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A Review on Microfabricated Engineered Particle Systems for Drug Delivery-PRINT

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Abstract- **The unique characteristics of perfluoropolyether (PFPE) to fabricate monodisperse, shape and size specific particles ranging from a nanometer to micrometer size regime is utilized by Particle Replication in Non-wetting Templates (PRINT). Precisely engineered particles are produced by lithographic techniques derived from the microelectronics industry that has the nano-scale precision and spatial resolution thereby working as a platform for drug delivery. Here we describe the strategy for formulation, a 'Top-down' approach and delivery of small molecules not restricted to biological therapeutics using the PRINT technology; where particle size, shape and chemistry has been involved in a great extent in enhancing the systemic particle distribution properties and independent control of surfaces functionality. Further, the particular interest due to pharmaceutical needs for increased control over dry powder drug delivery and high therapeutic indices towards respiratory drug delivery has an important application of PRINT technology. Construction of liposomes, dendrimers and colloidal precipitates at nano-scale has adavantages when we speak of engineered nature of particle production. The edge of PRINT technology lies in its cargo carrying capacity including small organic therapeutics, biomolecules, macro-molecules; allowing a nano scale range facile incorporation of water soluble drugs.**

Index terms- PRINT, Drug delivery, therapeutics, perfluoropolyether

I. INTRODUCTION

Human disease treatment using particulate drug delivery systems play an important role whereparticles such as liposomes, protein nanoparticles and PLGA microparticles are currently used in marketed drug products using a variety of dosage forms [1, 2]. In particular, particle aerosol inhalation therapyis commonplace for the treatment of respiratory disease. Inhaled therapy using pressurized metered dose inhalers (pMDI), dry powder inhalers (DPI) and nebulizers is an attractive route for treatment of respiratory diseases, allowing for local delivery of high concentrations of therapeutics in lungs and avoidance of systemic toxicities associated with oral or injectable therapies [3–6]. Despite the prevalence of aerosol therapy, direct drug delivery to thearea of disease remains surprisingly inefficient in parts due to the lack of control of particle properties including particle size indrug formulation. Although a wide array of devices are available in the market [7], dose delivery efficiencies for dry powder asthma inhalers range from 3% to 15% for children and 10% to 30% for adults, indicating that less than one third of the contained drug actually reach lungs. The most advanced pMDIs deliver only 60% of the inhaled material to central and intermediate bronchial airways [4].

The preparation of respirable particle with reproducible and tunable aerodynamic property remains a challenge [4,5]. Conventional fabrication of these pharmaceutical aerosols for DPIs is accomplished by techniques such as micronization (milling) or spray drying [8]. These formulation techniques result in polydisperse aerosol populations with large particle size distributions and limited control over particle shape. Additional formulation challenges arise from forming dry, non-agglomerating powders comprised of pure active ingredients, especially biologicals like siRNA, proteins and monoclonal antibodies (mAbs). Dry powder inhaled mAbs or siRNA therapies are not in market as of now. The unmet need for improved aerosol drug delivery technologies is large. Respiratory diseases includeasthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis and influenza are a significant cause of morbidity and mortality worldwide, with an estimated 10 million lungs disease related deaths in 2004 globally and with health care costs in the US alone of a projected $173 billion in 2010[9,10].

It is well demonstrated the use of roll-to-roll particle nanomolding technology by a 'Top-Down' approach, (PRINT, Particle Replication in Non-wetting Templates) to fabricate monodisperse, non-spherical particles with unprecedented control over size and shape [11–13] and highlights the benefit of this approach. This approach can have positive effect on drug delivery and particularly for respiratory drug delivery. In this review, we highlight other published studies that demonstrate the breadth and applicability of PRINT drug delivery technology for applications beyond delivery in the respiratory tract, including systemic delivery.

In previous efforts, PRINT nanoparticles and microparticles have been used to study the effects of particle size on cellular internalization and particle biodistribution *in vivo*. Gratton et al. [14] studied the effects of particle size and shape on cellular internalization and intracellular trafficking and demonstrated significant dependence on particle size and shape in both the internalization rate and internalization pathways of HeLa cells [14]. Interestingly, the authors demonstrated that rod-like particles show a higher internalization rate than diameters equivalent to cylindrical particles. Merkel et al.[15] hadexamined the role *in vivo,*that particle modulus plays in particle circulation thereby finding that hydrogelmicroparticleswith low-modulus have eliminated half-lives of greater than 90 hours [15]. Increasing the stiffness of

these particles by increasing hydrogel crosslink density can reduce the elimination half-life 30-fold and change the accumulation of these particles from spleen to lungs and liver. These two studies highlight the importance that flexible control of particle size, shape and chemistry affords drug delivery vehicles. Additionally, the PRINT manufacturing process supports preclinical and clinical studies.A Phase-I clinical study of a PRINT vaccine candidate has been initiated by Liquidia Technologies, demonstrating the production of GMP pharmaceutical materials at a scale relevant to clinical development using this novel nanofabrication process [16].

The outcome of implementing this particle engineering approach for dry powder fabrication is improved aerosol performance applicable to respiratory drug delivery, demonstrated by incorporation of a variety of pharmaceutically relevant compounds. *In vitro* results demonstrate that PRINT particle aerosols possess high respirable dose, high fine particle fraction and tunable particle aerodynamic diameter. *In vivo* canine deposition studies demonstrate the ability to influence dry powder delivery as a function of particle geometry. These results suggest that this tunable particle engineering approach is a versatile platform for enabling next generation respiratory drug delivery. In this review we highlight someof the utility of PRINT for the production of particles not limited to small molecule, protein and oligonucleotide drug delivery demonstrating that PRINT is a diverse formulation approach and should find applicability in oral, parental and topical dosage forms for multiple disease indications.

The nature of PRINT technology takes drug delivery for the first time into the uncharted realm of engineered drug therapies given its *á la carte* approach and versatility. PRINT allows the simultaneous control over all of the parameters that are essential in the rational design of conventional delivery vectors in nanomedicine. It allows for the precise control over particle size (20 nm to >100 μm) through use of an appropriately designed master template. The advanced imprint lithography of PRINT ensures replication of the identical master features to afford particles that are truly monodisperse. Particle shape is also controlled through the judicious choice of a master and geometries such as spheres, cylinders, discs and toroids with defined aspect ratios can be accommodated. The composition of particles made using PRINT is also readily tunable and amendable to the inclusion of a variety of organic matrices including albumin, hydrogels, PLLA, PLGA, etc. Moreover, the porosity, texture and modulus of the particles can be altered in a logical fashion through careful alteration of the matrix formulation. In view of the fact that PRINT is compatible with a wide range of chemistries and the gentle nature in which particles are fabricated.A variety of cargos can be carried using this technology. The inclusion of hydrophilic or hydrophobic therapeutic molecules, oligonucleotides, peptides, proteins, siRNA, contrast agents, radiotracers and fluorophores can be accommodated through inclusion in the particle matrix.

The concentration of such cargos in the particles can be exactly chosen to meet specific needs and standards since PRINT does not rely on the kinetic trapping of external molecules during particle fabrication as is the case with liposomes and micelles. Finally, particle surface properties are readily modified though either the matrix composition or post-functionalization with surface moieties. Thus, the surface of the particle can be decorated with peptides being targets; as well as aptamers, avidin/biotin complexes, antibodies, cationic/anionic charges and 'stealth' poly(ethylene glycol) (PEG) chains for steric stabilization. We believe that PRINT is the only technology that can independently design in these attributes to create truly engineered nanovectors for drug therapies. For the first time, key therapeutic parameters such as bioavailability, bio-distribution and target-specific cell penetration can be simultaneously designed into a therapy. In this review we have also documented the first *in vivo* study that was carried out of PRINT particles administered intravenously into healthy mice. The promising bio-distribution profile and bloodpharmacokinetics of 200 nm non-targeted radiolabeled PEG-based nanogels fabricated using PRINT methodology are discussed.

1. The Process of Manufacturing

Particle Replication in Non-wetting Templates (PRINT) utilizes the unique characteristics of perluoropolyether (PFPE) to fabricate monodisperse, shape and size specific particles ranging from the nanometer to micrometer size regime. In Figure1, step 1-2 (below), a liquid (red) is evenly distributed over the PFPE mold (green) using a roller. In step 3, the liquid is solidified by a variety of methods (photochemically or thermally cured, lyophilized, frozen, etc) In step 4, the particles are harvested from the mold using a sacrificial adhesive layer. In step 5, the particles are collected in solution aftedissolving the sacrificial adhesive.

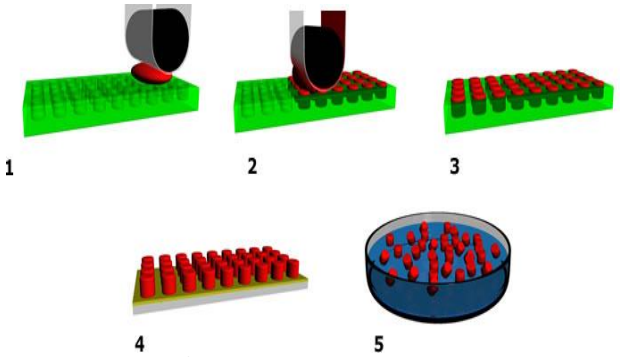

Figure 1: Process of synthesizing the the particles using the PRINT process**.**

In the PRINT process (above), the permanent silicon master template, using advanced lithographic techniques is fabricated. To the surface of the master template is then added the liquid PFPE fluoropolymer. The perfluoropolyether has a positive spreading coefficient which allowswetting the nano-scale features of the master template with extremely high fidelity. The master template after the fluoropolymer has been wet, is photochemically cross-linked and peeled away to generate a precise mold having nano-scale cavities. The low surface energy and high gas permeability of the PRINT mold enables the organic liquid precursor to the drug carrier particles to fill the cavities through capillary action, but it does not form an inter-connecting "flash" layer of liquid wetting the land area between the cavities. Such specific wetting and filling enables the fabrication of freestanding and harvestable particles that have the same precise shape of the silicon master template from which they were derived. Using a wide range of gentle chemistries the liquid in the mold cavities is converted to a solid then the array oforganic particles can be removed from the mold either by physical methods or by bringing the mold in contact with an adhesive layer. To harvest the PRINT particles, physical methods were used herein, which were then purified, characterized and radiolabeled for bio-distribution studies.

2.Examining effects of particle shape, size, modulus and surface chemistry on bio-distribution and clearance

Definitive bio-distribution maps that establish the interdependency of the deformability, shape, size and surface chemistry of nanoparticles *in vitro* and *in vivo* over length scales ranging from cells to tissues to the entire organism are needed by many different research communities. For example, fungal and bacterial pathogens are first and foremost recognized by their form or shape. However, the complete understanding of the role and significance of that form and shape is largely lacking. Indeed, some bacterial pathogens, including the bacteria *Salmonella*, *Shigella* and *Yersinia* and the gram-positive bacterium *Listeria monocytogenes* can induce their entry into non-phagocytic mammalian cells. Similarly, red blood cells and neutrophils are able to deform and undergo over 100 % strain (double in length) in order to navigate through various biological barriers that would prevent non-flexible objects from crossing. As such, nanofabricated tools (e.g. precisely defined particles) hold significant promise to provide insight into the fundamentals of cellular and biological processes. These tools can also yield essential insights into the design of effective vectors for use in nano-medicine, especially for the design of nano-particles for use as targeted therapeutics and imaging agents. Not much is in our knowledge about how the interdependency of size, shape, deformability and surface chemistry can influence the bio-distribution, cell-uptake and intra-cellular trafficking of micro-particles and nanoparticles. Beyond understanding the bio-distribution of particles delivered via parental routes (Figure 2), particle size, shape, deformability and surface chemistry should play a very significant role for understanding the mechanisms associatedwith particles that are inhaled either intentionally for use as a therapeutic or during environmental exposure. Understanding the role that mechano-biology plays as a function of shape, size and surface chemistry certainly lies at the core of how biological particles like neutrophils and red blood cells navigate their barriers. Ascertaining definitive bio-distribution maps through the use of precisely defined particle probes containing appropriate imaging beacons useful for quantification will undoubtedly lead to a set of rules that will be of immense use to science and to the application of nano-carriers to improve human health, treatment and diagnosis.

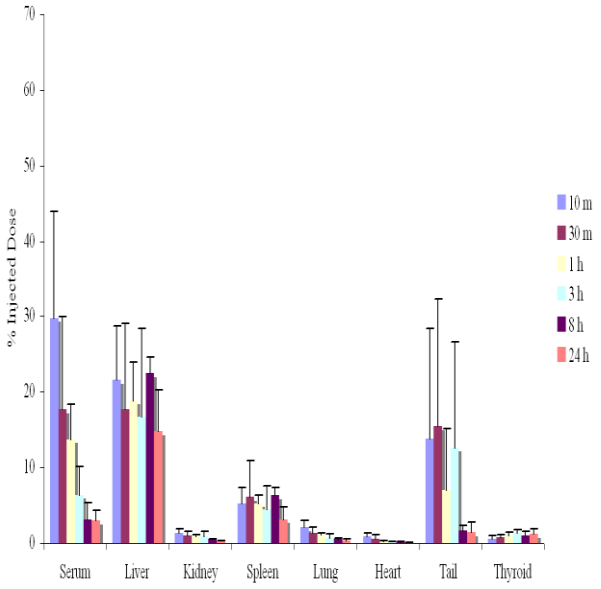

Figure 2: Bio-distribution of 125I-labeled 200 nm particles over 24 hours in healthy mice.

3.Imaging Modalities for a Dynamic View of Bio-distribution

It has been successfully designed, PRINT® particles that can be conjugated to 64 Cu, a long-lived ositron emitter useful for micro-PET/CT imaging. A work has been conducted in collaboration with the Stanford Center for cancer Nanotechnology Excellence Focused on Therapy Response and the CalTech/UCLA/Institute for Systems Biology Nanosystems Biology Cancer Center, which allowedmonitoring the bio-distribution of the PRINT® particles *in vivo*, in real time. Currently work is going on the incorporation of MR contrast agents as a cargo within PRINT® particles to complement the PET/CT results described below.

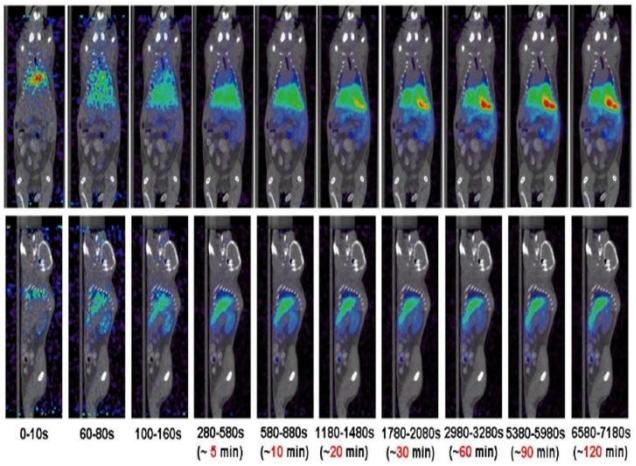

Figure 3: PET/CT result complemented using PRINT carrying a cargo of MR contrast agents.

MicroPET imaging with 64Cu-DOTA PRINT particles. Time resolved PET images consisting of a two hour dynamic scan was useful for drawing inference. The PET/CT images are overlayed. A mouse was injected with 136.2 μCi of 64Cu-labeled DOTA-nanoparticle and both the coronal view (top) and sagittal view (bottom) presented in Figure 3 was observed.

4.Drug Delivery

Potential therapeutics can be designed having biological activities using many peptides and proteins. The delivery of these peptide/protein drugs is one of the biggest obstacles to its clinical application. Taking advantage of the PRINT® technology, it is still under research how to deliver peptide/protein drugs into cells and maintain its biological activity.

5.Functional, Bio-absorbable Nanoparticles via PRINT®

Poly(L-lactic acid) (PLLA) and Poly(lactic acid-co-glycolic acid) (PLGA), first used for sutures, have more recently received attention as drug delivery matrices. PLLA and PLGA are bio-absorbable polymers that degrade hydrolytically at physiological pH and are then metabolized by the Krebs cycle. This makes them very attractive for drug delivery because there are no residuals after treatment. It has been found that both PLLA and PLGA particles are currently fabricated by either emulsion solvent evaporation methods or supercritical processing techniques. With these methods, spherical particles are generated, and while there is some basic control over size, the particles created are dispersed. Cargo encapsulation is also a potential challenge with these methods because the cargo typically has some affinity for the secondary phase and partitions out of the polymer phase before solidification. Finally, these methods tend to be solvent intensive, thereby harmful for the environment and increases processing costs.

Using the PRINT® technology platform, cleaner methods for making bio-absorbable particles with complete control over size and shape has been developed. These particles can be surface functionalized and can easily encapsulate a wide variety of cargos.

6.Nano-molding of Protein Particles

This research involves the nano-molding of proteins for the fabrication of protein PRINT® particles of monodisperse size and shape. Protein particles in lyophilised forms are generally highly dispersed in particle size, aggregated and often made through costly and complicated processes. There is little success in attempts to engineer mono-disperse, discrete protein particles using wet-milling, spray-freeze-drying, microemulsion or super critical fluid methods.A gentle, facile route is enabled by PRINT® technology to mono-disperse particles of 100% protein as small as 200 nm cylinders. Protein PRINT® particles of any shape and size are effortlessly achievable. Research has been proved a success in making PRINT® particles composed of albumin and albumin 0.5 wt % siRNA, and Abraxane, the gold standard therapeutic used in metastatic breast cancer.

7.Electric Field Assisted Delivery

Numerous diseases are localized in the body including cancer and cardiovascular disease. Although there aredrug therapies that have been developed, but the challenge is in providing a sustained, therapeutically relevant dose at the specific site of disease. PRINT® is a versatile technology platform that allows for the production of monodisperse, shape and size specific nanoparticles, which can easily encapsulate therapeutics and be tailored for a variety of release profiles thereby providing local, sustained and controlled therapeutic dosing. To deliver these particles to specific sites of disease this technology has been combined with iontophoresis, a method where electrical field is used to enhance the movement of charged molecules and particles into tissue. Severalresearch is going on of this therapeutic system, one in particular for the intra-luminal application ofnanoparticles to prevent bypass failure.

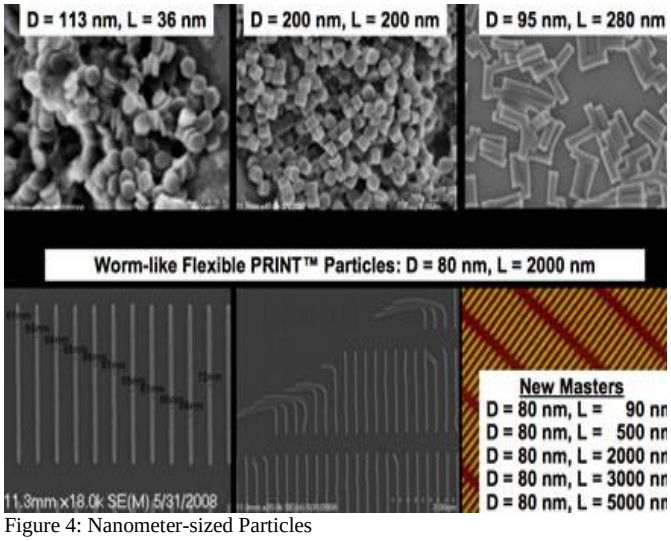

Figure 4: Nanometer-sized Particles

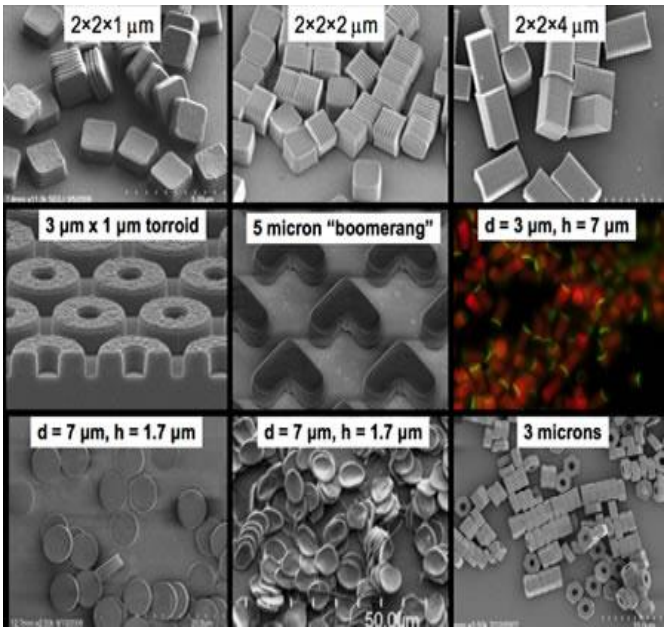

Figure 5: Micron-sized Particles

II. CONCLUSION

PRINT is the first general, singular method capable of forming organic nanoparticles in which critical design parameters can be precisely and independently tailored bringing a greater understanding of cause-and-effect to the field of nanomedicine. With the unprecedented ability of PRINT technology to control particle size, shape, composition, modulus, cargo and surface properties, questions such as the roles of size, shape and mechano-chemico functionality play on the bio-distribution of carriers *in vivo*and further understanding the detection, diagnosis, prevention and therapeutic strategies could be answered with a great deal of surety. As such, PRINT is a significant scientific and technological breakthrough, to revolutionize and accelerate our translational understanding, detection and treatment of disease allowing the fabrication of heretofore inaccessible populations of nanobiomaterials.In particular for respiratory drug delivery,high-performance aerosol particles are well developed by the PRINT process. Precise control over size and shape allows for defined aerodynamic properties, which, in turn, leads to enhanced aerosol performance and differential lung deposition *in vivo*. Micro-moulding is presented as a versatile strategy for formulating particle systems of small molecules, biologics, oligonucleotides and drug mixtures. Overall, micro-molding is a viable particle design strategy that may address challenges existing for respiratory drug delivery, dosage forms, hence constituting a high opportunity for the development of next-generation therapeutics.

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