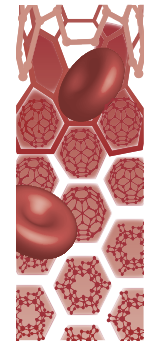
Interview

For reprint orders, please contact: reprints@futuremedicine.com

Nanomedicine



Drug delivery research and much more: interview with Dan Peer

Dan Peer*[1,2,3,4]

[1]Laboratory of Precision Nanomedicine, The Shmunis School of Biomedicine & Cancer Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

[2]Department of Materials Sciences & Engineering, Iby & Aladar Fleischman Faculty of Engineering, Tel Aviv University, Tel Aviv, Israel

[3]Center for Nanoscience & Nanotechnology, Tel Aviv University, Tel Aviv, Israel

[4]Cancer Biology Research Center, Tel Aviv University, Tel Aviv, Israel

*Author for correspondence: peer@tauex.tau.ac.il

First draft submitted: 3 April 2023; Accepted for publication: 24 April 2023; Published online: 18 May 2023

Keywords: controlled drug release • gene/drug delivery • immunology • infectious diseases • nanotoxicity • oncology

D Peer is a full professor and director of the Laboratory of Precision NanoMedicine at Tel Aviv University, where he also holds the position of vice president for research and development. He has published 160 papers so far, with over 20,000 citations and has registered many patents (135 granted and pending). In 2023, Peer was elected to the US National Academy of Engineering 'for developing innovative strategies for systemic, cell-specific delivery of RNA payloads'. He has also been involved in various biotechnology companies as a founder and member of scientific advisory boards.

How did you first get involved in the field of targeted delivery of RNA therapeutics?

It's a tough question. I did my education in the field of drug-delivery systems, developing liposomes, targeted liposomes. When I was a postdoc, I was introduced to the field of siRNAs. I knew about plasmid DNA, but siRNAs were fairly new. There was a challenge on developing good entrapment for siRNA or good strategies to deliver siRNAs. This is how I started.

Can you describe some highlights or a key turning point in your career since that beginning?

I think we did some pioneering work by understanding both the chemistry part of it and the biology. We were the first to show systemic delivery of siRNAs to immune cells *in vivo*, back in 2007. It seems to be not long ago, but this is how we started.

Then we were the first to show systemic cell-specific delivery of messenger RNA in an animal. That was also groundbreaking at that point because nobody could deliver mRNA in a cell-specific manner *in vivo*, in an animal. We're developing this in parallel to developing unique lipids, ionizable pH-sensitive lipids or entrapping nucleic acid. We're also developing strategies for cell-specific delivery. When you can combine those two, the lipid envelope for the RNA payloads, as well as the targeting moiety in different approaches, it becomes a whole story and then you can use it.

The next milestone that we achieved was the first, again, systemic cell-specific delivery of CRISPR-Cas mRNA with single guide RNA, and we're the first to show it could work in cancer *in vivo*. The next one, which was very recently, was the first bacterial mRNA vaccines. We're very much intrigued by the mRNA vaccines. We were challenged by the fact that nobody could develop a bacterial vaccine with mRNA. It took us a few years, but we published this very recently.

You mentioned about the first known mRNA vaccine for a bacterial infection. Can you explain a bit more about what made you investigate that area specifically?

You know that antibiotic resistance will be our next, next challenge in the world. Perhaps not in the next 10 years, but maybe in the next 20 years. We see more and more bacteria that are highly resistant, mostly in hospitals. Patients

Future Medicine

are going into the hospital with one disease and they're coming out, if they're coming out, with *Pneumococcus* or some other bacterium which are super resistant to antibiotics.

Therefore, we're challenged by this. We decided to develop a vaccine, at least to show a proof of concept that we can tackle a very aggressive bacterium. We use as a model *Yersinia pestis*, which is the etiological source of the Bubonic plague and the Pneumonic plague, but we used this as the model of the Bubonic plague, or the 'Black Death', in Europe in the 1300s. That's very challenging because it's very, very lethal – one bacterium can kill one person. This is something you cannot do in a regular academic lab, as you need special BL-4 labs to do those challenge trials. We then collaborated with the Israeli Institute of Biological Research, which has the facilities to perform those experiments. Now we're also working on some bacterial strains that have antibiotic resistance. They are less aggressive than this strain, but if we can make it for the plague bacteria, we probably can make it.

Can you talk more broadly about the other work your lab is doing?

We have several exciting projects. We're working on circular RNA, for rare genetic diseases such as Duchenne muscular dystrophy. We are working on some immune modulation strategies as well. We have some projects which are basic science, trying to understand mechanisms. We are increasing our arsenal of ionizable lipids. We have a very large lipid library; some of it was licensed by several companies. Hopefully, some of it will mature into clinical testing very soon. We very much like the field of drug discovery using RNA molecules.

We have a paper coming soon on a new target that we have identified for cancer. I cannot talk much about it because it is under embargo but it is something that is very logical but no proof of concept has been made over the years. It's a very interesting target, as many tumors have this in stable genome. Then the question is, what can you do with that? We've got some very convincing data that it might be a really important therapeutic target for cancer.

It sounds as though your research spans a variety of domains. Can you explain a bit more about that?

Everything you can do with RNA, I would say, but this is in a broad sense because many times we try to look for it from the biology standpoint. We don't have the right tools to study, for example, *in vivo* gene expression or *in vivo* gene knockdown or knockout. We have to develop those tools.

This is what we have been doing in the past 15 years. We've developed tools to study biology *in vivo* in the right environment and in the appropriate cells. I wouldn't say microenvironment but in the right environment. For example, gene expression or gene silencing, what will happen to other genes? What will happen globally to the cell? What will happen globally to the animal in different pathological conditions? We are not only delivery people; we use tools, and we develop tools for delivery of RNA payloads to study the biology and try to understand mechanisms.

One of the most exciting projects which we're running now in the lab is a trafficking project. We try to understand different chemistries. What will they do? How will they interact with some proteins intracellularly? How is the trafficking, how do the cells cope with extra RNA, with extra lipids? What is the degradation machinery? What is the expression machinery? What happens inside the cells, which is dependent on a formulation, and on specific lipids and on specific valence? It's a huge project, if you think about this, but it's a very basic biology project. Combining forces with experts in cell biology, in genetics, in chemistry, in microscopy gives you a very good picture of what's going on inside the cell.

You've described research at various stages of development & some of your lipid library being licensed. What from your research areas might be closest to becoming a product utilized in clinical practice?

Theoretically, the first one will be the vaccines but It's a variety of projects. I think that molecular medicines are really booming right now. I think this is the future; we are already living the revolution. I'm very happy.

11 to 12 years ago, 15 years ago, people told me in grants that I applied for that this can never be scaled up. Lipids, with RNA, with siRNAs as an example, could never be scaled up. mRNA, it's a lot of work; *in vitro* transcription can never be scaled up. Now, after 15 billion doses of COVID vaccines, whether manufactured by Moderna or by BioNTech-Pfizer, we've seen that there is no challenge anymore in the scale-up, at least not on the simple lipid nanoparticles with an RNA payload. Fifteen billion doses, just imagine that. There is no other example in humankind that you have either a vaccine or a drug that has been given to so many people around the globe. It's quite remarkable how this field has been changing and how it is booming.

Can you outline how this field of RNA therapeutics & vaccines overlaps & interacts with the field of nanomedicine?

I think it's basically the same. This is one example, probably the best example. You need to have a nanomedicine in order to deliver RNA vaccines. With small molecules or with peptide you probably can have other solutions. But in the case of nucleic acid, you must have a nanomedicine. The entire package was the nanomedicine, but it needs to be controlled by the nano size dimensions. Without nanomedicine, there will be no mRNA vaccines, no other types of nucleic acid which we see right now, reaching the clinic. We will see more and more of those. It may be different approaches, different materials, different strategies, but they will be in the nanosized area.

In 2013, you published an article in *Nanomedicine* about the grand challenges for siRNA. Looking forward, what would you say are some of the great challenges for RNA delivery now?

Standardization. The article was about the lack of standardization in the field. I think that at that point we still had a naive approach that everything needs to be standardized. But this naive approach became a reality because you need to have thresholds. If I do experiments in my lab, and they could not be reproduced by others in their lab, we have a problem. We needed standards. The journals also took this position.

Now many journals ask you to show your gating strategy if you do flow cytometry, to write exactly the resources of reagents and types etc. where they buy from – what is the catalog number? Also, the method needs to have standardization. One of the things that led to a dramatic breakthrough in the field of nucleic acid delivery was the introduction of microfluidic mixing strategies in the field. Then you have more homogenic preparation, which is very, very important, because if in my lab I have a system and in your lab you have exactly the same system, and we use the same reagent with the same compounds, it's very likely that we get the same results at the end.

These standardizations are essential to the field of nanomedicine because we are in a 20-year lag or even more from protein therapeutics, from antibodies, and the field of monoclonal antibodies is highly standardized already. This is what we need in the field of nanomedicine in order to see more and more inventions that begin in academia to become important influencers of clinical medicine.

Do you think that there are any other key factors for determining the path of RNA therapeutics in the next decade or so?

I think that the scope of standardization will grow. Where are your payloads from? Where are your nanomedicine compounds from? If they are lipids, which kind of lipids? Where can you achieve them and how pure they are? Purity is very important because, for example, if you don't have ionizable lipids as pure as possible, you might get degradation not only of lipids of the nanoparticle but also of the RNA at some point within the nanoparticles. Maintaining the RNA integrity, for example, in my field is super important. That's one of our challenges.

I think this is the future of medicine, of nanomedicine as well. There are many, many more things to do. We haven't discussed toxicity profiles so far. Those nanomedicines could be highly toxic. How can we reduce toxicity, liver toxicity, kidney toxicity, renal toxicity, lung toxicity, immune toxicity? Can we make this challenge into an opportunity?

This is another area to try to connect chemistry and understanding the biology with excellent cell biologists, biochemists and immunologists. I think that bringing them together to the table, all those people with different skills coming from diverse fields, as I have in my lab, gives you an overview, at least on a 30,000-feet level, to understand what's going on. But then you have to focus and go deeper into understanding basic mechanisms. Toxicity is one of our major, major challenges.

We need to increase the therapeutic window – it will make our life much easier. For example, lipid nanoparticles, if you're going to inject them systemically, will go to the liver eventually. A little bit of it could go to the spleen, a little bit to the lungs, but the real majority will go to the liver. Can we adopt a method or strategy that means less will go to the liver and more will go, for example, to tumors? This is another area with very important implications for the field.

I also think that people mostly publish what is very successful. They do not publish all their negative results. It's a different subject, but it's very important. We want to create an open discussion between scientists that will share even their worst results as long as they're reproducible. Because you can learn from those results. You can learn not to repeat the same mistakes and be very honest and open-minded in studying toxicity. Not many people study toxicity effects. It's not sexy. It's sexy to cure cancer. It's sexy to create new vaccines. It's sexy to treat amyotrophic

10.2217/nnm-2023-0121

lateral sclerosis. But it's not sexy to say that you understand the potential immune toxicity that happens because of cationic lipids. I think we need to be susceptible to people who study those fields, which by itself represents fundamental science.

Financial & competing interests disclosure

D Peer declares the following competing financial interest(s): D Peer receives licensing fees (to patents on which he was an inventor) from, invested in, consults (or on scientific advisory boards or boards of directors) for, lectured (and received a fee) or conducts sponsored research at TAU for the following entities: ART Biosciences, BioNtech SE, Earli Inc., Impetis Biosciences, Kernal Biologics, Newphase Ltd, NeoVac Ltd, Roche, SirTLabs Corporation, Teva Pharmaceuticals Inc. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Interview disclosure

The opinions expressed in this interview are those of D Peer and do not necessarily reflect the views of Future Medicine Ltd.