PRINT® Nanoparticle Manufacturing Scaleup for Pharmaceutical Applications

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ABSTRACT

In an effort to translate PRINT nanoparticle fabrication from batch processes to continuous manufacturing, Liquidia Technologies has harnessed the scale of roll-to-roll processing with the precision of microlithography to enable GMP production of gram to kilogram quantities of nanoparticle based therapeutic formulations. The in-house capabilities now enable fast turnover from single wafer masters to thousands of square feet of nanopatterned mold, to thousands of square feet of discreet nanoparticles of predetermimed shape, size and composition. An overview of the technical challenges, advancements, and ongoing research and development in the area of PRINT processing scale-up, as well as selected pharmaceutical application results, will be presented.

Keywords: PRINT, nanotechnology, nanoparticle, therapeutics, manufacturing,

1 INTRODUCTION

Nanomedicine, an offshoot of nanotechnology, refers to highly specific medical intervention at the molecular scale for treating and curing disease or repairing damaged tissues, such as bone, muscle, or nerves. At this size scale – about 500 nanometers or less –biological molecules and structures operate inside living cells. Nanotechnology research is still in its infancy, and it may be at least five years or more before the first generation of truly engineered nanoproducts become commercially available. The pharmaceutical industry continues to evaluate the potential of these new technologies to alleviate the burden of rising research costs, improve the speed and efficiency of the discovery process, and create high-value new generation therapeutics. While nanotechnology is widely seen as having huge potential, the pharmaceutical industry remains skeptical that success at the bench scale can successfully be translated into high volume products.

Based on seminal work in Professor Joseph DeSimone's laboratory at the University of North Carolina, a novel, top-down approach to nanoparticle fabrication has been invented.[1-3] This approach, called Particle Replication in Non-wetting Templates (PRINT®, Liquidia Technologies, Inc.), uniquely enables the precise control over particle size, particle shape, particle composition, particle cargo, particle modulus and particle surface properties. For the first time, key therapeutic parameters such as bioavailability, biodistribution, and target-specific cell penetration are able to be simultaneously designed into a particle. The PRINT technology brings the precision and uniformity of the semiconductor industry to nanomedicine to create novel, complex particles with simultaneous control over structure and function.

Since its inception, Liquidia Technologies, Inc. has been addressing manufacturing scale-up of the PRINT process using roll-to-roll processing, and the current state of the efforts are reported herein.

2 PRINT PROCESS SCALE-UP

Nanoparticle fabrication using the PRINT process involves several distinct steps: 1-Replicate rigid, nanopatterned "post" master with Fluorocur® (Liquidia Techologies, Inc.) resin to create non-wetting "mold" with nanocavities (image A in figure 1); 2-Fill nanocavities with matrix material, solidify using thermal or chemical means, and remove from nanocavities (images B through E in figure 1); 3-Purify, functionalize, and formulate resulting particles as needed using pharmaceutically acceptable methods (image F in figure 1). In order to achieve high-throughput, continuous PRINT manufacturing, translation of each of these areas to roll-to-roll and compatible processing methods has been achieved, aspects of which are addressed individually below.

2.1 Tooling and mold fabrication

The inherent size limitation of nanopattererned silicon wafers (12" diameter or less) has required the internal development of 2-dimensional scaling techniques to create large area, continuous mold containing nanocavities. The resulting proprietary techniques enable fabrication of tiled, continuous tools to be produced in-house from a single wafer, which are then used to produce thousands of square feet of high-fidelity mold for use in nanoparticle fabrication (Figure 2). Seams are routinely produced with step heights less than one micron, and seams approaching 100 nm have been demonstrated.

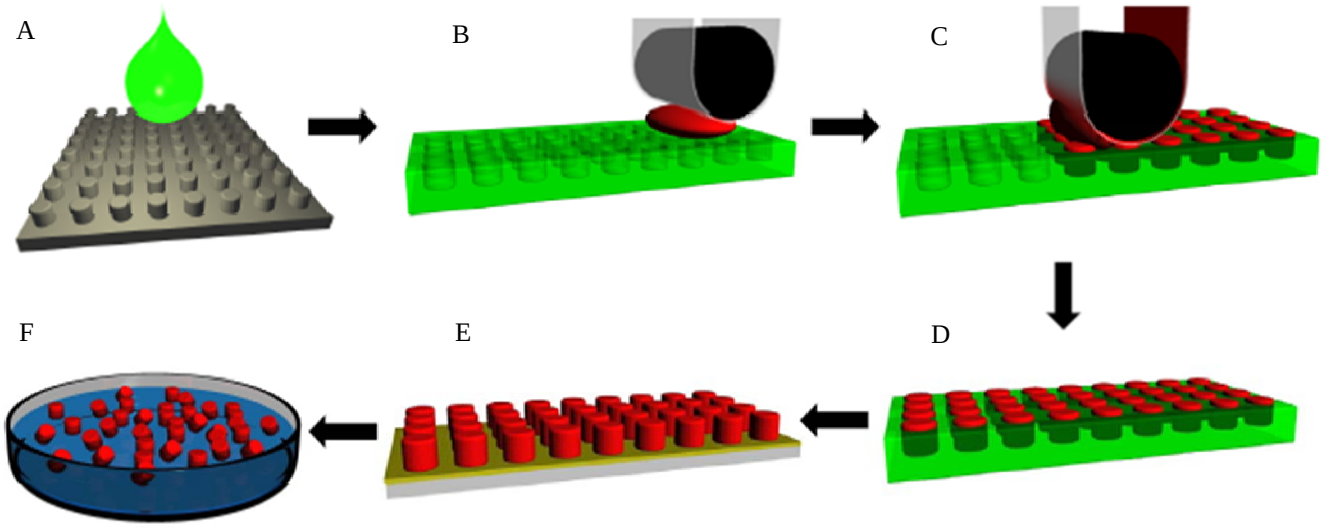

Figure 1. Depiction of PRINT process (clockwise from upper left image): A-Replication of rigid master via curing of Fluorocur resin to form "mold"; B-Application of matrix material to mold; C-Filling of cavities with matrix material; D-Solidification of matrix material; E-"Harvest" of discreet particles from mold; F; Collection of particles in suspension medium for further functionalization, purification, or other desired treatment.

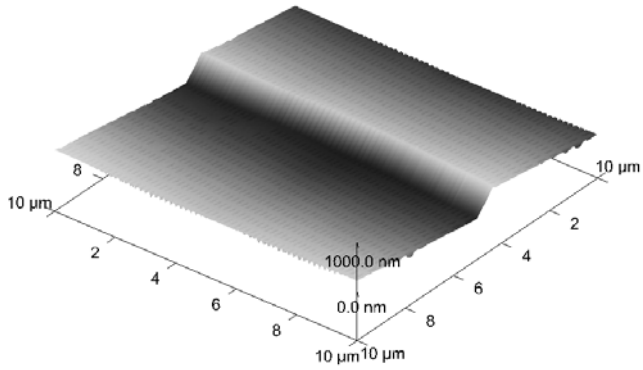

Figure 2. AFM image of 80x320 nm mold surface including seam of approximately 500 nm step height.

2.2 Nanoparticle fabrication

Enabled by the introduction of continuous Fluorocur mold material, translation of PRINT processing to roll-to-roll has utilized a proprietary and unique combination of known processing methods to achieve relatively high speed nanoparticle fabrication. Currently, approximately two square feet of particles in a two-dimensional array (Figure 3) can be produced per minute, corresponding to approximately 7 mg of 80x320 nm isolated, discreet particles (Figure 4) per minute. While this is a significant achievement for Liquidia, efforts are underway to improve throughput efficiency by 1000 fold.

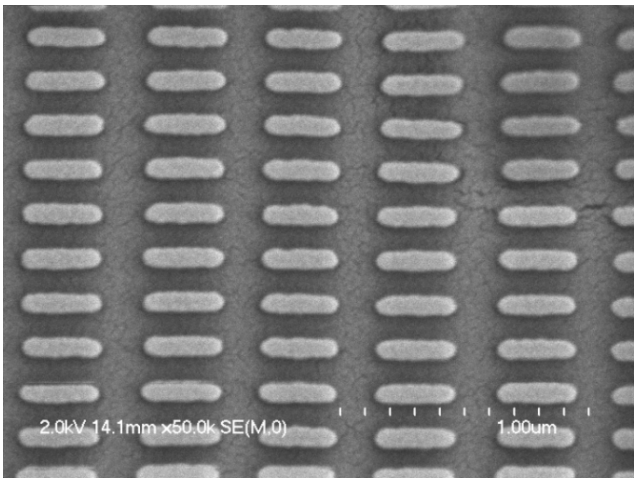

Figure 3. SEM image of 80x320 nm PLGA nanoparticles in a two-dimensional array

2.3 Formulation

In addition to the scale-up challenges facing new nanotechnologies such as PRINT, safety concerns and regulations inherent to the pharmaceutical industry must also be addressed. For consideration for human use, sterility of final formulated product must be achieved. It has been demonstrated at Liquidia that nanoparticles of a certain size can be sterilized by passage through a 0.22 micron sterile filter. Acceptable recovery yields have been demonstrated for nanoparticles as large as 80 nm by 320 nm, where the long particle dimension exceeds the nominal pore size of the filter, but due to the unique shape inherent to PRINT, passage through the filter in the correct

orientation enables simple, terminal sterile filtration. New, simplified variants of PRINT processing are also under development that will allow for aseptic processing for particles too large for terminal sterile filtration.

3 IN VIVO EFFICACY

Utilizing scaling advancements as described above, Liquidia has recently demonstrated the application of 80x320 nm PRINT processed particles as an adjuvant for influenza vaccines. By simple reconstitution of a lyophilized powder of surface-active 80x320 nm particles with a solution of commercially available trivalent influenza vaccine (Fluvirin®, Novartis Vaccines) before injection, a significant improvement in immunogenicity in mice was observed (Figure 5). These encouraging results warrant further investigation of PRINT processed nanoparticles in pharmaceutical applications as well as continued process research aimed at further throughput improvement.

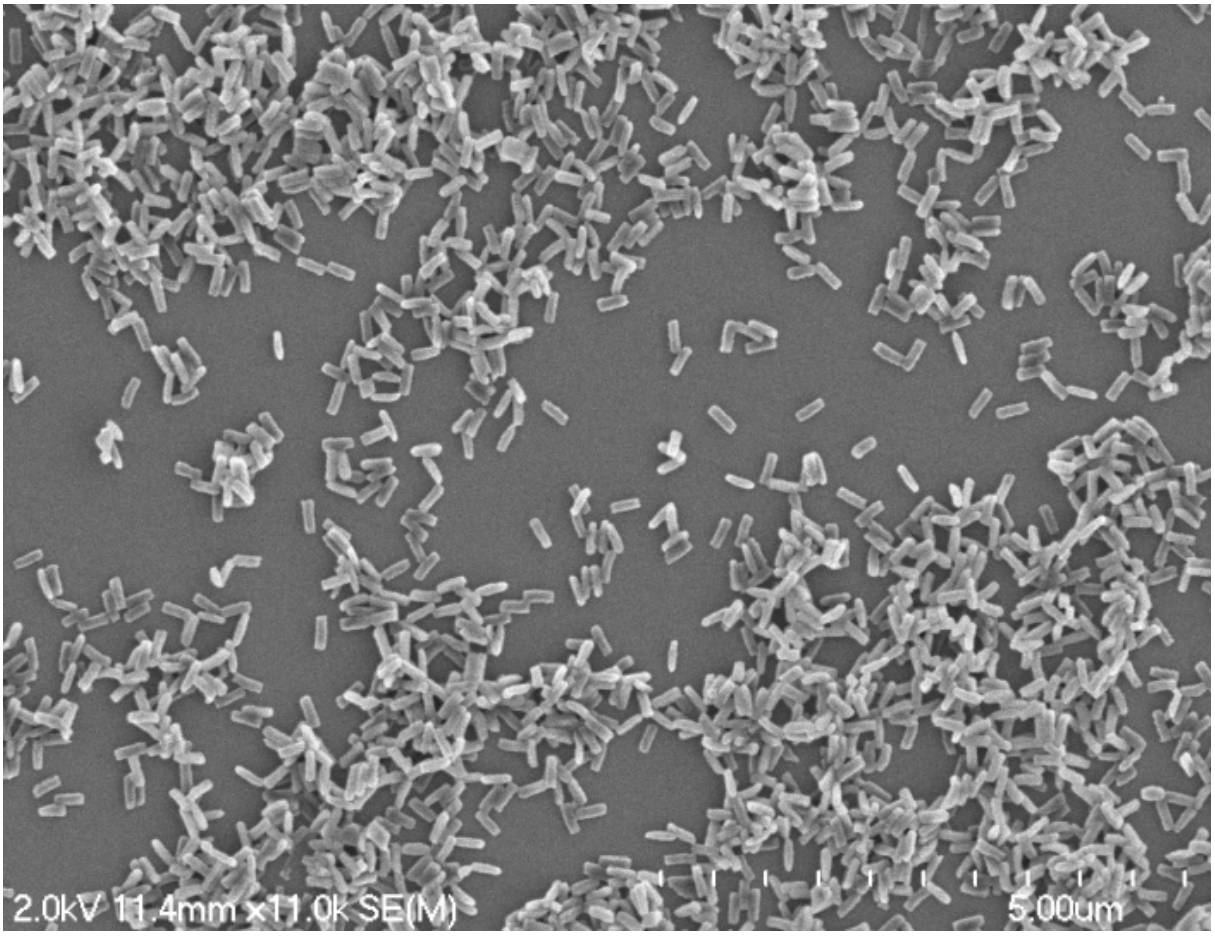

Figure 4. SEM image of 80x320 nm surface active PLGA based particles used as a vaccine adjuvant *in vivo*

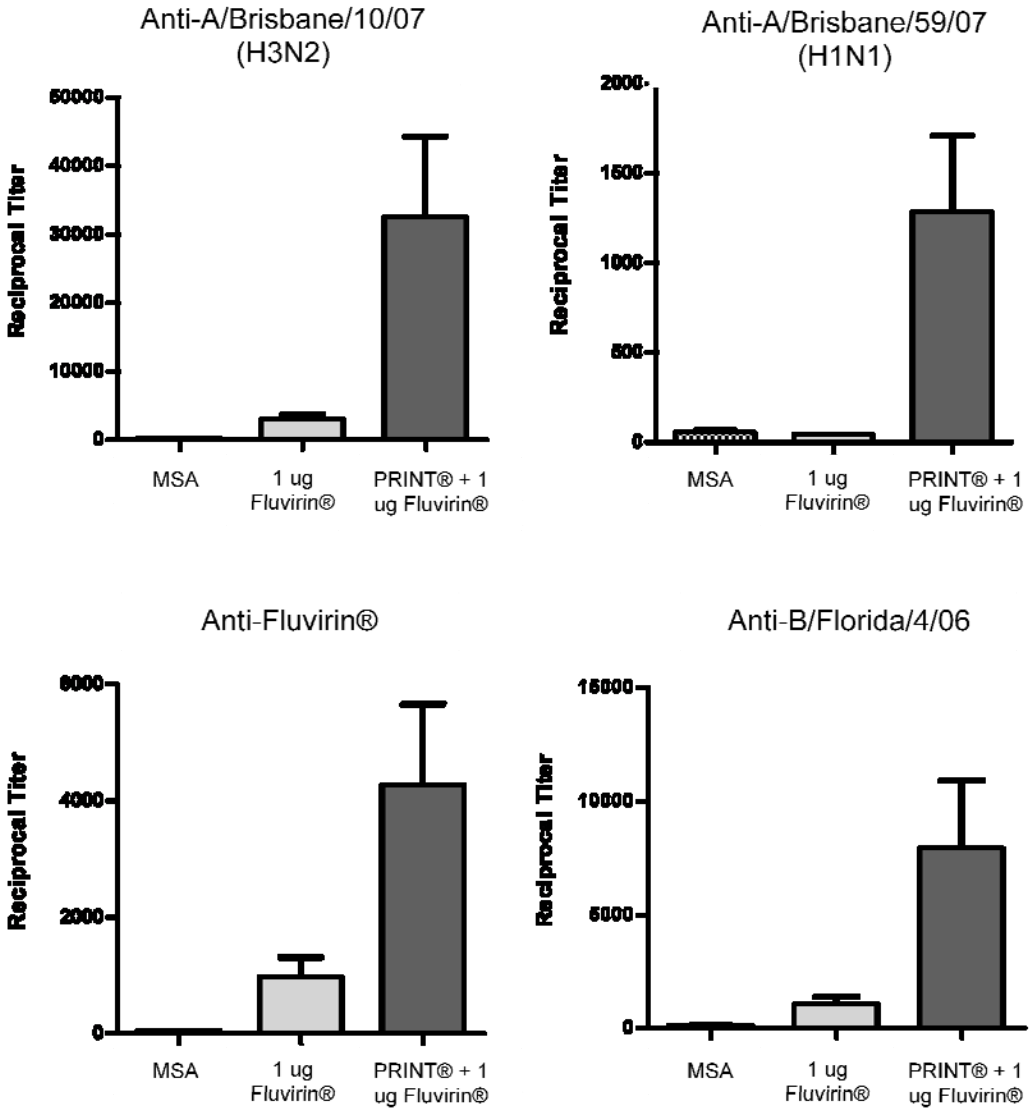

Figure 5. Mouse antibody response to commercial Fluvirin® influenza vaccine with and without use of surface active 80x320 nm PRINT® particles as adjuvant.

4 CONCLUSION

Liquidia Technologies has successfully translated the PRINT nanoparticle fabrication process from a low volume, batch scale technique to a continuous, high-throughput compatible process. This has been achieved by application of the well-established techniques of roll-to-roll processing combined with in-house research and development of tooling and molding techniques, and has enabled demonstration of PRINT processed nanoparticles in various potential pharmaceutical applications.

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