JUNE 2022 Volume 1, Issue 2

VACCINE INSIGHTS

SPOTLIGHT ON:

Advances in formulation and administration

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Advances in formulation and administration

INTERVIEW

Advancing adjuvants: what's new & what's next in vaccine formulation?

GSK's Derek O'Hagan shares his thoughts on the evolution of adjuvants, the importance of systems vaccinology, and the road ahead for RNA vaccines.

Charlotte Barker, Editor, *Vaccine Insights* speaks to **Derek O'Hagan**, Senior Advisor Vaccines R&D and GSK Fellow, GSK

DEREK O'HAGAN is Senior Advisor Vaccines R&D and GSK Fellow at GSK. He is a qualified pharmacist and former academic researcher, who has worked on vaccine delivery and adjuvants in the pharmaceutical industry since 1993. In the mid 1990s, he worked on the novel emulsion adjuvant MF59, which is now included in a licensed flu vaccine in more than 40 countries. Prior to joining GSK, he was Global Head of Vaccine Chemistry and Formulation Research for Novartis Vaccines and was part of the team that established the Novartis program on self-amplifying RNA vaccines, now active in GSK. He has served on the Board of Scientific Advisors for the Controlled Release Society and is a Fellow of the American Association of Pharmaceutical Scientists.

Vaccine Insights 2022; 1(2), 9–14

DOI: 10.18609/vac.2022.003

Q What led you to work in with vaccines?

DO: I'm something of a rarity – a formulation scientist who works exclusively on vaccines. My PhD was focused on vaccine delivery, and I'm still working on vaccine formulation and delivery 30 years later. Before moving into industry, I was a Lecturer in Pharmaceutical Sciences at the University of Nottingham, carrying out Medical Research Council and

"RNA vaccines … pose many challenges for formulation and delivery, including protecting the RNA against degradation, promoting uptake of the RNA into the relevant immune cells, and ensuring that the RNA can escape the endosome without being destroyed."

WHO-funded research into vaccines. Since then, I've worked in small and large biotech and big pharma, but always on vaccines.

Vaccines have kept me hooked because of their immense practical potential. Science is fascinating; I love science. But I wanted my work to make a difference in people's lives, and it's hard to think of a field with a greater overall impact than vaccines.

Q What are you working on right now?

DO: In terms of disease areas, my focus – and GSK's focus – is on key unmet medical needs. Prior to COVID, RSV was one of the largest unmet medical needs worldwide and GSK has vaccine candidates in late-stage clinical trials to protect infants and the elderly. Meningococcus is another big target for GSK and we're currently building combination vaccines to cover the diversity of strains.

Vaccine adjuvants have been an area of interest for me throughout my career, allowing me to work across many different projects. GSK uses the concept of 'adjuvant systems' – a 'delivery system' approach that will be familiar to a lot of pharmaceutical scientists. It can comprise an emulsion or a liposome, and its purpose is to deliver an immune-potentiating agent, to focus its effects, and minimize any potential for poor tolerability or reactogenicity.

I also work extensively on RNA vaccines, which pose many challenges for formulation and delivery, including protecting the RNA against degradation, promoting uptake of the RNA into the relevant immune cells, and ensuring that the RNA can escape the endosome without being destroyed.

Q What are the biggest gaps in our knowledge regarding adjuvants?

DO: For a long time, adjuvant mechanisms of action were unknown. Adjuvants existed and were used – insoluble aluminum salts and emulsions for example – but no one could really explain how and why they worked.

In the last 20 years, there has been an explosion in our understanding of the activation of innate immunity by pathogen-associated molecular patterns (PAMPs) interacting with toll-like receptors (TLRs). It became clear that many adjuvants in development were essentially PAMPs. A good example is monophosphoryl lipid A, which is in several GSK products, and was shown to be an agonist of TLR 4. We now know of at least 10 human TLRs, with TLR4

DOI: 10.18609/vac.2022.003

and 9 already being exploited by existing adjuvants, and adjuvants targeting TLR7 and 8 in development.

The TLR system is an ancient recognition system that exists in all mammalian species. It's how our bodies recognize that we are infected and activate innate immunity, or at least that was our understanding a few decades ago. Since then, many other receptor systems of innate immunity recognizing all kinds of viral RNA, DNA, and cell wall components, intracellularly and extracellularly, have been identified. Now, there is a whole new range of targets beyond TLRs.

There are also other mechanisms whereby adjuvants work, for example by impacting lipid metabolism. These are all now druggable pathways that can be exploited through upregulation, downregulation, and modulation. There is a lot of space in the adjuvant world beyond the adjuvants we currently have. It's not so much a gap, as it is an opportunity.

Q How is the concept of systems vaccinology changing the way we look at adjuvant development?

DO: It is a way to accelerate adjuvant development, which has been very slow in the past as we did not understand mechanistically how they worked. Systems vaccinology is a multi-omics approach to exploring mechanisms of action, safety, and tolerability in humans. Instead of doing massive clinical trials with hundreds of people and looking for one readout, it involves doing small trials with a few people and looking for hundreds of readouts using many different omics techniques. It allows predictions of efficacy, safety, and tolerability, and helps you move quickly into late development, by establishing a solid basis on which to build a subsequent clinical development program. These days, we have sophisticated techniques to understand exactly what is happening in terms of activation and stimulation, and to look at the downstream consequences and any adverse events that show up.

Part of systems vaccinology is systems serology, which looks at antibodies in a much more sophisticated way. In the past, people looked at whether antibodies neutralized or not, or if they bind or not. With systems serology, the functionality of the antibodies induced can be seen in a very detailed way. You can look at the Fc portion, not just the antigen-binding portion, of an antibody and see if has the right glycans to mediate the functionality needed to protect against the pathogen.

Q In a recent article, you said "we've likely reached a major tipping point supporting the extensive development and licensure of new vaccines containing emulsion adjuvants" [1]. Why do you think that point is now?

DO: For nearly 100 years, there was only one class of adjuvants – insoluble aluminum salts. The next generation of adjuvants that emerged was emulsions, at the end of the 20th century. It started slowly with one seasonal flu product, and a few million doses every year, but during the 2009 H1N1 pandemic, hundreds of millions of doses were administered. We now have a decade's worth of follow-up on those subjects, and we can confidently say

that emulsion adjuvants are safe and effective, and can be used in all populations, from young children to the elderly. It's that accumulated safety record that makes me think we've reached a tipping point.

Since emulsions have been available, there have been many trials trying to understand how they work and what advantages they bring. Particularly in flu and other pandemics, they give much more potent immune responses, greater breadth of response, and allow antigen sparing. All of this in 2009 for the flu pandemic, was like a trial run for the current pandemic.

GSK is working on several protein-based COVID-19 vaccines with emulsion adjuvants. We are in a partnership with Medicago to develop an adjuvanted virus-like particle vaccine, COVIFENZ®, which was recently approved by Health Canada. We are also seeking regulatory authorization for a vaccine developed with Sanofi and we are in clinical trials with SK Bioscience Co., which is funded by the Bill and Melinda Gates Foundation, to make a vaccine available to as many people as possible.

It will be interesting to see how more traditional recombinant proteins and emulsion adjuvants compare with RNA vaccines. RNA vaccines are fast to produce and have proven safe and effective, but more established technologies may have advantages. Protein-based vaccines with emulsion adjuvants have been used in hundreds of millions of people over decades so the degree of confidence about safety is high, and there is the practical element of them being fridge stable and not requiring frozen storage.

One of the big challenges for COVID-19 vaccines is SARS-CoV-2 variability and different strains emerging. In the flu setting, adjuvanted vaccines with emulsions are good at covering the breadth of viral diversity. Will that translate into COVID-19? There is some preclinical evidence that supports it.

Q What have been the biggest impacts of the pandemic on the vaccine industry?

DO: Undoubtedly, the biggest impact is RNA now being a proven technology. It had been around for 10–15 years, and it was being explored predominantly in cancer vaccines by BioNTech and Moderna, and in infectious disease programs like GSK's. But nobody was trying to run fast with RNA, other than in the oncology space, because it was unproven. A year on, it's an established, proven, safe, and effective technology. That is a radical change. Now, we need to figure out what else it is good for and in which situation it might be better than the established technologies. Many companies are now saying 'let's invest in RNA and see what it can do'.

Beyond that, the speed at which new vaccines reach the market has changed. It used to take a decade or longer to develop a new vaccine; then we did one within a year. Are we going to go back to taking a decade? I don't think so. All the accumulated lessons on how to speed up development will be utilized in future. The question is: what lessons from the pandemic are broadly applicable versus those that are unique to the pandemic situation?

Q What's your view on the potential of RNA vaccines?

DOI: 10.18609/vac.2022.003

DO: I have been working on RNA vaccine projects since 2009, when I contributed to one of the early programs in RNA established by Novartis. We were working with self-amplifying mRNA, which some people still believe will be the 'next big thing' in RNA vaccines.

"RNA is the best way to make vaccines very quickly, so in a pandemic RNA is the way to go."

What was clear to us even then was that it was going to be valuable for rapid response. When you have a pandemic outbreak and you want to make a vaccine very quickly, that's definitely where RNA will be the best option, as Pfizer, BioNTech, and Moderna have now shown with their rapid response to COVID-19. RNA is the best way to make vaccines very quickly, so in a pandemic RNA is the way to go. Beyond that, there is still debate.

Companies that exclusively use RNA sometimes push the idea that RNA is the new way to make all vaccines. However, you are not going to replace all the vaccines that already work well with RNA. They could fulfill some of the major unmet medical needs, such as a vaccine for RSV and an improved flu vaccine, but it is still to be proven.

The COVID-19 vaccines have shown RNA is safe, but the tolerability question is still an open one. Most people would say they are reactogenic, in that adverse effects are quite common. If there is a highly pathogenic pandemic virus circulating, with lockdowns and so on, people are willing to accept that they may be sick for a couple of days after a vaccine. But for something like annual flu, I think that is debatable. Plus, COVID-19 vaccines contain a single strain whereas flu is quadrivalent, so arguably you are going to have more antigen and RNA in there, which may mean it is more reactive.

Coming back to self-amplifying mRNA, there is a belief that you can use lower doses because it has amplification machinery that produces multiple copies of the RNA encoding the antigen. It is still to be proven, but that would mean much, much lower doses of RNA, with the same efficacy.

Q Are there any other exciting developments you can see on the horizon?

DO: I have worked predominantly on vaccines that prevent infectious diseases. What has been tried for many years, with limited success, is the concept of therapeutic vaccines. Can you modulate the human immune response to give therapeutic benefits for chronic infectious diseases – for example, HIV or hepatitis C virus?

Pre-pandemic, RNA was already being explored primarily as a therapeutic approach in oncology. In the literature, RNA has also been explored for modulating allergies and autoimmune diseases. The immune system is complex, and it often goes awry. Can we modulate the immune system to overcome some of the problems we suffer when it goes wrong? Can we exploit it to our therapeutic advantage? Can we overcome cancer? I believe we are going to see vaccines being used for much more than preventing infectious diseases.

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Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author has recieved company support for attending meetings and/or travel. The author also has numerous patents, planned, issued or pending. The author also is an employee of GSK, and owns stock in the company. The authors have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited.*

Revised manuscript received: *Apr 12 2022;* ***Publication date****: May 26 2022.*

DOI: 10.18609/vac.2022.003

VACCINEINSIGHTS

ADVANCES IN FORMULATION
& ADMINISTRATION

SPOTLIGHT

Closing the window of vulnerability: better vaccine adjuvants for neonates

Kiva Brennan
The National Children's Research Centre & Trinity College Dublin

"Ultimately, we need pediatric-specific vaccines if we are to best serve children's health."

VIEWPOINT

Vaccine Insights 2022; 1(2), 127–129
DOI: 10.18609/vac.2022.021

Although it is well known that immune responses differ dramatically with age, most vaccines are still developed with the adult immune system in mind. To protect the most vulnerable in our society, we need to consider their unique needs when developing vaccines and adjuvants.

On May 20, 2022, Charlotte Barker, Editor, *Vaccine Insights* spoke to Kiva Brennan, Postdoctoral Research Fellow, The National Children's Research Centre, and Trinity College Dublin, about pediatric-specific adjuvants. This article has been written based on that interview.

It is clear that the neonatal and pediatric immune system differs in important ways from that of adults and that can have an impact on responses to vaccines. The adult immune system detects pathogens via families of pattern recognition receptors (PRRs) on the cell surface, notably Toll-like receptors (TLRs), which recognize bacterial or viral antigens and direct our immune system to respond appropriately. While children are born with a full set of cell-surface receptors, the responses are often dampened in neonates and young children, leaving them more vulnerable to disease and less responsive to vaccination.

Childhood vaccination programs see infants vaccinated against a range of dangerous infectious diseases over the first year of life, but two or three doses are often required to get a good immune response. This leaves a window of vulnerability in a child's life before they are protected. A good example is the pneumococcal vaccine – children receive two to three doses of the vaccine in the first 13 months of life, but often don't show effective protection against pneumococcal disease until 18 months.

The requirement for multiple doses across many months is a particular challenge in lower- and middle-income countries (LMICs) and areas of conflict, where it may be difficult for parents to access clinics and authorities to maintain accurate vaccination records.

Most current childhood vaccines were designed, developed, and tested in adults. Historically, children were assumed to be 'small adults,' and the only concession to tailoring vaccines to their stage of life was to scale down the dosages. As in many areas of medicine, there has been a blind spot when it comes to the true stakeholders – those receiving the final product.

This blind spot is reflected in adjuvant development. Many pediatric vaccinations are still adjuvanted with Alum, which targets humoral or TH2-type responses. Over the last 20 years, as we continue to move towards safer and less immunogenic sub-unit vaccines, there has been a drive to develop adjuvants that encourage a broader TH1 response. These newer adjuvants have focused on PRRs such as the TLRs – this works well in adults, but less so in neonates and young children. One mechanism behind this difference, is the TLR-induced interferon (IFN) responses require endosome formation, which is blocked in neonates by downregulation of RAB11.

When I joined Professor Sarah Doyle's laboratory at Trinity College Dublin, I applied my background in TLR signaling and viral immune evasion to investigate the role of newly discovered PRRs in pediatric immunology. We examined the activity of various PRRs in neonatal cord blood and found that, unlike many PRRs, cytosolic nucleic acid (CNA) receptor activity is well conserved in neonates [1]. This makes evolutionary sense – when a baby is born, their microbiome is naïve. Therefore, many bacterial pathways on the cell surface are dampened to allow colonization, whereas cytosolic receptors remain intact to defend against viral pathogens.

CNA sensors can induce the interferon responses that TLRs lack in neonates. Combining CNA stimulation with Alum significantly boosts the immune response to Alum in neonatal cells, triggering greater proliferation of T-cells, IFN-γ, and interleukin (IL)-17 responses [2]. Our ongoing proof of principle study in neonatal mice will show whether the addition of a synthetic double-stranded RNA (Poly I:C) to existing Alum-adjuvanted vaccines will improve immune responses – potentially allowing fewer doses and earlier protection.

Other groups are exploring alternative pediatric-specific adjuvants. Ofer Levy's group

DOI: 10.18609/vac.2022.021

at Harvard University is looking at TLR7/8 – one of the few TLRs where the responses are intact and Ingileif Jónsdóttir's lab at the University of Iceland is doing exciting work with neonatal mouse models. The neonatal vaccination research community remains small, but I hope to see more researchers entering the field as the importance of tailoring vaccines to their intended recipients (whether children, adults, or the elderly) gains recognition.

Ultimately, we need pediatric-specific vaccines if we are to best serve children's health. There are certainly challenges – practical and ethical – in researching neonatal immunity and vaccination. But there is an increasing awareness that we need to find ways to do so, whether with umbilical cord blood cells, neonatal animal models, or carefully designed clinical trials.

UNICEF estimates that 1.5 million children under 5 years old die from vaccine-preventable diseases every year [3]. More effective pediatric adjuvants could allow us to vaccinate earlier and with fewer boosters, closing the window of vulnerability and saving lives.

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Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Prof Sarah Doyle's research group in TCD recieved funding from National Children's Research Centre, SFI, HRB, IRC, TCD. The author is named on the European Patent: "Vaccine adjuvants" with E Lavelle, S Doyle, C Harte, K Brennan, K Meade 18174947.4 – 1116 and US Patent: "Vaccine adjuvants". E Lavelle, S Doyle, C Harte, K Brennan, K Meade. 17/056,826. The author has no other conflicts of interest.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *This article was written based on an interview. On May 20 2022, Charlotte Barker, Editor, Vaccine Insights, spoke to Kiva Brennan.*

Submitted: *May 20 2022;* ***Revised manuscript received:*** *Jun 7 2022;* ***Publication date:*** *Jun 22 2022.*

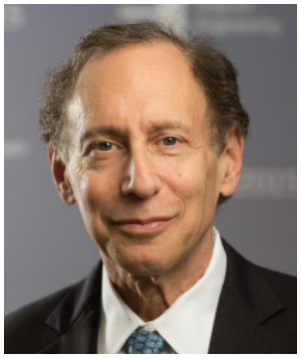
INTERVIEW

Engineering the next generation of vaccine delivery technology

Charlotte Barker, Editor, *Vaccine Insights* speaks to **Ana Jaklenec** and **Robert Langer** from MIT's Koch Institute

The Langer lab is one of the world's top research groups in biotechnology, materials science, and drug delivery. Vaccines have been a longstanding interest for the group. Their goal? To achieve global vaccine coverage by developing better delivery and manufacturing methods. Here, Robert Langer and Ana Jaklenec tell us about some of the most exciting projects underway in the lab, from self-boosting vaccines to microneedle patches.

ANA JAKLENEC (AJ) is a Research Scientist and Co-Principal Investigator in the Langer Lab, at the Koch Institute for Integrative Cancer Research at MIT. She has over 10 years of experience in the area of bioengineering, materials science, micronutrient, and vaccine stabilization and delivery. She has written over 30 articles in high-impact journals and has over 20 issued and pending patents worldwide. She is the recipient of the Ruth L Kirschstein National Research Service Award (NRSA) from the National Institutes of Health (NIH). Dr Jaklenec was elected to the American Institute for Medical and Biological Engineering (AIMBE) College of Fellows in 2022 for her work in controlled delivery of vaccines and heat-stable micronutrients for global health and was elected to the 2022 Control Release Society (CRS) College of Fellows.



ROBERT S LANGER (RL) is the David H Koch Institute Professor (there are 12 Institute Professors at MIT; being an Institute Professor is the highest honor that can be awarded to a faculty member). Dr Langer has written over 1,500 articles and has over 1,400 issued and pending patents worldwide. Dr Langer's patents have been licensed or sublicensed to over 400 pharmaceutical, chemical, biotechnology, and medical device companies. He is the most cited engineer in history (h-index 300 according to Google Scholar). Dr Langer has received over 220 major awards and is one of only three living individuals to have received both the United States National Medal of Science (2006) and the United States National Medal of Technology and Innovation (2011).

Vaccine Insights 2022; 1(2), 45–50

DOI: 10.18609/vac.2022.007

Q What are some of the most exciting projects you're working on right now?

AJ: Our goal from a research standpoint is to develop the next generation of vaccines, focusing on four key aspects: self-boosting vaccines, enhanced ability or ultra-stable vaccines, decentralized manufacturing, and needleless application and delivery.

"We're also extending the work to mRNA vaccines, and putting them into microneedle patches that are applied like a band-aid and can be easily shipped all over the world."

The COVID vaccine was developed and manufactured very quickly, but can we do better? Can we design vaccines that are broadly neutralizing and have an easier delivery, including everything from storage to administration?

The most important question is how do we vaccinate seven billion people? How do we reach all parts of the world, not just the US and Europe? We are so connected that infectious diseases can paralyze everyone unless we address them globally.

RL: When the Bill and Melinda Gates Foundation was getting started, one of the priorities was to solve the problem that people often do not come back for repeat injections, especially in the developing world. In 2017, Ana and our team published a new printing approach, making nanoparticles that 'pop' at pre-specified times based on the composition of the particles or the thickness of the shells, to release vaccines over time [1]. Over 200 days, we can give eleven discreet boosts with one injection, removing the need for repeat injections.

We're also extending the work to mRNA vaccines, and putting them into microneedle patches that are applied like a band-aid and can be easily shipped all over the world. We are doing that in collaboration with Mark Prausnitz, one of my former students and a professor at Georgia Tech.

Q What is it about vaccine delivery that attracts you as a research topic?

AJ: Working on delivery allows you to deliver anything, whether that is biologics or nucleotides. They are broad platforms and can be applied to different things, but the impact you can have with a vaccine is particularly large. There is something almost magical about stopping disease before it even begins – I find that inspiring.

RL: Even when I was a post-doc in 1974, we developed microparticle systems to deliver macromolecules like proteins and nucleic acids, and one of the first things that I thought about was vaccines [2,3]. When Bill Gates came to visit our lab in 2013,

he was thinking exactly the same way. Given the expertise of our lab, which has always been focused on materials and drug delivery, how could we make the greatest impact? Vaccines are the way to make a giant difference. Our lab has helped launch around 40 companies based on our work, including Moderna, which obviously had a big impact on the current pandemic.

Q Robert – as a co-founder of Moderna how did it feel when you heard that the COVID-19 vaccine was a success?

RL: Sadly, many people wanted Moderna to fail. There was a lot of skepticism. A well-known local newspaper, The Boston Globe, published a picture of me on the front page in May 2020 with the headline "This is not how you do science." By November 2020, we were able to announce data from a 30,000 patient trial, proving that the vaccine was around 95% percent effective. That result changed everything – for Moderna and for the world. It was a great feeling and very exciting but others at Moderna deserve most of the credit, not me.

Q Ana – can you tell me more about your work on self-boosting vaccines?

AJ: We use a microfabrication method termed SEAL (StampEd Assembly of polymer Layers). I tell people to imagine tiny coffee cups, smaller than a grain of sand – you put your coffee (vaccine) in and close the lid to seal it. Whatever is inside does not come out until you break the cup, or the lid comes off.

A nice aspect is that the formulation of the drug or vaccine that you put in is independent of the cup, and the cup opens up when the polymer degrades. You could use essentially any thermoplastic polymer, but we have been using the poly(lactic-co-glycolic acid) (PLGA) class. This material has been widely used for decades to make absorbable sutures and is safe both for children and adults. The degradation of the cup starts when it is injected into the body and becomes hydrated. Then, depending on how the molecular weight of the material, some will open up almost immediately, while others will open several weeks or months later. Usually, you need three shots of polio vaccine; using SEAL you can inject a mix of cups that open in one month, two months, and six months – replacing three injections with one.

Q What have been some of the biggest challenges so far with that approach, and how have you overcome those?

AJ: A big challenge was making them in the first place. They must have a core-shell structure, whilst still being small enough to be injected with a needle. We were trying to make spheres – we worked with leading 3D printing experts and many different labs, and we just kept failing. Then one day we had a meeting where someone said, "what if we made cubes?'. We tried methods used in the microelectronics industry and finally, it worked! The second

challenge is filling the particles – we had to work with a company that built custom robotics to fill the core and get drugs or vaccines inside.

RL: It is picotechnology, which is three orders of magnitude smaller than nanotechnology – pretty amazing!

AJ: We are about to complete a large animal study showing that an animal can be vaccinated with one injection of a self-boosting formulation, versus multiple standard shots, and be just as protected. Our goal, given the right resources, is to move into human trials sometime in the next couple of years.

Q How could your work on nanoparticles improve the delivery of RNA vaccines?

RL: We are working on both polymer and lipid nanoparticles. What has been done at Moderna and BioNTech is terrific but challenges remain with nanoparticles, including shelf-life and stability, targeting, optimal loading, and manufacturing challenges.

AJ: I agree with Bob that improving stability is important. Potency is also important, and that can be a function of kinetics, but also of targeting and adjuvancy.

There is also a mucosal aspect. What we are seeing with COVID vaccines, especially with new SARS-CoV-2 variants, is that vaccinated people can still become infected and shed the virus. A vaccine that triggers mucosal immunity could prevent that. Signaling molecules have been identified that can direct a vaccine to certain mucosal areas of the body. We are working on the area of enteric mucosal immunity, seen for example in *E. coli* or polio infections. We use a nanoparticle-based technology that targets the immune cells in the lymph nodes and signals them to migrate and activate the whole immunology cascade that imparts this mucosal immunity [4].

Q Ana – a paper you published in 2019 on tracking vaccination status made you a target for anti-vaxxers. What was the goal of the work?

AJ: While working on microneedle patches for vaccine delivery, we had discussions with the Gates Foundation about the challenges of medical record keeping in developing countries. In the US, we all have digital medical records, but in poor areas, with limited infrastructure, it is

"We are working on the area of enteric mucosal immunity... We use a nanoparticle-based technology that targets the immune cells in the lymph nodes and signals them to migrate and activate the whole immunology cascade that imparts this mucosal immunity."

DOI: 10.18609/vac.2022.007

very difficult to maintain accurate records. What is needed is some means to inform healthcare workers which vaccines somebody has already had, and which they may need, while maintaining the patient's privacy. We came up with a system using OR-like codes printed with near-infrared dye made up of nanocrystals called quantum dots. The dye is completely invisible to the naked eye and can be delivered alongside a vaccine via microneedle patches [5]. Healthcare workers could detect the patient's vaccination record using a specially equipped smartphone, without the need for a centralized database.

We felt this would actually be more private than a digital medical record, but the idea got pulled into a conspiracy theory about Bill Gates tracking people via microchips. Anti-vaxxers created a website to highlight our work and published my office address. It was shocking, but I felt that it was important to communicate the truth about the situation, so I gave several interviews explaining the real nature of the work. I hope that the huge number of people who have been vaccinated in a short space of time during the pandemic – and the difference that has been made to our lives – will help people see that vaccines are safe and effective.

Q What's next?

RL: With the Gates Foundation, we continue to work on microneedle patches, and we want to bring these into the clinic.

AJ: Self-boosting vaccines are something we are both passionate about, and we will continue that work with funding through the Gates Foundation. We have done work on vaccine printers with the Biomedical Advanced Research and Development Authority (BARDA), with the goal of decentralizing manufacturing so that vaccines can reach parts of the world with limited infrastructure. Ultra-stable vaccines would also make accessibility much better for all.

RL: We are also working on the possibility of oral vaccines. We published a paper in Science with Giovanni Traverso, an assistant professor at MIT, who has developed an oral pill that can inject mRNA, antibodies, or vaccines into the stomach or intestine [6].

Q What is the biggest lesson we can take from the COVID pandemic?

RL: Certainly, we have learned that vaccines and vaccine research are incredibly important.

AJ: I agree – we were taking vaccine research for granted prior to this pandemic. It was hard to start companies around vaccine technologies because they are prophylactic, inexpensive, and need to be distributed globally, so people did not see a big financial incentive. Now we know that vaccines can prevent the complete shut down of society across the globe.

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Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Langer R and Jaklenec A both receive funding from the Gates foundation. Jaklenec A is a consultant for Moderna and Langer R co-founded and also has equity in Moderna. For a list of entities with which Langer R is, or has been recently involved, compensated or uncompensated, see: https://www.dropbox.com/s/yc3xqb5s8s94v7x/Rev%20Langer%20COI.pdf?dl=0 . The authors have no other conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited.*

Interview conducted: *Mar 16 2022;* ***Revised manuscript received****: Apr 29 2022;* ***Publication date:*** *May 18 2022.*

DOI: 10.18609/vac.2022.007

VACCINEINSIGHTS

ADVANCES IN FORMULATION & ADMINISTRATION

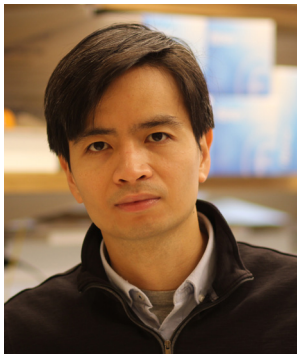
SPOTLIGHT

INTERVIEW

Developing a microneedle patch for vaccine delivery

Charlotte Barker, Editor, *Vaccine Insights* speaks to **Thanh Duc Nguyen**, Associate Professor, University of Connecticut

The COVID-19 pandemic has renewed longstanding interest in alternatives to injection for vaccine delivery. We caught up with Thanh Nguyen to find out more about a new microneedle patch that promises to deliver multiple doses of vaccine with a single application.



THANH NGUYEN is Associate Professor in the Department of Mechanical Engineering at the University of Connecticut (UConn), a position he has held since 2016. He completed his postdoc with Professor Robert Langer at Massachusetts Institute of Technology (MIT), where he developed a platform technology that can create three-dimensional microstructures of biomaterials such as biodegradable safe polymers (used in many FDA-approved devices) for applications in vaccine/drug delivery and medical implants. He obtained his PhD from Princeton University in the department of Mechanical and Aerospace Engineering (2013).

Vaccine Insights 2022; 1(2), 121–125

DOI: 10.18609/vac.2022.019

Q Your group works across a wide range of applications – what are the overarching themes?

TDN: Put simply, my work is driven by the desire to help people. I have a background in materials science and have worked in micromanufacturing, microfabrication, and biomaterials. When I started the group at the University of Connecticut, I wanted to combine

this knowledge to create a diverse biomaterials research program. We want to develop new materials and new manufacturing processes, with the overall goal to improve the quality of patients' lives.

Q When did you start working on vaccine delivery?

TDN: I entered the vaccine delivery field during my post-doc at the Massachusetts Institute of Technology (MIT) in 2013, where I worked on a Bill and Melinda Gates Foundation-funded project to make a single-injection vaccine. At that time, we were struggling to make a system that could deliver a vaccine repeatedly over a long period. I was first assigned to use 3D printing to create a micro-system which can achieve this via a single injection to release vaccines in a longitudinal manner, simulating the effect of multiple prime/booster injections. Unfortunately, the 3D printing technologies at the time were challenging to make a safe and pure polymeric system which is small enough to be injected through a normal syringe/needle. With colleagues at MIT, I came up with a new idea of using micro-molding and an additive polymer assembling process to fabricate a 3D micro-system which is small enough for injection, only contains safe polymeric micro-capsules, and can be controlled to deliver vaccines repeated over a long period of time. This process, which I called StampEd Assembly of Polymer Layer (SEAL), is inspired from the idea of Prof Bob Langer (my postdoc mentor at MIT) on developing something similar to 3D printing and based on my background of microfabrication and microelectronics [1].

> "When the COVID-19 pandemic happened, we quickly recognized this microneedle platform could be useful, not only in this pandemic but in preparation for the next."

However, the SEAL microsystems still rely on injections, which might not always be the ideal vaccination process. That is why, when I joined the University of Connecticut, we developed a new injection-free microneedle system, based on the earlier work with SEAL. We developed the second-generation of SEAL process – more scalable than the first version – to create arrays of tiny microneedles located on a supporting skin patch, similar to a bandage. We were able to use the patch to embed the microneedles into the superficial layers of skin (like an invisible tattoo, with no patch left on the skin after 5 mins of application) and show that the microneedles can be pre-programmed to repeatedly release Prevnar-13™ (a Pfizer vaccine against pneumococcal bacteria) over approximately 2 months. This triggers a high antibody titter and protects animals (rats) from infection with the deadly bacteria [2].

When the COVID-19 pandemic happened, we quickly recognized this microneedle platform could be useful, not only in this pandemic but in preparation for the next.

We were lucky to be the only academic group that received funding from BARDA to develop a vaccine patch during the pandemic [3]. Our microneedle system gave us the same immunogenicity as multiple subcutaneous injections that would need to be repeated over a long period. This single-time application of the microneedle is now being tested with the

DOI: 10.18609/vac.2022.019

COVID-19 vaccine, and we have shown neutralizing activity against SARS-CoV-2.

Q In your view, what makes microneedle patches ideal for needle-free vaccine delivery?

TDN: Microneedle patches could revolutionize the way that we vaccinate people. The current method of injections into the arm causes many problems that interfere with effective global immunization. Injecting vaccines with needles is painful, costly, and has poor compliance. People must travel to medical centers, sometimes repeatedly over months for booster shots. Booster shots also pose a big problem, as they require scheduling and return, as well as extra cost. This creates a huge burden, not only on the economy but also on the patient. This is especially a problem in developing countries. Another problem is vaccine storage at low temperatures and the need for cold chain facilities.

"Microneedle patches could revolutionize the way that we vaccinate people. The current method of injections into the arm causes many problems that interfere with effective global immunization."

The microneedle patch solves many of these problems. It can be shipped to people over large distances, without the need for cold-chain facilities. It can be applied to the skin like a sticking plaster, without any pain. It does not require trained personnel and only requires a one-time skin application – we can program the patch so it can deliver the vaccine repeatedly over time.

Q How are the patches produced?

TDN: To assemble the patches, we make the microneedle shell and insert the vaccine as a dry powder with an excipient for stability into the core, before capping it. It is similar to 3D printing, in that the shell and the core are assembled layer by layer, allowing the creation of a sophisticated structure. The shell is made of poly(lactic-co-glycolic acid), a biodegradable polymer used for erodible surgical sutures. We can control the molecular weight of this shell so that it is degraded at different time points, allowing the vaccine to be released [2].

For translational use, we need to carry out further investigation into scaling up the manufacturing process. However, the technology is based on a computer chip manufacturing process, so we believe that it can easily be scaled up and automated.

Q Is there any indication that microneedle patches might confer longer-lasting protection than traditional injections?

TDN: Our microneedles have a two-fold benefit. First, these tiny microneedles embed in the superficial layer of skin. This superficial layer hosts many dendritic and Langerhans

cells that trigger immune responses and help to boost the antibody level in the blood. This will increase the efficacy of the vaccine, even three- or four-fold compared to a subcutaneous injection of the same dose. Researchers have shown this dose-sparing benefit from the use of transdermal microneedles for flu vaccines [4].

The second – and unique – benefit of our microneedles is the ability to release the vaccine instantaneously and longitudinally over a long period to ensure that people will not miss any vaccine booster doses. There is no need remember the vaccine schedule or worry about storing the vaccines in cold storage conditions to avoid vaccine degradation. This single-time microneedle patch with built-in booster doses will sustain a high antibody level in the blood, thus creating an effective and long-term immune protection.

Q How does the side effect profile compare to injections?

TDN: We have tested the safety of these microneedles extensively on rat skin, using different vaccine antigens and even with adjuvants like Alum [2]. Alum is known to cause skin irritation in intradermal subcutaneous-injection vaccines, but even with the adjuvant, we found no skin irritation or any significant side effects from these rat studies, compared to the use of multiple bolus injections in the conventional vaccination process. We even did some preliminary testing with vaccine-free microneedles in a large animal model (pigs) and also found no side effect of the microneedles. To affirm this safety profile, we do intend to test different kinds of adjuvants and re-confirm safety of the vaccine microneedles in large animal models before moving to clinical trials.

Q What stage is the work at now and what's next?

TDN: The process has been optimized at laboratory scale. We have tested the microneedle system with different types of vaccine antigens, such as the pneumococcal vaccine, the COVID-19 antigen, and even mRNA vaccines. We have also used it to release small molecule drugs and antibodies. It is a platform that allows us to easily work with different types of antigens, drugs, and biologics.

We now have the data to show the safety and efficacy of the platform in a small animal model [2]. The next step is testing in a large animal model to confirm the safety and efficacy before we bring it to clinical trials. I recently founded a company called Single-Time Microneedles to commercialize and scale up the process of creating this microneedle patch.

Q What motivated you to become an entrepreneur?

TDN: This transition was also inspired by my mentor, Prof Langer. I wanted to create a highly transitional and impactful research program which can provide practical solutions for humanity. Academics can work on fundamental science but moving into clinical trials needs a lot of capital investment. We need to get help from industry to bring the product all the way from the lab to the end-user – and that is my ultimate goal.

DOI: 10.18609/vac.2022.019

BARDA also encouraged me to set up Single-Time Microneedles to allow us to raise funding and take this next step. We were luckily among the less than 3% of startup applicants to be selected into an extensive incubator program, gBETA Medtech (Gener8tor), which trains us on entrepreneurship skills/knowledge and allows us to meet and network with many great mentors, investors, and other founders to get support on moving to clinical use [5].

The biggest challenge for me is always to form a good team with motivated and collaborative people. Entrepreneurship is no different from academia in this respect. University of Connecticut has a terrific network for academic faculty to commercialize research products. And through wonderful mentors like Prof Langer, we can always build a great team.

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author's lab and UConn have been receiving research funds from NIH, BARDA, USDA, USAID, and MERCK. The author has founded two companies SingleTimeMicroneedles Inc. and PiezoBioMembrane Inc. The author has no other conflicts of interest.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited.*

Submitted: *Apr 4 2022*; ***Revised manuscript received:*** *Jun 2 2022*; ***Publication date:*** *Jun 21 2022.*