce efficiently and consistently in the lab.

Depending on the virus and vaccine type, growth conditions may need to be optimized and vaccines modified with additives.[5] For example, many vaccines include adjuvants, which are chemicals that help stimulate a larger immune response.

If the WHO is involved, a set of standards is developed that helps vaccine manufacturers measure how much vaccine they produce and ensure that dosing remains correct and consistent throughout their stock.

Vaccine Manufacturing

While hundreds of companies worldwide produce vaccines for many diseases, this production is usually on a small scale and highly specialized. Astonishingly, only two companies in the world are dedicated to global distribution of their vaccines.[6] This seems to be in part because global distribution is costly and highly regulated, making it unprofitable for small companies. The largest challenges associated with vaccine production and distribution are in legal and regulatory issues.

Legally, companies are unable to sell or distribute vaccines to a country until approval of the vaccine is obtained. Requirements for approval range from adequate manufacturing processes and facilities to allowing regular

inspections of production practices.[6,7] Each country or region has its own set of rules and requirements, making the approval process for globally-distributed vaccines arduous and costly.

Once approval is obtained, vaccine production can be scaled up. The most common manufacturing method for many vaccines, including the flu vaccine, uses chicken eggs to grow vaccine viruses, which can then be purified from the egg white.[8] Clearly, this process is not without limitations, one of which is that millions of eggs must be harvested to create vaccines for large populations, creating a shortage of eggs and driving up the price. This is especially problematic in developing countries. Thus, cell-based production using synthetic biology and recombinant DNA vaccines are becoming increasingly widespread as a cheaper and more accessible method.

To ensure safety, each batch of vaccines are carefully tested for sterility and protein concentration according to the WHO standards. It can then be packaged for use in humans. In most countries, clinical studies must be done in a small group of people before the vaccine can be made available for the public. For the flu vaccine, this has largely become routine, but is a major hurdle for manufacturers of novel vaccines.

At the brink of a possible epidemic, the testing and regulatory process is often sped up, with WHO and governments shunting extra money into vaccine development. In the best case scenario, WHO estimates that a vaccine can be completed, tested, and distributed in five to six months;[5] however, some diseases are harder to crack than others, and the timeline may be longer for viruses like Zika.

Even for influenza, the most well-characterized vaccine, the process is difficult. Each yearly flu shot consists of different strains and chemical components specific to that year. Almost immediately after a year's flu season, the research restarts. Researchers begin selecting the most probable strains of a virus for the upcoming year and start preparing vaccine production by March. Release of the year's vaccine may be as early as July, with distribution coming soon after.

Race for a Zika Vaccine

Zika virus is a flavivirus, similar to dengue fever, yellow fever, and West Nile virus.9 First isolated in monkeys and mosquitoes in 1947 in Uganda's Zika Forest, it has since appeared in multiple forms in other African nations, Southeast Asia, the Pacific Islands, and South America. It is most commonly transmitted to humans by mosquitoes, though a few cases of sexually-transmitted Zika virus have been documented in the most recent outbreak. Symptoms usually appear as a low-grade fever, rash, eye irritation, or joint pain, though only about 20% of patients experience noticeable symptoms.

While Zika is generally not a serious risk to children and adults, it has been associated with miscarriage or severe birth defects in children whose mothers were infected during pregnancy.[9] According to one Brazilian study, of 42 pregnant women shown to be infected with Zika, 29% showed fetal abnormalities on ultrasound imaging. Beyond minimal studies of this kind, little is known about the spectrum of outcomes associated with the virus, and the biological chain of events leading to birth defects like microcephaly are not well understood. Additionally, there is some evidence that exposure to Zika virus can increase risk of Guillian-Barré syndrome, a severe autoimmune disorder in which the immune system attacks the nervous system, in some patients causing permanent nerve damage leading to perpetual weakness, numbness, and fatigue.

As soon as the risks associated with the latest outbreak were identified, researchers began to work on a variety of preventative solutions, from developing vaccines to genetically modifying the carrier mosquitoes.[10] At the same time, governments have discouraged travel to affected areas and global health organizations have worked to educate the public, especially pregnant women, about the potential risks. Education campaigns have stressed the importance of birth control and protection from mosquito bites. Still, there is a signifi-

cant need for a vaccine effective for pregnant women in high-risk regions.

While effective vaccines exist for yellow fever and other viruses from the flavivirus group, no viable Zika vaccine has been produced at this time. As of May 2016, however, 18 different companies and research groups are working towards developing a Zika vaccine.[9] These groups are developing vaccines of varying types, including inactivated, recombinant, and DNA vaccines.

One group is using two different approaches to develop two parallel vaccine candidates. India's Bharat Biotech started working on a Zika vaccine in November of 2014 as an extension of their work on dengue fever and other flaviviruses, before the most recent outbreak began.[11]

Their first candidate is an inactivated vaccine, which contains whole Zika virus particles that have been "killed" using chemicals, heat, or radiation so that they can no longer replicate or cause infection; however, because they contain most of the Zika DNA and proteins, they trigger a similar immune response to the natural virus.[11] One major advantage of an inactivated vaccine is that it doesn't require refrigeration and can easily be freeze-dried for transport, making it ideal for use in developing countries.[3] Inactivated viruses are known for their safety and genetic stability compared with live viruses and the efficient immune response they produce. However, one disadvantage is that they require booster shots later in life to maintain a patient's immunity.

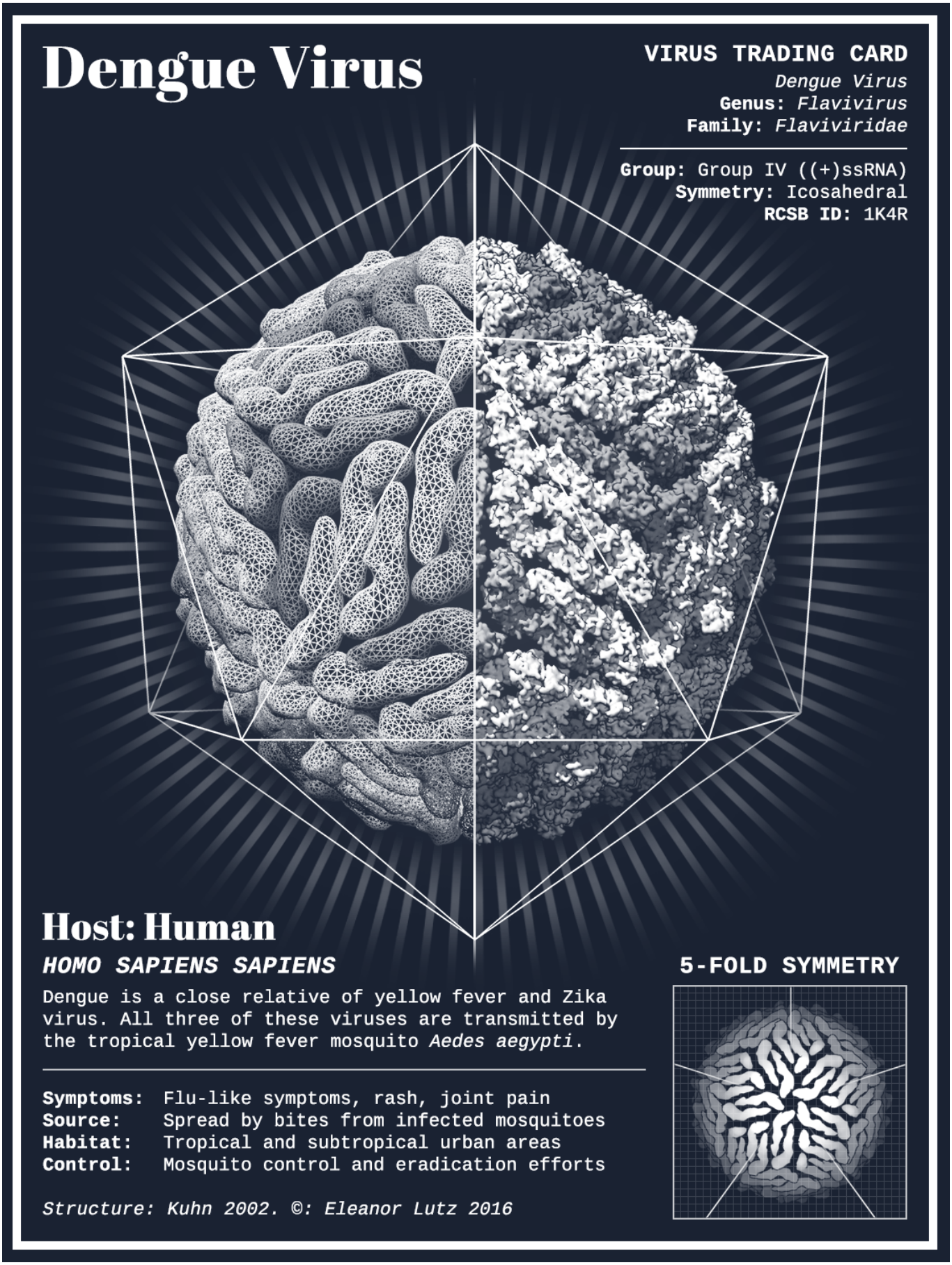
The second vaccine being developed by Bharat Biotech is a recombinant vaccine, which is produced by inserting the Zika DNA into an innocuous viral vector, a virus genome that has been "attenuated" and thus does not cause disease in humans.[11,12] Essentially, the recombinant Zika vaccine does not contain the full Zika virus, but contains certain regions of DNA targets from the virus that can activate the specific immune system. Recombinant vaccines avoid several obstacles to production and potential safety risks. For example, they generally have less risk of a side effects and reactions than live or inactivated viruses. Recombinant vector vaccines closely resemble a natural infection and can stimulate a similar immune response. Additionally, recombinant viruses can reproduce in the body and there is some thought that booster vaccines later in life will be unnecessary, as the patient will continually have both the virus and compatible immune components in their bodies long past the initial vaccine.

Both of these vaccines are still undergoing animal and human trials, which are long and painstaking in order to ensure patient safety. According to Dr. Krishna Ella, CEO of Bharat Biotech, it may be years before either vaccine can hit the market.[11] The exact timeline depends significantly on how much support they can garner from the World Health Organization and other groups, and the approval process from national health administrations (such as the FDA in the United States).

Pennsylvania-based company Inovio Pharmaceuticals is another company working towards a Zika vaccine.[13] Theirs is a DNA vaccine, in which DNA is injected into the body and taken up by some of the patient's cells, which then express proteins that the DNA codes for on their surfaces and induce an immune reaction against them.[14] Thus, the body's own cells essentially become vaccine manufacturing plants, producing both the antigens and the resulting immune response. In mice, this vaccine has proved promising, producing a robust antibody and T cell response. The company plans to begin testing the vaccine in humans by the end of 2016, but it will likely be a few years before it makes its way to market.

Summary

Although the public may not often appreciate the rigorous process vaccines undergo prior to distribution, vaccine research has a huge impact on society. In a world with constant evolution of new and potentially deadly virus strains, bioengineers and epidemiologists are on the front lines, working quickly to identify pathogens and develop new vaccines while ensuring the safety and efficacy of their products.



Courtesy of Eleanor Lutz

Dr. Suzie Pun

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