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A Scalable Microfluidic Platform for the Development of Lipid Nanoparticles for Gene Delivery

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Extended Abstract

Microfluidic devices have been broadly used to produce nucleic acid-delivery nanoparticles for genetic medicine because they offer control, reproducibility and scalability of the nanoparticle precipitation process to overcome a significant challenge in the translation of these therapeutics [1-5]. Control over process parameters afforded by microfluidics, allows optimization of nanoparticle quality and encapsulation efficiency [2]. Automation improves the reproducibility and optimization of formulations. The continuous nature of the microfluidic process is inherently scalable, allowing optimization at low volumes to conserve scarce or costly materials, and seamless scale-up of optimized formulations by employing multiple microfluidic mixers performing identical unit operations in parallel.

In this study, we present a scalable microfluidic platform for producing nanomedicines. The platform includes a system designed for production under cGMP conditions employing 8 parallel microfluidic mixers capable of producing a 25 L formulation of RNA lipid nanoparticles (LNP) in ~4 h. Seamless scale up of production was demonstrated by producing test batches of siRNA-LNPs against the blood clotting protein Factor VII (FVII) on each of 3 systems designed for different stages of nanomedicine development. The physico-chemical characteristics were determined by DLS, and HPLC, and *in vivo* efficacy was measured by assaying serum FVII levels in murine models.

With a system designed for bench-scale formulation development we produced 10 mL batches of siRNA LNPs of avg. diameter ~60 nm (PDI <0.1) with encapsulation efficiency >95 %. No differences were observed in physicochemical properties of these particles when batch sizes were scaled-up by 10x on a pre-clinical scale-up system or by 100x with a system employing 8 microfluidic chips arrayed in parallel. The particles exhibited consistent lipid composition and N/P ratio within the target specifications. In addition, nanoparticles manufactured across the microfluidic platform showed a similar dose-dependent gene knockdown achieving >90 % reduction in protein levels at a dose of 1 mg/kg. These studies demonstrated the seamless scale-up of nanoparticle formulations across the platform with the potential for producing large scale, clinically relevant volumes, of lipid nanoparticles. The system employing 8 parallel mixers can prepare up to 25 L of product under 4.5 hours at 12 mL/min per mixer and incorporates a disposable fluid path that eliminates the need for costly and time consuming cleaning validation.

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