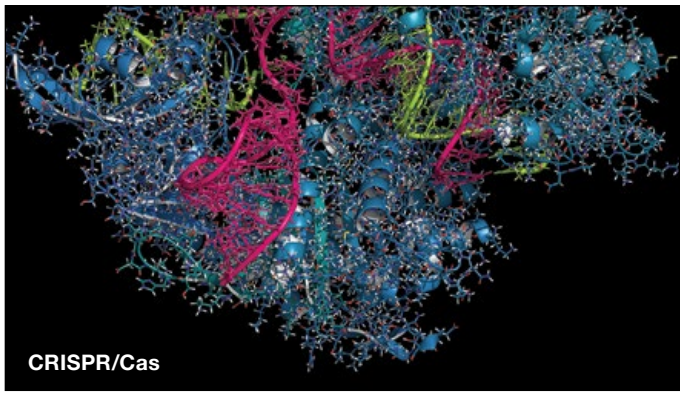
CRISPR/Cas

# Synthetic biology's clinical applications

Engineered systems of genes and other molecular components created through synthetic biology make medical treatments more effective and promise cures for a range of health problems. Perhaps equally important, recent technologies make it easier for a broader range of scientists to apply synthetic-biology approaches that drive expanding clinical applications, from designing new diagnostics and building molecularly engineered tissues to developing new drugs and vaccines. **By Mike May**

Synthetic biology provides scientists with an arsenal of new tools to accurately and efficiently modify the molecular workings of cells to gain medical advantages. According to Jim Collins, Termeer Professor of Medical Engineering and Science at Massachusetts Institute of Technology (MIT) in Cambridge, "Synthetic biology brings together engineering and molecular biology to model, design, and build synthetic gene circuits and other biomolecular components and uses them to rewire and reprogram organisms for a variety of purposes." The clinical uses of synthetic biology already cover a wide range of areas, including diagnostics and treatments. Further, these clinical applications will likely expand more rapidly over the next few years because of the easy-to-use gene editing tools now available.

In 2012, molecular biologist Martin Jinek (now at the University of Zurich in Switzerland) and his colleagues published an article about clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems, which make it possible for any molecular biologist to edit an organism's DNA (scim.ag/1piiXv7). This system has quickly supplanted previous editing modalities such as zinc finger nucleases. "This is the most widely used genome editing tool," says Collins. "It's starting to move synthetic biology toward nonexperts, and it caught on because it works remarkably well in many organisms and is very easy to use."

Synthetic biology is already playing a role in new clinical applications. For example, Collins and his colleagues have modified the genetic machinery from a cell using synthetic biology and embedded it in paper that can be freeze-dried for storage. This process has made possible the development of a paper-based diagnostic that detects pathogens in saliva or blood. Collins points out that the genetic elements embedded in the paper work just like they would in a living cell. He adds that these paper-based diagnostics can be engineered to "detect antibiotic resistance or viral infections such as Ebola." Such diagnostics could be quickly engineered to track public-health concerns. Going beyond diagnostics, the examples below highlight a variety of clinical research projects and emerging treatment options based on synthetic biology—a field that continues to expand into new therapeutic areas.

## Two-stage twist

The value of a more efficient means for engineering DNA cannot be overemphasized in synthetic biology. In the United Kingdom, for example, scientists at **Touchlight Genetics** developed a two-step process to synthesize DNA that can be used in biological products. "This enzymatic process enables large-scale, high-yield synthesis of DNA—in the grams per liter range—without bacterial fermentation," says Lisa Caproni, group leader of research applications at Touchlight. The resulting product is called "doggybone DNA" (dbDNA) because of its shape.

Caproni emphasizes that this process overcomes several shortcomings of traditional DNA synthesis. For example, the method provides a ready-to-use product in which DNA does not need to be manufactured in and separated from bacterial material, such as *E. coli*. This is advantageous because DNA produced in bacteria can include genetic information that spurs antibiotic resistance—which is not at all desirable for medical treatments—and as Caproni says, may present "unnecessary practical and regulatory hurdles when used [in humans]." She adds that "some desired DNA sequences are found to be incompatible with growth in bacteria." For example, toxic genes cannot be produced inside any cell. The Touchlight technology sidesteps these challenges.

This technology can be used in several clinically related applications. "The process produces stabilized linear DNA that can be used both in the biomanufacturing of therapeutic DNA products, such as DNA vaccines and DNA-based gene therapy products, and in the creation of a variety of biological products, including therapeutic antibodies and viral vectors," Caproni explains. "In both cases, large quantities of highly pure DNA are required, and it is apparent that the provision of DNA at this scale and purity is expensive, and is often a bottleneck in product development." Because no bacterial steps are



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required, large amounts of dbDNA can be made quickly, which makes it easier and more economical to create therapeutic products.

### Working together

The breadth of synthetic biology, especially when applied clinically, makes teamwork a necessity for this field. For example, geneticist George Church of Harvard University, synthetic biologist Drew Endy of Stanford University, and microelectronics expert Joseph Jacobson of MIT founded **Gen9**, a company in Cambridge, Massachusetts that advertises itself as a "high-throughput supplier of synthesized genes."

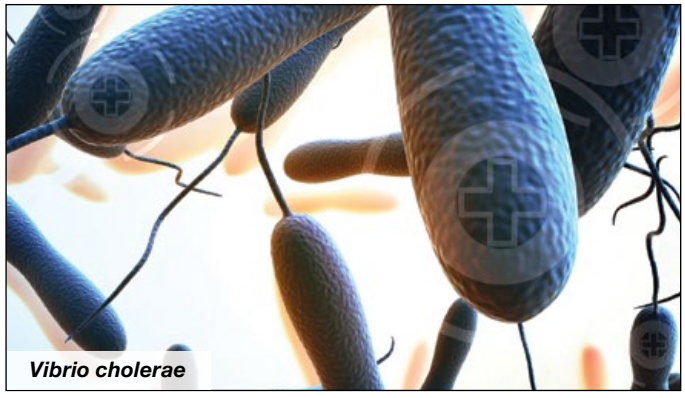
Historically, scientists synthesized genes in pieces composed of 200 or less base pairs—the four building blocks of DNA—largely to reduce the odds of errors. Church, Endy, and Jacobson teamed up to create the BioFab platform, a chip-based process for gene synthesis that can synthesize hundreds of thousands of base pairs. This process allows researchers to work with nonribosomal peptide-synthetase (NRPS) enzymes, for example. These enzymes come from a long gene cluster that is "critical in identifying new antibiotics," says molecular biologist Devin Leake, vice president of R&D at Gen9. Developing new antibiotics is especially important to Leake because he is allergic to the old standby, penicillin.

Researchers can also apply the Gen9 technology to other clinical opportunities. "We're seeing a lot of interest in protein engineering, like engineering antibodies," Leake says, noting that Gen9 enables researchers to precisely define DNA content that is then synthesized to make antibody variants. "Researchers can modify the amino acids that they want, like maybe including a hydrophobic region in a pocket of an antibody," Leake explains. "Then, changing just one amino-acid residue might give the antibody new properties, and we can explore the entire sequence space." That sort of control could be used to engineer compounds to custom fit disease targets, for example. Moreover, many drug candidates can be tested because the BioFab platform can build gene libraries of millions of variants that can then be screened for safety and effectiveness.

### Building the tools

Some of the most intriguing clinical uses of synthetic biology are still in their infancy. For instance, scientists at UK-based **GlaxoSmithKline** (GSK) plan to use synthetic biology to create living systems that make small molecules, like aspirin, that typically come from chemical rather than biological processes. To enhance this capability, GSK licensed CodeEvolver, a protein-engineering technology from California-based **Codexis**. GSK uses this synthetic-biology platform to create unique enzymes for use in manufacturing drugs that work faster or more efficiently.

"We are focused on engineering biology as an improvement or replacement of traditional chemistry in the manufacture of our medicines," says Doug Fuerst, GSK's technology development leader. Using biological systems to make chemical compounds, Fuerst explains, can improve the quality of the compounds and reduce the cost. "Using this enzyme evolution approach opens the chemical reaction space that is difficult to access with traditional chemical approaches," explains Mark Buswell, head of GSK's advanced manufacturing technologies. In fact, the biological approach is the only way to catalyze some reactions.




**A microbe could be genetically engineered to detect a particular pathogen and kill it, for example, the bacteria *Vibrio cholerae* that causes cholera.**

Once a reaction process is engineered through the enzymes, it can be put into cells, so that each cell performs like a drug-making factory. "We start with the ability to control the enzymes," Buswell says. Eventually, he hopes to use that enzyme knowledge to harness biochemical pathways to make drugs in cells instead of reactors.

Eventually, this approach might be used in human cells. Buswell calls this "blue-sky thinking," but a person's own cells might one day be engineered to make the drug required to treat an illness. As Buswell points out, "We're not actively doing that."

### Living therapies

Some companies, however, are already turning synthetic biology into cell-based treatments. As MIT's Collins explains, "The field is beginning to expand toward engineering therapeutic microbes—living therapeutics." For example, a microbe could be genetically engineered to detect a particular pathogen and kill it, for example, the bacteria *Vibrio cholerae* that causes cholera. "Such a microbe would function both as a living diagnostic and a living therapeutic," Collins says.

Similarly, a bacteriophage—a virus that infects bacteria—could be engineered to treat bacterial infections. Such a virus might also be used to resensitize a bacterium that had grown resistant to antibiotics. As a result, the bacteria could be made susceptible once again to the antibiotic treatment. With the growing problem of antibiotic resistance, this synthetic-biology technique would be very useful in treating infectious diseases.

Although Collins says that these applications continued>

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are "very early on and more on the promising side than execution," he sees them moving ahead, and he plays several roles in advancing such technologies. For instance, **Synlogic** in Cambridge, Massachusetts (where Collins is a scientific cofounder), is engineering microbes to treat phenylketonuria, an inherited disease that results in high blood levels of the amino acid phenylalanine and can cause mental disorders if not treated.

In addition, Cambridge-based **EnBiotix**, where Collins is also a scientific founder, is engineering EPP-001, a bacteriophage that causes target bacteria to secrete an enzyme that destroys a bacteria-based biofilm. Collins and his colleagues hope to use EPP-001 to treat infections in prosthetic joints.

## Subverting *Salmonella*

It turns out that bacteria can have both good and bad sides. For example, the Centers for Disease Control and Prevention reports that *Salmonella* infects about 1 million people every year in the United States, 380 of them fatally—but this bacterium is not all bad. Scientists at **Prokarium** in the United Kingdom use genetically altered *Salmonella* to deliver vaccines. "We've tamed the *Salmonella*. We have engineered it to retain its ability to enter the body's immune cells but also to prevent it from causing diseases," says Prokarium's chief executive officer Ted Fjällman. Prokarium uses the bacteria to deliver a vaccine orally. "It enters through the gut lining, is engulfed by immune cells, and then it starts making vaccine," Fjällman says. "It's like a bioreactor in your body."

So far, this technology has been tested in humans in the United Kingdom, the United States, and Vietnam. In addition, it has been tested as a vaccine against diarrhea, hepatitis B, and typhoid—however, it remains in clinical testing. Nevertheless, Fjällman says that this platform "can deliver almost any protein vaccine."

Despite the promise of this platform, much work lies ahead for Fjällman and his colleagues. Although Prokarium purchased the typhoid vaccine from **Emergent Biosolutions** in Gaithersburg, Maryland, this vaccine-*Salmonella* combination must still undergo proper clinical testing.

If this technology proves safe and effective, it will offer many benefits. In addition to the advantage of oral delivery, the *Salmonella*-based vaccine could be thermally stable at 37°C for weeks. "That is very helpful for developing regions," Fjällman says. "This is a huge displacement technology if we can make it happen."

## Tailoring tissues

The basis of synthetic biology goes beyond genes and proteins. For instance, **OxSyBio** in the United Kingdom developed 3D printing technologies to construct biological materials or to make materials that mimic them. In 2013, Hagan Bayley and his colleagues at the University of Oxford described these tissue mimics (scim.ag/10ClLDf). The 3D process can control the size of the droplets, which are 30–50 μm across. By printing tens of thousands of droplets only picoliters in volume, Bayley and his colleagues generated cell-like compartments separated by lipid bilayers. Modification with proteins made the bilayers behave like biological membranes, which even allowed electrical communication similar to that occurring in neurons. The authors concluded that "printed droplet networks might be interfaced with tissues, used as tissue engineering substrates, or developed as mimics of living tissues."

Bayley's printing technology can be extended to include living cells that produce functional tissues for use in medical research and, eventually, in clinical applications. This research is being pursued at OxSyBio. "These printed tissues are very similar to biological tissues, and we anticipate that they can be used in toxicology or drug screening," says OxSyBio's director Mike Molinari. In addition, the cells in these tissues can be genetically engineered to further specify tissue properties.

The most advanced clinical applications for printed tissues lie in the future, but Molinari sees several possibilities for the near-term. For example, he says, "Tissue patches might be used to repair damaged heart tissue." He adds, "While the printing of complete synthetic organs cannot be dismissed, organ repair is a more plausible prospect. We will start with small pieces and then migrate to larger and more complex tissues." Eventually, Molinari hopes for even more grandiose applications: "One day," he says, "we might even provide on-demand printing in the operating theater."

The ultimate scope of clinical applications for synthetic biology remains to be seen. Today's application in diagnostics, drug discovery, and tissue engineering should soon grow more extensive and spawn opportunities that are currently unimaginable. Indeed, synthetic biology has the potential to radically change the way clinicians manage disease and to help us live, longer, healthier lives.

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