Laser confocal micrographs show pollen grains bearing complex surface topologies that have been micropatterned with a flowable "stamp."

objects bearing discontinuities in surface slope and protruding features (peaks) of high height-to-width ratio that hang over the surface present challenges. Alternatively, liquid carriers (such as a transfer film floating in water onto which an object is dipped) can be used to integrate functional components onto objects of complex geometry. However, it is difficult to deposit arrays of small components that are not first formed on a thin transferrable film with precision on complex geometry, because the movement of each array element is relatively restricted on a film compared with a liquid carrier. By comparison, printing and pick-and-place processes are more versatile regarding an object's geometry, but require the component material to be printable or graspable. This also requires the object to be digitally mapped in 3D, adding even more time and cost to manufacturing.

To overcome some of the constraints in additive-based surface modification processes associated with use of only solid or liquid carriers, Zabow describes a transfer technique for an array of functional components that are arranged in a complex geometry on the target (such as a periodic pattern of components, conforming to a curved surface). The method uses a sugar mixture as a pourable and dissolvable carrier and a process similar to that used in making hard candy. A heated sugar and corn syrup mixture is allowed to cool, but before it solidifies, it is poured over the components to be integrated onto the surface, forming a meltable "stamp." Zabow starts with a pouring and solidification step (casting) in which the sugar-based carrier is poured at low temperature over the functional components (including microscale metal, polymer, and glass elements) that have been prearranged in a desired pattern on an initial surface. The components—now embedded in a hardened sugar mixture "stamp"—are then transferred by slowly melting the stamp over the target object (hence, reflowing). After the deformed sugar mixture cools and resolidifies, the sugar mixture is washed away using water. Because the process uses a carrier that undergoes a phase change, it provides the control of solid carriers with the geometry matching of liquid carriers. Thus, the technique removes some constraints of solid, fluid, and contact mechanics associated with water and solid stamp-based transfer methods.

Zabow demonstrated the technique on a wide range of objects, including those with protruding features that overhang the surface, as well as on component and object materials such as metal disks, ellipses, and rings; polymer and glass microspheres; and hydrogels. The precision of the method was also demonstrated by attaching an array of thousands of 1-μm disks onto a pin head, which surpasses the boundaries of current transfer techniques.

The author also transferred desired functionality onto multiple biological surfaces including individual hair fibers, plant seeds, and animal cells. The ability to deposit conformal arrays across a range of micro- to macroscale objects that bear complex geometry using a biocompatible, water-based, low-temperature process may be transformative to various disciplines, including tissue engineering, as well as technologies such as biomedical devices, metamaterials, and sensors. For example, functionality integration with commonly used biomedical components (needles, fibers, tubings) could enable the design and manufacture of diagnostic and therapeutic tools, such as bioelectronic therapeutics (*7*–*9*).

There are many different directions to further develop the reflow-transfer technique of Zabow by considering fundamental principles in rheology (deformation of flowing films) and phase equilibria (optically or chemically driven phase transition). The process also opens the door to questions about quality and reproducibility. How the placement precision of the integrated functional components can be optimized and determining the limits on functional components that can be transferred are also questions to be explored. ■

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ACKNOWLEDGMENTS

B.N.J. acknowledges the support of GlycoMIP, a National Science Foundation Materials Innovation Platform funded through Cooperative Agreement DMR-1933525. B.N.J. also acknowledges the support of the National Science Foundation through CBET-2144310.

10.1126/science.ade6722

MEDICINE

The influenza universe in an mRNA vaccine

An mRNA–lipid nanoparticle vaccine protects animals from 20 influenza lineages

By **Alyson A. Kelvin**[1,2] *and* **Darryl Falzarano**[1,3]

The greatest challenge to preventing the next influenza pandemic is the extensive diversity within the influenza virus family (*1*). At present, 20 lineages of influenza A and B viruses have been identified, each containing numerous strains (*2*, *3*). Current influenza vaccines, composed of four influenza viral antigens, provide little protection beyond the viral strains targeted by the vaccines. Universal influenza vaccines that can protect against all 20 lineages could help to prevent the next pandemic (*4*). Designing and manufacturing a vaccine that can provide such broad protection has been challenging, but the demonstration of the feasibility of mRNA–lipid nanoparticle COVID-19 vaccines offers a possible strategy (*5*). On page 899 of this issue, Arevalo *et al.* (*6*) report an influenza vaccine, using mRNA–lipid nanoparticle technology incorporating representatives of all 20 influenza virus lineages, that protected mice and ferrets from diverse influenza viruses. This provides a pathway to a universal influenza vaccine.

Influenza viruses are an ever-constant public health threat because circulating viruses continue to evolve, and new viruses spill over from animal reservoirs. The 20 influenza virus lineages over both A and B viruses are defined by 20 different hemagglutinin (HA) proteins. Current approved seasonal vaccines focus immune responses on the surface HA protein of circulating influenza viruses. The HA protein is responsible for host cell receptor binding, thus facilitating infection (*7*). Neutralizing antibodies, directed toward the HA receptor binding domain can block influ-

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enza viruses from binding host cells, thereby providing protection from infection.

The most commonly used influenza vaccine is the split virion protein vaccine. These vaccines are produced in chicken eggs to grow selected viruses, which are subsequently inactivated with detergent to break up the viral particle. For seasonal vaccines, a maximum of four viral antigens are prepared in this manner. However, this platform has a limited ability to induce broadly protective responses. Recognizing the challenge of targeting 20 lineages at once, previous universal vaccine strategies have largely focused on exploiting conserved regions of viral proteins and manipulating the host immune responses to focus on these regions (*3*). Universal vaccine targets have included the internal nucleoprotein (NP) and the less accessible yet conserved regions of surface proteins, such as the matrix 2 (M2) protein and the stalk region of HA (*3*, *4*). Although more highly conserved, these proteins or protein domains are often difficult to produce, are poorly immunogenic, and elicit immune responses without blocking infection.

Arevalo *et al.* took an alternate approach to universal vaccine design by leveraging the mRNA–lipid nanoparticle platform to include mRNAs that encode HA proteins representing each influenza virus lineage in a single formulation. Although a good seasonal vaccine target, HA has so far been suboptimal as a single-antigen universal vaccine candidate (*7*). Using a brute-force approach, representative HA molecules of each of the 20 lineages were chosen for the mRNA vaccine. Selection was based on phylogenetic analysis and the likelihood that a particular HA would be a threat to humans. In mice and ferrets, vaccination elicited specific antibodies toward each of the 20 different HA targets in the vaccine. These findings highlight the flexibility of the mRNA vaccine platform to encompass a high number of vaccine antigens without interference among the various mRNAs.

Immunization of ferrets and mice also provided protection from both a virus that matched the vaccine components as well as mismatched (or heterologous) influenza viruses. Notably, lung infection of vaccinated ferrets with a matching virus strain was completely blocked, outperforming previous egg-based split virion vaccines (*8*, *9*). Additionally, the universal mRNA vaccine candidate elicited antibodies to the conserved HA stalk region, which is associated with broadly reactive antibodies and cross-protection. Because the HA antibodies elicited by the mRNA vaccine were against both conserved regions and the receptor binding domain, each antibody is hypothesized to cover other strains within each lineage. This was demonstrated by a heterologous influenza virus challenge whereby mice and ferrets vaccinated with the 20-lineage mRNA vaccine were protected from severe disease and mortality despite having evidence of viral infection in the lungs. Nonneutralizing antibodies and antibody-dependent cellular cytotoxicity (ADCC) were identified as mechanisms of protection. Conversely, T cell depletion studies suggested little involvement of cell-mediated immunity.

These results indicate that people vaccinated with a universal mRNA vaccine would also be protected from severe disease through B cell–dependent mechanisms if a completely new influenza virus strain were to emerge. Moreover, because most people above the age of 6 months have previously been infected with influenza viruses or are vaccinated, it is essential to determine whether a previous exposure could influence the outcome of immunization with a universal mRNA vaccine (*10*–*12*). To this end, Arevalo *et al.* showed that a previous infection with influenza H1N1 virus in mice did not alter vaccine-associated immunogenicity or protection.

"Using a brute-force approach, representative hemagglutinin molecules of each of the 20 lineages were chosen for the mRNA vaccine."

The strengths of the mRNA platform for pandemic vaccine production include flexibility of antigen design, increased numbers of potential viral targets, speed of production, and inexpensive and scalable manufacturing (*13*). These strengths are important when designing and producing vaccines for a highly diverse, unpredictable family of viruses that can easily spread globally in a matter of weeks (*3*, *14*). The current production timeline of the standard egg-based influenza split virion protein vaccines is 6 months, which is not sufficient to stop the next potential pandemic virus in the targeted time frame of 100 days, as recommended by the Coalition for Epidemic Preparedness Innovations (*3*, *15*). Formulating and manufacturing 20-valent vaccines on the current split virion vaccine platform would be nearly impossible. The high amount of protein needed for each component would potentially be unfavorable to the vaccinee and, as Arevalo *et al.* demonstrated, would not induce a balanced immune response to each of the 20 proteins. Currently, it is not clear why immunogenicity after mRNA vaccination remained stable as the number of targets increased. The authors hypothesized that this lack of immunodominance may be the result of the induction of long-lived germinal center reactions that regulate B cell clonal expansion, B cell maturation, and antibody focusing. It is also possible that the initial events after immunization may provide equal opportunity for antigen presentation. mRNA vaccines can be taken up by both muscle cells and antigen-presenting cells (APCs), whereas protein vaccines rely specifically on APCs for uptake. This may result in less opportunity for all antigens to initiate robust immune responses.

Although the 20-lineage universal mRNA vaccine or another mRNA influenza vaccine can quickly be scaled up to prevent the next pandemic, questions remain regarding the regulatory and approval pathway of such a vaccine that targets viruses of pandemic potential but that are not currently in human circulation. Hence, estimating vaccine effectiveness and developing a target product profile for a vaccine against viruses not yet identified is not straightforward. Clearly, discussions of this nature should not wait until the next pandemic virus emerges. Additionally, it is unknown how far this high-valency vaccine model can be extended and whether additional antigens from each lineage can be included to further increase protection or decrease the need to update the vaccine. Critics may argue that increasing the number of vaccine targets will increase influenza virus evolution to circumvent vaccine immunity. But this scenario seems unlikely because the multivalent vaccine from this study was associated with preventing viral infection and replication in vivo. These results suggest that this vaccine will most likely decrease the potential of new viral strains to emerge. Addressing both the limits of mRNA components and clarifying a pathway to approval are essential to the optimization and use of truly universal vaccines. ■

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10.1126/science.adf0900

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Science, **378** (6622), .
DOI: 10.1126/science.adf0900