A nanotech fix for nicotine dependence

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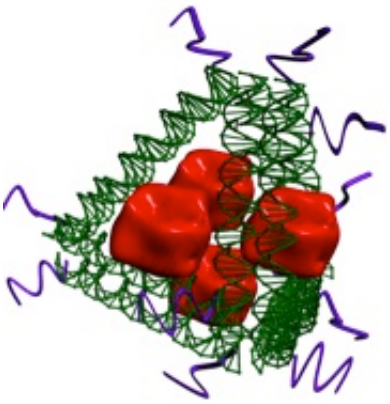

Tetrahedron DNA-scaffolded nicotine vaccine. A tetrahedral DNA structure is seen in green, with attached vaccine components including nicotine haptens, adjuvants and T-helper epitopes.

A chemical component present in the nightshade family of plants is one of the world's most tenaciously addictive substances. It is the nicotine contained in tobacco and found in high concentrations in cigarettes. Smoking remains a global scourge; in the U.S. it is the leading source of preventable death.

Yung Chang and her colleagues at Arizona State University's Biodesign Institute have launched an ambitious new project, designed to attack nicotine dependence in a radically new way. The research effort, pursued under a new $3.3 million grant from the National Institute of Drug Abuse, will attempt to design a vaccine conferring immunity to nicotine,

using nanoscale structures assembled from DNA.

"The DNA nanostructure enables rational design and construction of synthetic vaccines, because of its precision control over the placement of various antigenic components," Chang says. "This approach may offer a new strategy to improve the efficacy of many different vaccines."

Chang, an immunologist, has been developing a method for incorporating key vaccine components onto self-assembling, nanoscale carrier molecules. She has worked on honing the technique along with nanotechnology innovator Hao Yan, a researcher at Biodesign's Center for Single-Molecule Biophysics who holds the Milton D. Glick Distinguished Chair in the Department of Chemistry and Biochemistry.

Joining the interdisciplinary team are ASU researchers in the fields of immunology, organic and DNA structural chemistry (Sidney Hecht) and bioinformatics (Li Liu and Sudhir Kumar), as well as specialists in the pharmacology of nicotine dependence from the University of Minnesota, led by professors Paul Pentel and Mark LeSage.

By the end of the three-year project, the group hopes to identify promising candidates for a new nicotine vaccine and advance them toward Investigational New Drug submission. The researchers emphasize that if their DNA nanotechnology approach proves successful, it could plausibly be applied to the development of future vaccines against any target of interest, including other drugs of abuse, infectious agents or tumor antigens, thereby opening an entirely new chapter in vaccine development.

Deadly lure

In the universe of addictive drugs, nicotine reigns supreme in terms of the numbers of people affected. In the U.S. alone, cigarette smoking

causes some 443,000 fatalities per year – exacting a greater human toll than the human immunodeficiency virus (HIV), illegal drug use, alcohol use, motor vehicle injuries, suicides and murders combined, according to the Center for Disease Control.

While various elements in cigarettes and other tobacco products account for their severe adverse health effects (including coronary heart disease, stroke, vascular disease, peptic ulcers, chronic lung diseases and lung cancer, and fetal brain damage and morbidity), it is the nicotine that produces potent dependency.

Existing methods – from patches to 12-step programs and chewing gum to experimental drugs – have been explored in efforts to curb nicotine dependence but the results have been less than stellar. One reason the addictive cycle is notoriously difficult to break is that only a single slip-up in abstinence from smoking is sufficient to re-infuse the brain with enough nicotine to reestablish cravings and drug-seeking behavior.

Seeking immunity

Innovative efforts have been under way for over 30 years to harness the body's immune system to combat various drug addictions, including nicotine. The basic idea is to stimulate an immune response that would recruit antibodies capable of binding with nicotine. In this way, most or all ingested nicotine molecules would remain sequestered in the bloodstream, incapable of reaching their targets in the brain, thereby stripping them of their addictive capacity.

The approach thus far has met with mixed results. Though animal studies and human trials have demonstrated a clear correlation between high levels of nicotine antibodies and reduced nicotine dependence, vaccine effectiveness in inducing abstinence has so far been disappointing. Arranging vaccine components in just such a way as to target the

immune system's B cells, bind with them, enter the cellular interior and induce effective immunity remains a significant challenge.

The technique under study accomplishes this feat through the rational arrangement of vaccine components onto nanoscale structures, using the base-pairing properties of DNA – the biological carrier of the genetic code. The Yan lab has been on forefront of the design and fabrication of elaborate 2-D and 3-D DNA nanostructures and the rapidly advancing field is poised to enter the biomedical arena.

The use of programmable DNA nanostructures provides high precision and delicate control over the vaccine's essential ingredients, potentially improving immunogenicity, efficacy and safety. Chang and her group will fabricate three different candidate DNA nanostructures as platforms for the vaccine's active constituents. Two of these will be nanostructures comprising 8-arm and 12-arm branched DNA scaffolds, while the third is a DNA tetrahedral structure (see Figure 1).

Attached to each of these nanostructures will be several crucial vaccine components:

- Nicotine particles known as haptens will stud the nanostructure, acting to provoke an immune response. Haptens are small molecules capable of binding with antibodies.
- An adjuvant will attach to the DNA structure. Adjuvants are additives commonly used in vaccines to improve their immunogenicity. The adjuvant applied in this case is known as CpG ODN—recognized in prior research as a strong stimulator of B cell activity.
- T-helper epitopes will complete the vaccine structure. These are antigen components recognized by the immune system and necessary for the recruitment of helper T cells, which work in conjunction with B cells to generate antibodies to a target

antigen.

The techniques of DNA nanotechnology being pursued, many of them developed in the Yan lab, allow for precision fine-tuning of nicotine haptens, adjuvants and T-helper epitopes, as well as the DNA platforms these constituents reside on in the candidate vaccine.

The proper display of nicotine on the DNA surface in particular is critical for the process of recognition by nicotine-specific B cells of the immune system. The tetrahedral DNA structure contains up to 36 discreet positions for NIC: Th-epitope:adjuvant complexes. Should greater density of nicotine haptens, epitopes or adjuvants be required, an alternate technique – known as DNA origami – may be used, providing up to 90 sites for antigenic components.

Further, the self-assembling nanoscaffolds can be fabricated in a high-throughput fashion with high reproducibility. The researchers believe the completed vaccine candidates will more faithfully mimic naturally occurring immune cascades, producing a more effective vaccine. For this to occur however, the vaccine structure must be internalized into the cell. This activity will be monitored through the use of fluorescent dyes, which can tag molecules for microscopy.

For each of the three DNA nanostructures under investigation, two different adjuvants will be tested, along with one non-immunization control. The ratios of hapten, adjuvant and epitope will also be varied and tested for immunogenicity on a trial and error basis. The vaccine candidates will first be evaluated in a mouse model, with the most successful being tested in rats.

In addition to measuring blood and brain levels of nicotine following

vaccination, the test subjects will be evaluated for the self-administration of nicotine, which is considered the gold standard in terms of vaccine effectiveness.

With combined effort from a multidicsplinary team, the group's aim is to identify two to three vaccine candidates that can be moved toward further clinical testing.

Provided by Arizona State University