Scientists at Columbia University are reaping the benefits of automated process optimization with Sunshine

The challenge

The immune synapse governs essential cell fate decisions that dictate a range of adaptive immune responses. Understanding and manipulating the tightly controlled biological signals within this synapse is essential for enabling the development of novel therapies that can reprogram immune cells. Until recently, advances in this field have been grounded primarily in protein engineering, but pioneering researchers are now starting to explore the potential of materials science to accelerate the development of novel immunomodulatory nanomedicines.

Figure 1: Sunshine: Formulation optimization and scale-up.

Dr Santiago Correa is an Assistant Professor of Biomedical Engineering at Columbia University in the City of New York, and established his Nanoscale Immunoengineering Lab at the start of 2023. The new group works at the interface of materials science, nanotechnology and immunology, engineering multifunctional lipid nanoparticles (LNPs) directly from biological matter, for example using liposomes to crosslink polymer networks. This gives rise to self-assembling, injectable immunomodulatory nanovesicles with biomimetic properties, with the aim of redirecting the body's immune response to fight cancer, autoimmune diseases or infections. For instance, these nanotherapies can be used to guide immunostimulatory cytokines towards tumor cells, decorating cell surfaces and stimulating a highly localized immune response against the cancer. This approach holds promise for the design of targeted, effective and well-tolerated chemotherapies in the future.

Bottlenecks in nanoparticle development

Many nanoparticle formulation platforms are designed for scaling up production in preparation for moving a formulation into preclinical and clinical trials, making them unsuitable, and often unaffordable, for academic applications. Instead, research laboratories generally perform nanoprecipitation by hand, making tiny batches of up to 15 milligrams of nanoparticles at a time. Manual nanoprecipitation is understandably extremely time consuming and, of far greater concern, can result in high batch-to-batch variability.

Santiago commented: "I wanted to find a way to increase the throughput of our polymer nanoparticle (PNP) and LNP development process and avoid laborious hand pipetting tasks wherever possible during formulation screening. We are a small team, so it's really important that we maximize our valuable lab time, so I knew that a manual precipitation approach would not be suitable for our upcoming projects.

Before purchasing Sunshine (**Figure 1**), I made a DIY microfluidic mixing system composed of syringe pumps, vials and a mixing device. This enabled us to produce gram-scale amounts of each type of nanoparticle – a huge step up from what we were previously able to achieve by hand. This higher output made it possible for us to process our formulations on the tangential flow filtration instrument, which had not previously been a viable option, due to our small sample volumes.

Higher throughput, lower variability

However, despite the boost in productivity, the team acknowledged some limitations to their homemade set-up, such as the lack of connectivity between separate components, and the need to program each pump individually – a complex and arduous task. In addition, the initial DIY system did not produce any data logs or reports for long-term monitoring or troubleshooting, and could not completely eliminate the variability between batches.

"These issues were hindering the lab's ability to scale up production for potential preclinical studies," Santiago explained. "This motivated me to search for a commercially available automation platform that would reproducibly generate larger quantities of nanoparticles in a shorter space of time, ultimately accelerating progress towards future preclinical trials."

The solution

Microfluidic mixing (**Figure 2**) has become a leading way to reproducibly generate nanoparticles within both the academic research and pharmaceutical sectors, and the value of the technique for developing highly effective therapeutics was further highlighted by its role in the rapid generation of large quantities of life-saving vaccines against the SARS-CoV-2 virus during the recent global pandemic. The outbreak therefore stimulated the commercialization of cost-effective microfluidics systems – such as the Sunshine LNP process developer chosen by Santiago – that are user friendly and more suitable for lab-scale research.

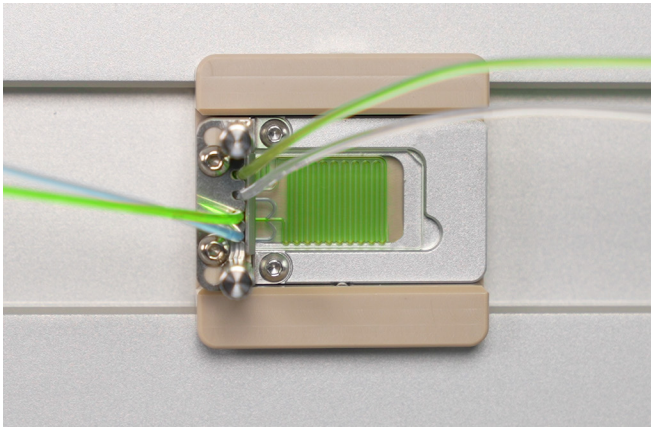

Figure 2: Microfluidic mixing with Unchained Labs' Sunny 490 Trident T.

"I'm fascinated with how nanotechnology can be integrated into larger macroscopic biomaterials, and I'm interested in characterizing how these materials interact with immune cells within the body. I was drawn to the idea of an easy-to-use, automated microfluidics system that would streamline both PNP and LNP development, even when working at a smaller scale. We also wanted to be able to set up a queue of different LNP formulation experiments, helping us to rapidly fine tune our product through LNP optimization. I discussed my lab's specific research needs and practical limitations extensively with Unchained Labs, and we compared the company's offerings with platforms from alternative providers. These detailed conversations quickly convinced me that Sunshine was the right choice for my group."

Figure 3: Loading the sample loops with reagent ahead of running an automated set of experiments.

The results

Santiago and his team are still early-stage users of the Sunshine (**Figure 3**), but they have already used the platform to successfully generate several different LNP formulations. Following on from this success, the group is now working on developing additional nanoparticles using the Sunshine.

"The Sunshine platform has given us substantially better consistency, reproducibility and yield in LNP formulation than our previous, homemade nanofabrication set-up, and eliminates many of the hands-on processes, so less of our bandwidth is

occupied by tedious tasks. This extra time has freed us up to plan what's next, and to experiment with alternative parameters, helping us to broaden the scope of our research."

"Beyond LNP formulation screening and LNP optimization, we are also interested in exploring the use of Sunshine for the inline modification of nanoparticle surfaces, enabling the precise and economical generation of engineered hydrogel systems. We hope to scale up production of the successful hydrogels at some point in the not-too-distant future, in order to generate enough biomaterial for in vivo immunomodulatory studies. The flexibility of Sunshine gives us the potential to support our future innovations when we are ready to take the next big step in our exciting research," Santiago concluded.

Dr Santiago Correa

Dr Santiago Correa is an Assistant Professor of Biomedical Engineering at Columbia University in the City of New York, and established his Nanoscale Immunoengineering Lab at the start of 2023.

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